From these results, it is concluded with respect to alkali-extracted main polysaccharides of the cell wall from *A.cylindrospora*, *M.mucedo*, and *R.nigricans* that (1) these polysaccharides contain the same componental sugars but in different molar ratios, (2) teichoic acid or similar substance, a well-known bacterial cell wall component, is not detected, (3) phosphorus content is very low (*cf.* the presence of high content of phosphorus was described in the cell wall of *Mucor rouxii*),[4] (4) a large part of D-glucuronic acid would be the branching point or exist as (1→3) linkage, (5) majority of D-galactose and L-fucose exist in a form oxidizable with periodate oxidation, and (6) in the Smith-type degradation of the materials, quantitative comparison between consumed galactose and produced glycerol suggests that a large portion of the glycerol are derived from (1→2)- or (1→6)-linked galactose.

The detailed structure of these cell wall polysaccharides will be discussed in a following paper.

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Synthesis of Methyl O-Acetyl-griseoluteate[1]

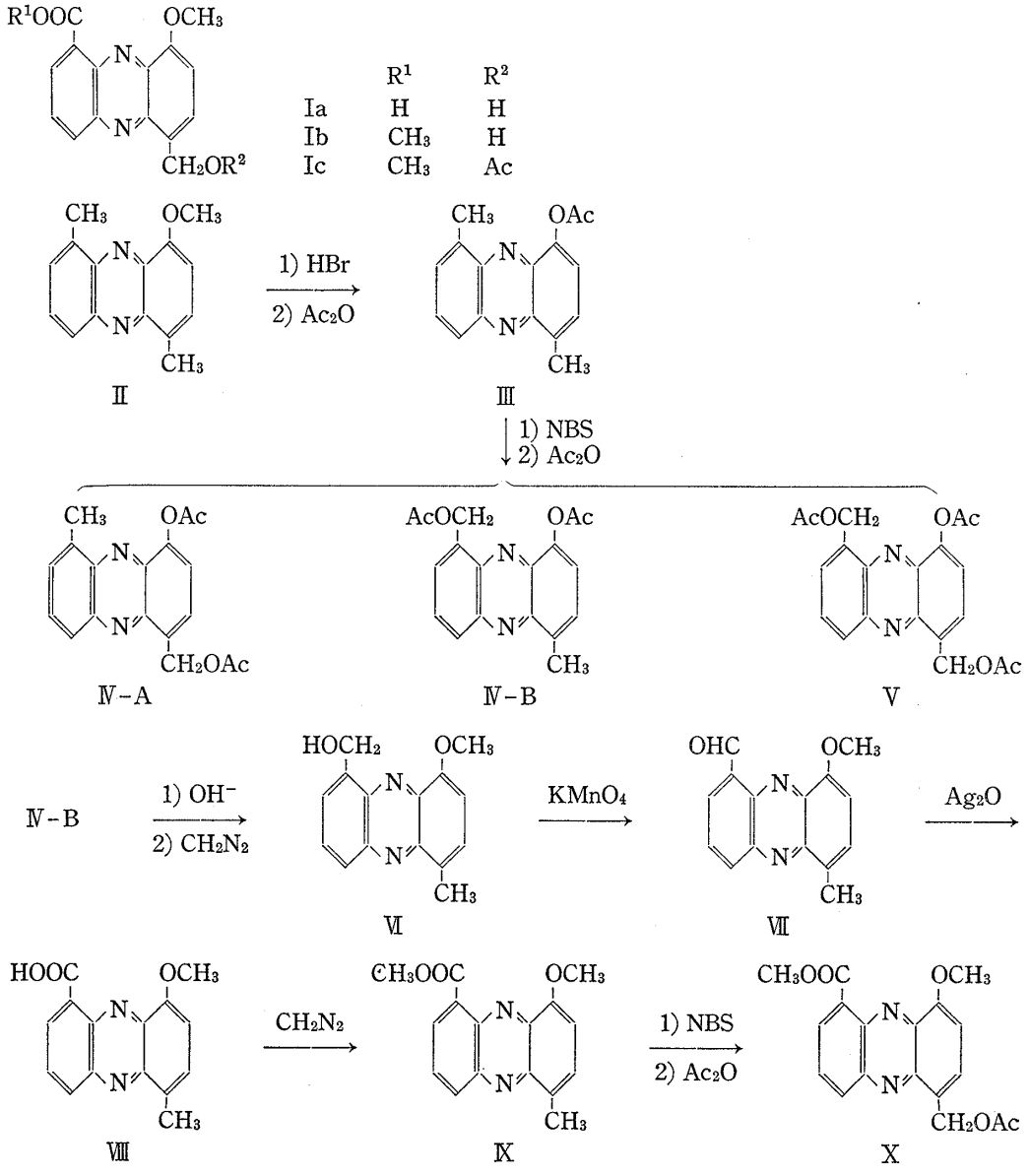
In 1958, S. Nakamura elucidated the structure of griseoluteic acid (Ia),[2] a hydrolysis product of an antibiotic griseolutein B[3] obtainable from *Streptomyces griseoluteus* and prepared a methyl ester (Ib) and a methyl ester acetate (=methyl O-acetyl-griseoluteate) (Ic) as the crystalline derivatives.

As the continuation of our synthetic studies on phenazine derivatives,[4] we have attempted the synthesis of griseoluteic acid derivatives, which has now been accomplished and is the subject of the present communication.

As the starting material, 4-methoxy-1,6-dimethylphenazine (II)[2c] was chosen. Demethylation of II with hydrogen bromide followed by acetylation with acetic anhydride and sodium acetate afforded 4-acetoxy-1,6-dimethylphenazine (III), $C_{16}H_{14}O_2N_2$,[5] mp 184°. IR

1) Studies on phenazines. Part XXXI.
2) *a*) S. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **6**, 539 (1958); *b*) S. Nakamura, *ibid.*, **6**, 543 (1958); *c*) S. Nakamura, *ibid.*, **6**, 547 (1958).
3) T. Osato, K. Maeda and H. Umezawa, *J. Antibiotics* (*Japan*), **4**, 34 (1951).
4) Series of "Studies on Phenazines." The latest publication, Part XXX: K. Ueda and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1521 (1968).
5) All the compounds given with the chemical formulae gave satisfactory analytical values. IR spectra were taken in Nujol mull unless otherwise stated. NMR spectra were measured at 60 Mc in $CDCl_3$ solution with tetramethylsilane as an internal standard. Chemical shifts were reported in τ-scale from internal tetramethylsilane. The mass spectra were taken with the Hitachi RMU-6D mass spectrometer.

cm^{-1}: 1767, 1203. NMR: 7.16 (3H, s), 7.15 (3H, s) (C_6H_5-C$\underline{H}_3$), in high yield. On bromination with one mole of N-bromosuccinimide either using benzoyl peroxide as a catalyst or under irradiation (100W high pressure Hg lamp) followed by acetolysis with acetic anhydride and sodium acetate, III furnished a di-acetoxymethyl derivative (V), $C_{20}H_{18}O_6N_2$, mp 165°. IR cm^{-1}: 1770, 1741 (sh), 1728, 1244, 1190. NMR: 4.18 (2H, s), 4.11 (2H, s) (C_6H_5-C$\underline{H}_2$-OAc), and a mixture of mono-acetoxymethyl derivatives (IV) as revealed by gas chromatography (GLC) (2 peaks on SE-30 at 200°) and by thin-layer chromatography (TLC) (an elongated spot on silica gel, Camag D-5, with different coloration between upper and lower parts as detected by ceric sulfate-sulfuric acid). Although the complete separation of two components of IV was with considerable difficulty, the repeated column chromatography (silicic acid, Mallinckrodt, and chloroform) has enabled us to isolate 1-acetoxymethyl-4-acetoxy-6-methylphenazine (IV-A), $C_{18}H_{16}O_4N_2$, mp 180°. IR cm^{-1}: 1767, 1746, 1256, 1210. NMR: 7.16 (3H, s), 4.11 (2H, s), from the earlier eluate and 1-methyl-4-acetoxy-6-acetoxymethylphenazine (IV-B), $C_{18}H_{16}O_4N_2$, mp 153°. IR cm^{-1}: 1767, 1737, 1219, 1199. NMR: 7.14 (3H, s), 4.21 (2H, s), from the later eluate. The assignment of both compounds was performed on the basis of the NMR analysis, particularly in terms of the comparison of the chemical shifts due to the methylene hydrogenes (benzylic position) of several analogous compounds, and the detail of which will be presented in near future.



On alkaline hydrolysis followed by diazomethane methylation, IV-B yielded smoothly 1-methyl-4-methoxyphenazine-6-methanol (VI), $C_{15}H_{14}O_2N_2$, mp 211°. NMR: 7.23 (3H, s), 5.94 (3H, s) (C_6H_5–$OC\underline{H}_3$), 4.69 (2H, s) (C_6H_5–$C\underline{H}_2$–OH), which lacks the carbonyl absorption band in its infrared (IR) spectrum. The hydroxymethyl derivative (VI) was then oxidized with potassium permanganate in acetone at reflux to afford an aldehydic compound (VII), $C_{15}H_{12}O_2N_2$, mp 211°, IR cm^{-1}: 1690. NMR: 7.22 (3H, s), 5.88 (3H, s), −2.03 (1H, s; assigned to the aldehydic hydrogen and was unaffected by the addition of D_2O), Mass Spectrum: 252 (M^+), in good yield. Further treatment of VII with potassium permanganate surprisingly was found to terminate mostly with the recovery of the starting aldehyde and with the trace amount of an acidic product. However, the conversion of the aldehyde function to an acid was successfully effected by silver oxide. Thus, treatment of VII in aqueous silver nitrate solution with alkali at room temperature for 1 hour furnished 1-methyl-4-methoxyphenazine-6-carboxylic acid (VIII), $C_{15}H_{12}O_3N_2$, mp 256°. IR cm^{-1}: 1720. NMR: 7.20 (3H, s), 5.89 (3H, s), in high yield. The methyl ester (IX), NMR: 7.21 (3H, s), 5.84 (6H, s), of VIII was subsequently subjected to N-bromosuccinimide bromination under irradiation followed by acetolysis as above giving a methyl ester acetate, mp 153°. IR (KBr) cm^{-1}: 1737, 1698, 1283, 1248. NMR: 7.88 (3H, s) (-OCOC$\underline{H}_3$), 5.91 (3H, s) (-COOC$\underline{H}_3$), 5.86 (3H, s) (C_6H_5-OC$\underline{H}_3$), 4.18 (2H, s) (C_6H_5-C$\underline{H}_2$-O-). The final product thus obtained was identified with methyl 1-acetoxymethyl-4-methoxyphenazine-6-carboxylate (X),[2a] mp 152°, prepared from the authentic sample of methyl griseoluteate (Ib) cordially supplied by Prof. S. Nakamura, in all respects (mixed mp, IR, TLC, NMR and Mass spectra). It should be noted here that in general on N-bromosuccinimide bromination[6] of the anisol derivatives, benzoyl peroxide tends to catalyze the nuclear bromination while under irradiation as in the present case the benzylic bromination takes place, which will be detailed in our full paper.

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Feasibility of Gas Chromatography for Ultra-micro Analysis of Aluminum in Biological Materials

Recently, gas chromatography has been extended to the study of inorganic fields such as metal chelates.[1] However, very little has been known for the application of gas chromatography to the analysis of ultra-micro amounts of metals which are present in biological materials. Meanwhile, the presence and the location of aluminum in human bodies have attracted much attention,[2] although biological significance of aluminum has not been clarified in detail. These facts described above stimulated us to investigate gas chromatographic analysis of aluminum in biological materials.

1) See for instance: R.S. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, Oxford, 1965.
2) I.H. Tipton, "Metal-Binding in Medicine," ed. by M.J. Seven, L. Audrey Johnson, J.B. Lippincott Company, Philadelphia, 1960, pp. 27—42.