Addition reactions of the C4'-C5' double bond of griseolic acid were investigated. C4'-C5' Dihydrogriseolic acid was obtained by reduction of the adduct having halogen at the 4'-position. The ring juncture of the two five-membered rings of the C4'-C5' dihydro derivatives was of all-“cis” configuration. Acetolysis of the protected dihydrogriseolic acid gave the corresponding 1'-acetoxy sugar derivative. Reaction of this sugar derivative with silylated bases gave guanine and uracil derivatives of the dihydrogriseolic acid. The cyclic nucleotide phosphodiesterase (PDE)-inhibitory activity of the C4'-C5' cis dihydrogriseolic acid derivative was weaker than that of griseolic acid. The uracil derivative of C4'-C5' cis dihydrogriseolic acid completely lost the inhibitory activity against both adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) PDE, whereas the guanine derivative showed reduced inhibitory activity against cAMP PDE, but retained its activity against GMP PDE. It was also apparent that the C4'-C5' trans dihydro derivative which was obtained as a minor product from the same culture broth of griseolic acid had almost the same inhibitory activity as griseolic acid.

Keywords—griseolic acid; base-exchanged derivative; adenosine 3',5'-cyclic monophosphate; guanosine 3',5'-cyclic monophosphate; inhibitory activity; cAMP phosphodiesterase; cGMP phosphodiesterase

Introduction

Griseolic acid (1) is a new type of nucleoside which was isolated from the culture broth of Streptomyces griseoaurantiacus SANK 63479.2) Its structure was subsequently determined as 1 by X-ray crystallographic analysis.3) We have reported synthetic procedures for griseolic acid derivatives having different substituents at the N1-, C6-, C2'- or C7'-position and their phosphodiesterase (PDE)-inhibitory activities.1,4,5) These studies revealed that the modification at the C6 or N1 position caused marked changes of the PDE-inhibitory activity, whereas substitution of the C2' or C7' position with various functional groups had little effect on the PDE-inhibitory activity. Furthermore, it became clear that 4',5'-dihydro-7'-deoxygriseolic acid (2, trans C4'-C5' dihydro derivative) which was isolated as a minor product from the same culture broth as griseolic acid showed almost the same PDE-inhibitory activity as that of griseolic acid.6) Model building studies of griseolic acid (1) and the trans C4'-C5' dihydro derivative (2) showed that the three-dimensional structures of the base moiety and the fused five-membered sugar moiety of these two compounds were very similar to each other and also to that of adenosine 3',5'-cyclic monophosphate (cAMP). On the other hand, the cis C4'-C5' dihydro derivative had a
completely different three-dimensional structure. Thus, we were very interested in the PDE-inhibitory activity of dihydro derivatives of griseolic acid at the C4'-C5' double bond. This paper describes the synthesis of cis C4'-C5' dihydro griseolic acid and its base-exchanged guanine and uracil derivatives. The PDE-inhibitory activities of these compounds were determined, and the role of the C4'-C5' double bond of griseolic acid in the PDE-inhibitory activity is discussed.

Results and Discussion

Synthesis

Synthesis of 4β,5'-Dihydrogriseolic Acid Derivative (Fig. 2)——It has been reported that the addition reaction to the vinyl ether bond can be carried out in the presence of an acidic catalyst such as mineral acid, p-toluenesulfonic acid, phosphoryl chloride or acid ion exchange resin.7) As griseolic acid has a vinyl ether moiety in its molecule, it was expected that griseolic acid would also undergo an addition reaction. When 3a was heated at room temperature for 3 d in acetic acid containing 4% hydrogen halide, a complex decomposed mixture of sugar and base moiety was obtained. However, when this reaction was carried out under restricted conditions, the desired reaction proceeded. For example, 3a gave 4a in 37%
yield when it was heated at 50°C for 15 min in acetic acid containing 4% hydrogen bromide. The adduct of acetic acid to the C4'-C5' double bond (5a) was also obtained by this reaction in the yield of 7.6%. In the case of reaction of 3b at 80°C for 2 h in acetic acid containing 4% hydrogen chloride, 4b was obtained in 46.5% yield. In addition 5b was also obtained in the yield of 6%.

Reduction of the halides (4a–4c) with zinc in an aqueous acetic acid or tri-n-butyltinhydride gave a 4',5'-dihydrogriseolic acid derivative (6a or 6b). Deprotection of 6a and 6b with aqueous alkaline solution gave 7a and 7b. The structures of these compounds were identified from the nuclear magnetic resonance (NMR) spectrum, elemental analysis and mass spectrum (MS). The ring juncture of the sugar moiety of 7a and 7b will be discussed later. Compound 6a was also obtained by medium-pressure catalytic reduction of 3a using platinum oxide in acetic acid at room temperature in poor yield. In addition, reduction of 3a with palladium on carbon in dilute hydrochloric acid at atmospheric pressure gave 6a quantitatively. None of the trans dihydro derivative was detected in the reaction mixtures of these reduction reactions. This suggests that reduction of the C4'-C5' double bond occurred from the same side as the adenine ring.

**Synthesis of Base-Exchanged Derivatives of 4',5'-Dihydrogriseolic Acid (Fig. 3)**

Attempts to exchange the base moiety of protected griseolic acid by using various acidic cat-
alysts were unsuccessful. In addition, acetolysis\(^9\) which usually yields the 1'-acetoxy sugar residue of a protected nucleoside, gave a complex mixture in the case of acylated griseolic acid. The reason for this seems to be addition reactions to the C\(^{4'}\)-C\(^{5'}\) double bond of griseolic acid. Accordingly, in the cases of 6a and 6b, a good result of acetolysis was expected. While in the case of 6a, acetolysis did not proceed smoothly, 6b gave the desired 1'-acetoxy sugar derivatives. That is, the 1β-acetoxy derivative (8a) and its α anomer (8b) were obtained in the ratio of 9 : 1 in good yield when 6b was allowed to stand at room temperature for 14 h in a mixture of acetic acid and acetic anhydride in the presence of concentrated sulfuric acid. It is considered that the formation of an acyloxonium ion by the 2'-acetoxy group of 6b caused the production of 8a as a main product. The sugar derivative (8a) was reacted with bis(trimethylsilyl)uracil at room temperature in the presence of tin tetrachloride to yield the uracil derivative (10),\(^{10-13}\) after deprotection with a 1 N aqueous solution of sodium hydroxide. The stereostructure was investigated in detail by decoupling and nuclear Overhauser effect (NOE) experiments in the NMR spectrum. These analyses are described in the next section.

The sugar derivative 12 was also obtained by the same method as 8a from compound 11. When 12 was reacted with bis(trimethylsilyl)acetylguanine in the presence of trimethylsilyl triflate under the same reaction conditions as used for 9, 9-guanino (13a) and 7-guanino (13b) derivatives were obtained after hydrolyzing the protecting groups of the products. The structures of these compounds were identified from the NMR and ultraviolet (UV) spectra, elemental analysis and fast atom bombardment (FAB) MS.

**Determination of Stereostructure of Compound 9** — The stereostructure of compound 9 was investigated in detail with \(^{1}H\)-NMR by means of decoupling and NOE experiments.\(^{14}\) The chemical shift of each proton of compound 9 and the decoupling data are shown in Fig. 4. The NOE data are shown in Fig. 5. Irradiation at 1'-H gave a 1.5% increase in the intensity of the 2'-H signal and irradiation at 4'-H gave a 5.2% increase in the intensity of the 3'-H signal. From these results, the configurations of 1'-H and 2'-H, 2'-H and 3'-H, 3'-H and 4'-H were determined as trans, cis, cis. In addition, irradiation at 1'-H unexpectedly caused a 4.5% increase in the intensity of the 7'-H signal. This fact suggests that 1'-H and 7'-H are located in close proximity. It became clear from a model building study that the uracil ring of compound 9 takes the β-configuration and the ring juncture of A and B is cis, and thus 1'-H, the A-ring, the B-ring, the 7'-carbon and 7'-H form a highly folded structure like a cage. On the other hand, the A-ring and B-ring of griseolic acid (1) and the trans dihydro derivative (2) form a planar structure. These conclusions are supported by the fact that 1'-H and 2'-H of 1 and 2 do not couple with each other, whereas the coupling constant of 1'-H and 2'-H of compound 9 was 6.3 Hz, which suggests that the dihedral angle of the two protons is about 135°. This relatively large coupling constant between 1'-H and 2'-H was also observed in compounds 4a—c, 5a—b, 6a—b, 7a—b, and 13a—b, which were supposed to have cis configuration between the A-ring and B-ring.
TABLE I. PDE-Inhibitory Activity of Griseolic Acid Derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>1a</th>
<th>1b</th>
<th>2</th>
<th>7a</th>
<th>7b</th>
<th>10</th>
<th>13a</th>
<th>13b</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 (µM) cAMP</td>
<td>0.16</td>
<td>0.32</td>
<td>0.14</td>
<td>12.6</td>
<td>188</td>
<td>558</td>
<td>50.0</td>
<td>73.0</td>
</tr>
<tr>
<td>cAMP/cGMP</td>
<td>0.25</td>
<td>2.46</td>
<td>0.22</td>
<td>0.23</td>
<td>55.13</td>
<td>1.65</td>
<td>33.3</td>
<td>1.11</td>
</tr>
</tbody>
</table>

PDE-Inhibitory Activity (Table I)

The test was carried out essentially according to Pichard and Cheung.\textsuperscript{15}) The details were reported previously.\textsuperscript{4,5)}

Naturally occurring 4',5'-dihydro-7'-deoxygriseolic acid (trans dihydrogenated form, 2 in Fig. 1) had the same PDE-inhibitory activity as that of griseolic acid, whereas the cis dihydro derivative (7a) showed only about 1/80 (cAMP PDE) and 1/85 (guanosine 3',5'-cyclic monophosphate (cGMP) PDE) of the activity. The cis dihydro hypoxanthine derivative (7b) showed about 1/1180 (cAMP PDE) and 1/26 (cGMP PDE) of the activity of 6-deamino-6-hydroxygriseolic acid (1b). These results revealed that trans dihydrogenation of griseolic acid caused little change of the three-dimensional relationship between the base moiety and the dicarboxylic acid residue. On the other hand, cis dihydrogenation caused a great change of this relationship. That is, the cis dihydrogenated derivatives have a highly folded stereostructure and can not bind tightly with the PDE binding site. This tendency was even more marked when the base moiety of the cis dihydrogenated derivative was changed to uracil. Thus, compound 10 had no inhibitory activity against cAMP or cGMP PDE. In contrast, the cis dihydrogenated guanin-9-yl derivative (13a) retained inhibitory activity against cGMP PDE, but showed 1/312 times weaker activity against cAMP PDE than that of griseolic acid.

From these results, it became apparent that uracil could not substitute for either adenine or guanine in the binding site of PDE. In contrast, hydroxanthine seems to be able to substitute for guanine but not adenine.

Conclusion

It has been reported that the structure of griseolic acid is an extended one, as determined by X-ray crystallographic analysis,\textsuperscript{3)} and it also became clear from model building studies that griseolic acid (1) and the trans dihydrogenated natural product (2) had almost the same three-dimensional structure. In contrast, the cis dihydrogenated derivatives, which were first synthesized in this work, had completely different stereostructures from the natural compounds (1 and 2). The stereostructure of this type of compound was defined as a highly folded one by NMR analysis. It also became apparent that the PDE-inhibitory potency showed a good correlation with the three-dimensional structure of the griseolic acid derivatives. That is, the PDE-inhibitory activities of griseolic acid (1) and the trans dihydrogenated derivative (2) were almost the same. In contrast, cis dihydrogenated derivatives showed extremely low inhibitory activity against cAMP PDE. However, it is very interesting that cis dihydrogenated guanin-9-yl derivatives retained the same inhibitory activity against cGMP PDE as that of griseolic acid.

Consequently, it is expected that guanine derivative of griseolic acid which has the intact double bond would show strong inhibitory activity against cGMP PDE. Synthetic studies of guanine derivative along this line are in progress in this laboratory.

Experimental

General—Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. 1H-
NMR spectra were obtained with a Varian EM-390 spectrometer (90 MHz) and with a JEOL GX-400 spectrometer (400 MHz), and the chemical shifts are expressed in ppm from tetramethylsilane as an internal standard: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; br, broad. UV spectra were obtained using a Hitachi 200-20 spectrophotometer. Thin-layer chromatographies (TLC) were carried out on Merck silica gel F254 pre-coated TLC plates, layer thickness 0.25 mm, and spots were visualized by UV irradiation or by spraying with 30% aqueous sulfuric acid followed by heating. Ordinary chromatography was performed by the rapid chromatography method using Merck silica gel (Kieselgel 60 Art. 9385).

**Dimethyl O2',O7'-Diacetylgriseolate (3a)** — In a round-bottomed flask, 10 g (24.55 mmol) of dimethyl griseolate was dissolved in 150 ml of pyridine, and 33 ml (0.32 mmol) of acetic anhydride was added under ice-cooling. The mixture was allowed to stand at room temperature for 2 h. At the end of this time, 15 ml of water was added under ice-cooling and the solvent was evaporated off under reduced pressure. The residue was dissolved in 400 ml of methylene chloride and the resulting solution was washed with 400 ml of a 1 N aqueous solution of hydrochloric acid, 400 ml of water and 400 ml of a saturated aqueous solution of sodium bicarbonate in that order. The solution was extracted twice with methylene chloride. The methylene chloride extracts were dried over anhydrous magnesium sulfate and the solvent was evaporated off under reduced pressure to give 6.70 g (55.6%) of 3a as crystals. **Anal. Calcd.** for C20H20N4O11: C, 48.78; H, 4.09; N, 11.38. Found: C, 48.91; H, 3.89; N, 11.85. UV $\lambda_{max}$ methanol nm (c): 258 (15300). NMR (DMSO-d6) $\delta$: 2.98 (1H, d, J = 15.0 Hz, 5'-H), 3.57 (1H, d, J = 15.0 Hz, 5'-H), 5.38 (1H, d, J = 3.0 Hz, 3'-H), 7.89 (1H, s, 1'-H), 8.34 (1H, s), 3.80 (3H, s, CH3), 3.69 (3H, s, CH3), 2.19 (6H, s, CH3CO).

**Dimethyl O2',O7'-Diacetyl-6-deamino-6-hydroxygriseolate (3b)** — Sodium nitrite (2.55 g, 36.96 mmol) was added to a solution of 1.82 g (3.70 mmol) of 3a in a 80% (v/v) aqueous solution of acetic acid (100 ml) under ice-cooling, and the mixture was allowed to stand for 16 h in a tightly stoppered vessel. TLC at this stage showed that the starting material remained in the reaction mixture. A further 1 g of sodium nitrite was added and the mixture was allowed to stand for 3 h. The residue obtained by evaporation of the solvent under reduced pressure was dissolved in acetone. Toluene was added to the mixture and then distilled off. This process was repeated three times. The residue was dissolved in a mixture of water and chloroform. The organic layer was washed with an aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride and then dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a pale brown glass-like substance. This substance was purified by silica gel column chromatography and dissolved in a small quantity of acetonitrile. An appropriate amount of benzene was added to the solution and the mixture was allowed to stand. The resulting white crystals were collected by filtration to give 1.28 g (70.4%) of 3b as fine white crystals. **Anal. Calcd.** for C20H20N4O11·H2O: C, 48.78; H, 4.11; N, 11.35. UV $\lambda_{max}$ methanol nm (c): 243 (12700), 248 sh (12500), 270 sh (4300). NMR (DMSO-d6) $\delta$: 5.22 (1H, d, J = 3.0 Hz, 5'-H), 5.62 (1H, d, J = 6.0 Hz, 2'-H), 5.73 (1H, s, 7'-H), 6.13 (1H, dd, J = 3.0, 6.0 Hz, 3'-H), 6.88 (1H, s, 1'-H), 8.18 (1H, s), 8.34 (1H, s), 3.80 (3H, s, CH3), 3.69 (3H, s, CH3), 2.19 (6H, s, CH3CO).

**Addition of HBr to 3a**

**Synthesis of 4a and 5a** — Compound 3a (2.45 g, 4.99 mmol) was suspended in anhydrous acetic acid containing 4% (v/v) hydrobromic acid (50 ml). The mixture was heated with stirring at 50°C for 20 min under protection from moisture. The solvent was then distilled off. Acetone and toluene were added to the residue and then distilled off; this was done three times. The residue was dissolved in a mixture of 50 ml of ethyl acetate and 30 ml of an aqueous solution of sodium bicarbonate. The organic phase was separated and washed, in turn, with 30 ml of a 5% (w/v) hydrobromic acid (50 ml). The mixture was heated with stirring at 50°C for 20 min under protection from moisture. The solvent was then distilled off. Acetone and toluene were added to the residue and then distilled off; this was done three times. The residue was dissolved in a mixture of water and chloroform. The organic layer was washed with an aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride and then dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a pale brown glass-like substance. This substance was purified by silica gel column chromatography and dissolved in a small quantity of acetonitrile. An appropriate amount of benzene was added to the solution and the mixture was allowed to stand. The resulting white crystals were collected by filtration to give 2.07 g (71.0%) of 3a as crystals. **Anal. Calcd.** for C20H20N4O11·H2O: C, 48.78; H, 4.11; N, 11.35. UV $\lambda_{max}$ methanol nm (c): 243 (12700), 248 sh (12500), 270 sh (4300). NMR (DMSO-d6) $\delta$: 5.22 (1H, d, J = 3.0 Hz, 5'-H), 5.62 (1H, d, J = 6.0 Hz, 2'-H), 5.73 (1H, s, 7'-H), 6.13 (1H, dd, J = 3.0, 6.0 Hz, 3'-H), 6.88 (1H, s, 1'-H), 8.18 (1H, s), 8.34 (1H, s), 3.80 (3H, s, CH3), 3.69 (3H, s, CH3), 2.19 (6H, s, CH3CO).

**Addition of HCl to 3b**

**Synthesis of 4b and 5b** — Compound 3b (4 g, 8.12 mmol) was placed in a two-necked flask fitted with a cooler and the flask was purged with nitrogen gas. Next, 4% (v/v) hydrogen chloride in acetic acid (40 ml) was added and the mixture was heated at 80°C for 2 h. At the end of this time, the solvent was distilled off under reduced pressure. The residue was dissolved in a mixture of toluene and methylene chloride prior to each distillation. The residue was extracted with methylene chloride...
and washed three times with a saturated aqueous solution of sodium bicarbonate. The extract was dried over anhydrous magnesium sulfate and then evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography eluted with 4% (v/v) methanol in methylene chloride, affording 2.0 g (46.5%) of 4b and 270 mg (6%) of 5b.

4b: Anal. Calc'd for C_{20}H_{21}ClN_{4}O_{11}: C, 45.42; H, 4.00; Cl, 6.70; N, 10.59. Found: C, 45.12; H, 6.67; N, 10.55. UV \( \lambda_{\text{max}} \) (nm): 248 (9500). NMR (CDCl_{3}) \( \delta \): 2.75 (1H, d, J = 15.6 Hz, 5'-H), 3.47 (1H, d, J = 15.6 Hz, 5'-H), 5.35 (1H, d, J = 4.2 Hz, 3'-H), 5.57 (1H, s, 7'-H), 6.32 (1H, dd, J = 4.2, 5.9 Hz, 2'-H), 6.55 (1H, d, J = 5.9 Hz, 1'-H), 8.18 (1H, s), 8.47 (1H, s), 4.12 (3H, s, CH_{3}), 4.25 (3H, s, CH_{3}), 2.47 (3H, s, CH_{3}CO), 2.65 (3H, s, CH_{3}CO).

5b: Anal. Calc'd for C_{22}H_{22}N_{4}O_{13}: C, 47.83; H, 4.35; N, 10.14. Found: C, 48.08; H, 4.58; N, 9.85. UV \( \lambda_{\text{max}} \) (nm): 248.5 (10200). NMR (CDCl_{3}) \( \delta \): 2.75 (1H, d, J = 15.6 Hz, 5'-H), 3.21 (1H, d, J = 4.9 Hz, 3'-H), 5.77 (1H, s, 7'-H), 6.03 (1H, dd, J = 4.9, 7.3 Hz, 2'-H), 6.49 (1H, d, J = 7.3 Hz, 1'-H), 8.07 (1H, s), 8.16 (1H, s), 3.80 (3H, s, CH_{3}), 3.86 (3H, s, CH_{3}), 2.07 (3H, s, CH_{3}CO), 2.13 (3H, s, CH_{3}CO), 2.34 (3H, s, CH_{3}CO).

Addition of HBr to 3b

Synthesis of 4c—Compound 3b (500 mg, 1.02 mmol) was added to 4% (w/v) hydrobromic acid in acetic acid (5 ml) and the mixture was dissolved by ultrasonication for 30 min. The solution was allowed to stand for 64 h at room temperature and the solvent was distilled off under reduced pressure. Distillation was done three times, each time first adding acetone and toluene to the residue. Ethyl acetate (30 ml) was added to the residue and the mixture was subjected to ultrasonic vibration. Insolubles were separated by filtration and dissolved in 30 ml of ethyl acetate and a 5% (w/v) aqueous solution of sodium bicarbonate. The organic phase was washed with 20 ml of a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure and the residue was purified by silica gel column chromatography to give 60 mg (10.3%) of 4c in the form of a white powder.

4c: Anal. Calc'd for C_{20}H_{21}BrN_{4}O_{11}: C, 41.25; H, 3.78; Br, 13.72; N, 9.62. Found: C, 41.14; H, 4.08; Br, 13.57; N, 9.40. UV \( \lambda_{\text{max}} \) (nm): 244 (14200), 249 sh (13800), 270 sh (6000). NMR (CDCl_{3}) \( \delta \): 2.98 (1H, d, J = 15.6 Hz, 5'-H), 3.17 (1H, d, J = 15.6 Hz, 5'-H), 5.35 (1H, d, J = 4.2 Hz, 3'-H), 5.57 (1H, s, 7'-H), 6.32 (1H, dd, J = 4.2, 6.6 Hz, 2'-H), 6.53 (1H, d, J = 6.6 Hz, 1'-H), 8.17 (1H, s), 8.47 (1H, s), 4.13 (3H, s, CH_{3}), 4.24 (3H, s, CH_{3}), 2.48 (3H, s, CH_{3}CO), 2.64 (3H, s, CH_{3}CO).

Dimethyl O^\cdot\O^\cdot-Diacetyl-4',5'-dihydrogriseolate (6a)—Compound 4a (572 mg, 1.00 mmol) was dissolved in 10 ml of acetone. Next, 80% (v/v) aqueous acetic acid (10 ml) and 690 mg (10.55 atom) of zinc powder was added and the mixture was stirred at room temperature for 4.3 h. At the end of this time, the solvent was distilled off and the residue was dissolved in a mixture of 10 ml of water and 20 ml of ethyl acetate. The solution was adjusted to pH 1 by the addition of 1 N hydrochloric acid and the insolubles were filtered off. The organic phase was washed successively with 20 ml of a saturated aqueous solution of sodium chloride and 20 ml of a 5% aqueous solution of sodium bicarbonate, and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure and the residue was purified by silica gel column chromatography to elute with 3% (v/v) aqueous methanol and methylene chloride to give 129 mg (26.2%) of 6a in the form of a colorless caramel-like substance. Anal. Calc'd for C_{20}H_{22}O_{11}N_{4}: C, 48.68; H, 4.67; N, 14.19. Found: C, 48.53; H, 4.67; N, 13.96. UV \( \lambda_{\text{max}} \) (nm): 258 (13700). NMR (CDCl_{3}) \( \delta \): 2.47 (1H, d, J = 15.6 Hz, 5'-H), 5.11 (1H, d, J = 4.9 Hz, 3'-H), 5.77 (1H, s, 7'-H), 6.03 (1H, dd, J = 4.9, 7.3 Hz, 2'-H), 6.49 (1H, d, J = 7.3 Hz, 1'-H), 8.07 (1H, s), 8.16 (1H, s), 3.80 (3H, s, CH_{3}), 3.86 (3H, s, CH_{3}), 2.07 (3H, s, CH_{3}CO), 2.13 (3H, s, CH_{3}CO), 2.34 (3H, s, CH_{3}CO).

Dimethyl O^\cdot\O^\cdot-Diacetyl-6-deamino-4',5'-dihydroxygriseolate (6b)—(i) Compound 4b (500 mg, 0.95 mmol), 10 mg (0.06 mmol) of 2,2'-azobisobutyronitrile, 20 ml of benzene and 3.1 ml of tributyltin hydride were added to a reaction vessel in that order, and the mixture was refluxed with stirring under a nitrogen atmosphere for 2 h. The solvent was then distilled off and the residue was purified by silica gel column chromatography eluted with 3% (v/v) methanol in methylene chloride to give 350 mg (74.9%) of 6b.

(ii) Compound 3b (600 mg, 1.22 mmol) was subjected to Parr catalytic reduction at room temperature and at 50 psig for 6 h using 70 ml of acetic acid and 600 mg (2.84 atom) of platinum oxide. At the end of this period, the vessel was purged with nitrogen and then the reaction mixture was filtered. Water was added to the filtrate, which was extracted three times, each time with 50 ml of methylene chloride. The organic phase was collected and dried over anhydrous magnesium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography eluted with 70% (v/v) benzene in acetone to give 450 mg (74.7%) of 6b. Anal. Calc'd for C_{20}H_{22}O_{11}: C, 45.9; H, 4.49; N, 11.33. Found: C, 48.36; H, 4.72; N, 11.07.
UV \( \lambda_{\text{max}} \) (v/v) aqueous methanol nm (\( e \)): 248.7 (10400). NMR (CDCl\(_3\) in the presence of D\(_2\)O) \( \delta \): 2.4—3.0 (2H, m, 3'-H), 5.1—5.4 (2H, m, 3' and 4'-H), 5.88 (1H, s, 7'-H), 5.94 (1H, dd, \( J = 4.5, 7.5 \) Hz, 7'-H), 6.30 (1H, d, \( J = 7.5 \) Hz, 1'-H), 7.97 (1H, s, 6.23 (1H, s, CH\(_3\)), 3.80 (3H, s, CH\(_3\)), 2.088 (3H, s, CH\(_3\)CO), 2.26 (3H, s, CH\(_3\)CO).

4'β,5'-Dihydrogriseolic Acid (7a)—Compound 6a (80 mg, 0.16 mmol) was dissolved in 0.2 N aqueous solution of sodium hydroxide (5 ml) and dissolved by ultrasonic vibration for about 10 min. The solution was allowed to stand for 2 h and then its pH was adjusted to a value of 2.3 by the addition of 1 N hydrochloric acid. The reaction mixture was purified by chromatography through an RP-8 prepacked column (Merck) eluted with 10% (v/v) aqueous acetonitrile to afford 25.5 mg (83.7%) of 7a.

6-Deamino-4'β,5'-dihydro-hydroxygrisecolic Acid (7b)—Compound 6b (350 mg, 0.71 mmol) was dissolved under ice-cooling in 20 ml of 1 N aqueous sodium hydroxide and the solution was allowed to stand at room temperature for 2 h. At the end of this time, the reaction mixture was adjusted to pH 1 with hydrochloric acid under ice-cooling. This mixture was subjected to RP-18 reverse-phase column chromatography eluted with a mixture of 3% (v/v) acetonitrile, 0.3% (v/v) acetic acid and water to give 140 mg (51.7%) of 7b.

Dimethyl 1'-Acetoxy-O2',O7'-diacetyl-1'-deadenino-4'β,5'-dihydrogriseolate (8a)—Concentrated sulfuric acid (2 ml, 0.04 mmol) was added to a solution of 500 mg (1.01 mmol) of compound 6b in 100 ml of a 4:1 (v/v) mixture of acetic acid and acetic anhydride, and the mixture was allowed to stand at room temperature for 14 h in a nitrogen atmosphere. Sodium acetate (15 g) was added to the reaction mixture and the solvent was evaporated off under reduced pressure. The residue was dissolved in a saturated aqueous solution of sodium bicarbonate and the solution was extracted three times with methylene chloride. The methylene chloride extracts were combined and dried over anhydrous magnesium sulfate. The solvent was then evaporated off under reduced pressure. The residue was purified by silica gel column chromatography eluted with a 1:1 (v/v) mixture of cyclohexane and ethyl acetate. Evaporation of the solvent from the second fraction gave 292 mg (69.0%) of 8a.

Dimethyl 1'-Acetoxy-O2',O7'-diacetyl-1'-deadenino-4'β,5'-dihydrogriseolate (8b)—The first fraction separated from the column chromatography described in connection with the synthesis of 8a was concentrated by evaporation under reduced pressure to give 33 mg (7.8%) of 8b.

Dimethyl 1'-Acetoxy O2',O7'-diacetyl-1'-hydroxy-4'β,5'-dihydrogriseolate (8c)—In a two-necked flask, 70 mg, (0.17 mmol) of 8a, 0.4 ml of bistrimethylsilyl uracil, and 20 ml of 1,2-dichloroethane were placed under an atmosphere of nitrogen. Sodium acetate (15 g) was added to the reaction mixture and the solvent was evaporated off under reduced pressure, and the residue was dissolved in a saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with methylene chloride three times. The methylene chloride extracts were combined and dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography eluted with methylene chloride containing 5% methanol to give 65.5 mg (83.2%) of dimethyl O2',O7'-diacetyl-1'-deadenino-4'β,5'-dihydro-1'-β-(uracil-1-yl)grisecolic acid (9).
7.5 Hz, 5-H), 11.30 (1H, s, NH).

**Dimethyl 1'-β-Acetoxy-O'-O'-dibenzoyl-1'-deadenino-4',5'-dihydrogriseolate (12)**—Concentrated sulfuric acid (2 ml, 0.04 mmol) was added under ice-cooling to a solution of 400 mg (0.65 mmol) of compound 11 (synthesized as described in the synthesis of 6b), dissolved in 80 ml of a 4:1 (v/v) mixture of acetic acid and acetic anhydride, and the mixture was allowed to stand at room temperature for 14 h. The reaction mixture was then mixed with 15 g of sodium acetate and concentrated by evaporation under reduced pressure. The residue was dissolved in a mixture of methylene chloride and a saturated aqueous sodium bicarbonate solution and extracted three times with methylene chloride. The methylene chloride extracts were combined and dried over anhydrous magnesium sulfate and the solvent was evaporated off under reduced pressure. The residue was purified by silica gel column chromatography, eluted with a 2:1 (v/v) mixture of cyclohexane and ethyl acetate to give 243 mg (69.3%) of 12.

11. **Anal.** Calcd for C32H29N5O12·1/2H2O·1/2benzene: C, 56.00; H, 4.83; N, 9.33. Found: C, 56.14; H, 4.50; N, 10.23. **NMR** (DMSO-d6) δ: 2.54 (1H, dd, J = 6.1, 15.2 Hz, 5'-H), 2.98 (1H, dd, J = 1.7, 15.2 Hz, 5'-H), 5.14 (1H, dd, J = 4.0, 5.1 Hz, 3'-H), 5.49 (1H, dd, J = 2.2, 5.1 Hz, 2'-H), 8.88 (1H, s, 7'-H), 5.06—5.10 (1H, m, 4'-H), 6.52 (1H, d, J = 2.2 Hz, 1'-H), 3.58 (3H, s, CH3), 3.81 (3H, s, CH3), 2.12 (3H, s, CH3CO), 7.15—8.16 (10H, m, benzoyl). MS m/z: 585 (M + 43).

2-Amino-6-deamino-2-dehydro-6-hydroxy-4',5'-dihydrogriseolic Acid (13a)—Compound 12 (200 mg, 0.37 mmol) and 200 mg (0.59 mmol) of bistrimethylsilyl-N'-acetylguanine was placed in a two-necked flask under an atmosphere of nitrogen and dissolved in 40 ml of 1,1-dichloroethane. Trimethylsilyl trifluoromethanesulfonate (0.4 ml, 0.002 mmol) was added under ice-cooling and the reaction mixture was allowed to stand at room temperature for 4 d, then worked up in the same manner as described in the synthesis of compound 12. It was purified by silica gel column chromatography eluted with methanol containing 3% (v/v) of methanol to give 54.8 mg (22.0%) of dimethyl 6-deamino-6-hydroxy-2-aminocinnamoyl-4',5'-dihydro-O',O'-dibenzoylgriseolate (isolated from the second fraction. **Anal.** Calcd for C32H29N5O12·1/2H2O·1/2benzene: C, 56.00; H, 4.83; N, 9.33. **UV**: 232 (28300), 260 sh (17300), 282 sh (13000), 253 (17700), 275 (13300). **NMR** (DMSO-d6) δ: 6.52 (1H, d, J = 2.2 Hz, 1'-H), 8.32 (1H, s, 8-H), 3.67 (3H, s, CH3), 3.78 (3H, s, CH3), 2.18 (3H, s, Ch3CO), 7.3—8.3 (10H, m, benzoyl), 11.73 (1H, s, NH), 12.33 (1H, s, NH).

**Dimethyl 6-deamino-6-hydroxy-2-acetylamino-4',5'-dihydrogriseolate (13b)**—In the reaction described for the synthesis of 13a, 102.0 mg (41.0%) of dimethyl 1'-deadenino-1'-β-(N2-acetylguanine-7-yl)-4',5'-dihydrogriseolate was isolated from the first fraction of silica gel column chromatography. **Anal.** Calcd for C32H29N5O12·1/2H2O·1/2benzene: C, 56.00; H, 4.83; N, 9.33. Found: C, 55.71; H, 5.03; N, 9.09. **UV** λmax (nm): 232 (28300), 260 sh (17300), 282 sh (13000), 253 (17700), 275 (13300). **NMR** (DMSO-d6) δ: 6.52 (1H, d, J = 6.0 Hz, 1'-H), 6.13 (1H, dd, J = 6.0, 3.3 Hz, 2'-H), 5.01—5.20 (1H, m, 3'-H), 5.38—5.62 (1H, m, 4'-H), 2.62—2.88 (2H, m, 5'-H), 5.87 (1H, s, 7'-H), 8.50 (1H, s, 8-H), 3.66 (3H, s, CH3), 3.79 (3H, s, CH3), 2.19 (3H, s, CH3CO), 7.2—8.2 (13H, m, benzoyl+1/2benzene), 11.53 (1H, s, NH), 12.19 (1H, s, NH).

In the same manner as described for the synthesis of 13a, 28.0 mg (53.8%) of 1'-β-(N2-acetylguanine-7-yl)-1'-
deadenino-4'β,5'-dihydrogriseolic acid was obtained from 80 mg (0.12 mmol) of the compound obtained above. Anal. Calcd for C_{16}H_{17}N_{5}O_{10}·3H_{2}O: C, 38.95; H, 4.66; N, 14.20. Found: C, 38.62; H, 4.51; N, 14.39. NMR (D_{2}O): 6.02 (1H, d, J=6.6 Hz, 1'-H), 4.65-4.79 (3H, m, 2'-, 4'- and 7'-H), 5.11—5.16 (1H, m, 3'-H), 2.47 (1H, dd, J=6.4, 15.1 Hz, 5'-H), 2.67 (1H, dd, J=15.1, 1.6 Hz, 5'-H), 8.22 (1H, s, 8-H), 2.11 (3H, s, CH_{3}).

Using 24 mg (0.05 mmol) of the compound obtained above, a similar reaction to that used in the synthesis of 13a was carried out at room temperature for 2 d. Worked-up gave 16 mg (73.7%) of 13b. Anal. Calcd for C_{14}H_{15}N_{5}O_{9}·3H_{2}O: C, 37.26; H, 4.69; N, 15.51. Found: C, 36.98; H, 4.88; N, 15.72. UV \text{H}_{2}O \text{ max nm (s): } 246 \text{ sh (6400), 285 (7800)}; \text{0.1 N NaOH: } 282 (6800), 264 (7100). NMR (D_{2}O) \text{ dd: } 5.92 (1H, d, J=7.3 Hz, 1'-H), 4.4—4.66 (3H, m, 2'-, 4'-and 7'-H), 4.99—5.01 (1H, m, 3'-H), 2.36 (1H, d, J=15.8, 6.5 Hz, 5'-H), 2.48 (1H, dd, J=15.8, 1.6 Hz, 5'-H), 8.05 (1H, s, 8-H).

Acknowledgment The authors wish to express their thanks to Dr. H. Nakao, the Director of the Chemical Research Laboratories, for his encouragement and advice, and to Drs. T. Hiraoka and T. Miyadera for their valuable suggestions.

References and Notes
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