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Studies on Griseolic Acid Derivatives. V.[1] Synthesis and Phosphodiesterase Inhibitory Activity of Substituted Derivatives of the Hydroxy Group at the 2′- or 7′-Position in Griseolic Acid

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Substituted derivatives of the hydroxy group at the 2′- or 7′-position in griseolic acid (**1**) were synthesized by selective substitution in order to study the relationship between structure and inhibitory activity against adenosine 3′,5′-cyclic monophosphate (cAMP) or guanosine 3′,5′-cyclic monophosphate (cGMP) phosphodiesterase (PDE). All of the derivatives which had a configurationally inverted substituent at the 2′-position instead of the hydroxy group were almost equal to natural griseolic acid in cAMP PDE inhibitory activity, except when the substituent was an amino group. On the other hand, substitution of 7′-OH with inversion tended to reduce the inhibitory activity a little.

Keywords——griseolic acid; 2′-substituted griseolic acid derivatives; 7′-substituted griseolic acid derivatives; adenosine 3′,5′-cyclic monophosphate; guanosine 3′,5′-cyclic monophosphate; phosphodiesterase; cAMP phosphodiesterase inhibitory activity; cGMP phosphodiesterase inhibitory activity

Introduction

Severin *et al.*[2] have reported the structure–activity relationship between adenosine 3′,5′-cyclic monophosphate (cAMP) derivatives and their inhibitory activity against phosphodiesterase (PDE). However, this inhibitory activity depends on two parameters; namely, the affinity of the cAMP derivative for the receptor site of PDE and the hydrolyzability by PDE. Therefore, the relationship based only on the affinity for the receptor site cannot be investigated. On the other hand, griseolic acid (**1**) cannot be a substrate of PDE, but inhibits the hydrolyses of cAMP and guanosine 3′,5′-cyclic monophosphate (cGMP) competitively.

Consequently, we planned to study the inhibitory activity against PDE using griseolic acid derivatives and thus to establish the structure–activity relationship based only on the affinity. We have already reported synthetic procedures for griseolic acid derivatives which are partially or fully acylated at the N^6-, $O^{2'}$- or $O^{7'}$-position and the PDE inhibitory activity of the products.[1] It was revealed that acylation of the amino group of the adenine moiety greatly reduced the inhibitory activity. On the other hand, acylation of the hydroxy groups at the 7′- and 2′-positions had relatively little effect on the inhibitory activity.

In the present work, we have synthesized derivatives of griseolic acid (**1**) with various substituents at the 2′- or 7′-position instead of OH, and have investigated the structure–activity relationship for PDE inhibitory activity.

Results and Discussion

Synthesis

The following methods are available introducing a variety of substituents into the sugar

moiety of a purine-type nucleoside[4]: (a) by using 2-acetoxyisobutylyl bromide,[5] (b) by using a *ortho* ester as an intermediate,[6] (c) by using a cyclonucleoside, in which the 8-position and sugar moiety are cyclized, as an intermediate,[7] (d) by using a 2′,3′-epoxide as an intermediate, (e) *via* a methanesulfonylated or toluenesulfonylated compound,[8,9] (f) *via* a trifluoromethanesulfonylated compound,[9-11] (g) by direct halogenation, (h) *via* a ketone which is synthesized by oxidation of the hydroxy group, (i) by addition across a double bond which is introduced by dehydration.[9] Methods (a) and (b) can introduce only a restricted range of substituents. In method (c), the purine compound must be substituted at the 8-position first, so this method needs many steps and is not convenient. Moreover, this method cannot give substitution at the 7′-position. Method (d) cannot be used because griseolic acid does not have a hydroxy group at the 3′-position and at the position next to 7′. Method (e) is inferior to method (f) in reactivity. As for method (g), the reactivity is low and only a halogen atom can be introduced. Method (h) can only introduce a hydroxy group. Method (i) cannot be employed as it is difficult to introduce a double bond into griseolic acid because of structural strain. For the above reasons, we synthesized the derivatives substituted at the 2′- or 7′-position according to method (f).

The structures of all derivatives were confirmed by nuclear magnetic resonance (NMR) spectroscopy and elemental analyses unless otherwise stated and yields were calculated based on the immediately preceding starting material.

Synthesis of Derivatives Substituted at the 2′-Position

We synthesized these derivatives according to the method shown in Fig. 1. Griseolic acid (**1**) was converted to the $O^{2'}$-benzoylated derivative (**2**) by the method already reported.[1] Then, we attempted to synthesize the dimethyl ester derivative (**3**). Takahashi *et al.* have reported the esterification of the carboxy group in griseolic acid with diazomethane,[12] but, this method is not suited to mass production because the $O^{2'}$-methylated derivative is formed as a by-product. As a result of detailed investigation using griseolic acid as a starting material, we found that the dimethyl ester could be synthesized in good yield by allowing griseolic acid to stand with benzoyl chloride in methanol. This reaction was thought to occur by way of the mixed anhydride followed by methanolysis. Thus, we synthesized **3** in good yield from **2** using this method. The $O^{7'}$-pyranyl derivative (**4**) was obtained in 88% yield by reacting **3** with 2,3-dihydropyran in the presence of *p*-toluenesulfonic acid in dioxane.[13] The methyl ester function was not considered to be a good protecting group in these syntheses, because the desired compounds, with various substituents at the 2′-position in griseolic acid, might not be obtained because of decomposition at the stage of removing the methyl groups. We therefore selected the benzhydryl group, which was easily removed under acidic conditions, as the protecting group. Compound **4** was allowed to stand in 1 N aqueous sodium hydroxide to remove the benzoyl group at the 2′-position and the methyl groups. Except in salt form, this compound is not stable and the pyranyl group was easily lost. Consequently, this compound was subjected, without purification, to benzhydrylation with diphenyldiazomethane to give **5** in 46% yield. Compound **5** was allowed to react with trifluoromethanesulfonyl chloride in the presence of dimethylaminopyridine to give **6** in 71% yield. Compound **6** was allowed to react with sodium azide in the presence of hexamethylphosphoramide and the pyranyl group of this compound **7** was converted to give **8** in 96% yield.[14] This compound was visualized on a thin the benzhydryl groups were removed to give **7** in 18% yield. According to Fukukawa *et al.*,[11] compound **7** was converted to give **8** in 96% yield.[14] This compound was visualized on a thin layer chromatography (TLC) plate by spraying with a solution of cysteine–sulfuric acid followed by heating. Compound **6** was allowed to react with anhydrous lithium halogenide (*e.g.*, lithium iodide, lithium bromide, and lithium chloride) in hexamethylphosphoramide and the pyranyl group and the benzhydryl groups were removed in the same manner as

described above to give **10a**, **10b**, and **10c** in 23.4%, 36.5% and 14.2% yields through **9a**, **9b**, and **9c**, respectively.

Compound **9a** was allowed to react with *n*-butyltin hydride in the presence of azoisobutyronitrile (AIBN) in benzene under reflux, and the benzhydryl groups were removed in the same manner as described above to give **11** in 69.2% yield (Fig. 1).

If an acetoxy group could be introduced at the 2′-position, the corresponding arabino-type derivative could be synthesized by removing the acetyl group. Thus, we investigated the substitution reaction of the acetoxy group with sodium acetate under various reaction conditions, but all the reaction mixtures turned brown and complicated decompositions occurred without giving the desired compound. Furthermore, all efforts to synthesize an arabino-type compound with sodium benzoate instead of sodium acetate were in vain, because the same type of decomposition again occurred. This complicated decomposition was thought to result from the acetoxy group, substituted at the 2′-position, migrating toward the

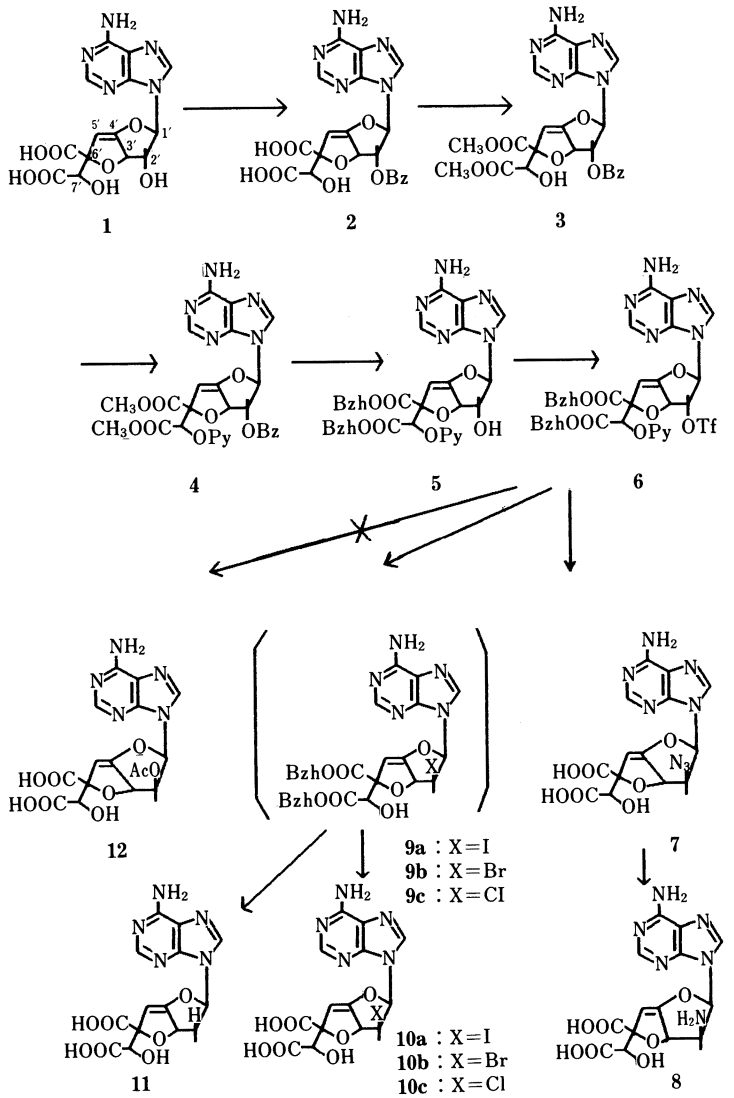

Fig. 1

3′- or 4′-position through a mechanism similar to that of the acyl migration reaction.

Synthesis of Derivatives Substituted at the 7′-Position

We synthesized these derivatives according to the methods shown in Figs. 2 and 3. Compound **13**, which has already been reported,[1] was allowed to react with trifluoromethanesulfonyl chloride in the presence of dimethylaminopyridine in methylene chloride to give **15** in 80% yield (Figs. 2 and 3).

Compound **15** was allowed to react with well-dried sodium azide in the presence of hexamethylphosphoramide to give **16** in 55% yield. The benzhydryl groups of compound **16** were removed with trifluoroacetic acid and the benzoyl group was removed by using a 20% methanolic solution of ammonia to give **17** in 49% yield.

According to the manner of Fukukawa *et al.*,[11] compound **16** was converted to **18** in 43% yield. This compound was visualized on a TLC plate by spraying with a solution of cysteine–sulfuric acid followed by heating. The benzhydryl group and the benzoyl group of compound **18** were removed in the same manner as described above to give **19** in 66% yield. Compound **15** was allowed to react with well-dried anhydrous lithium chloride or lithium bromide in anhydrous dimethylformamide under protection from moisture to give **20a** in 70% yield or **20b** in 53% yield, and each compound was deprotected in the conventional manner to give **21a** in 82% yield or **21b** in 57% yield. Compound **20b** was allowed to react with *n*-butyltin hydride (2.5 eq) in the presence of AIBN in anhydrous benzene, after which the benzoyl group and the benzhydryl groups were deprotected in the conventional manner to give **22** in 82% yield. This compound was identical with the natural product, the structure of which was

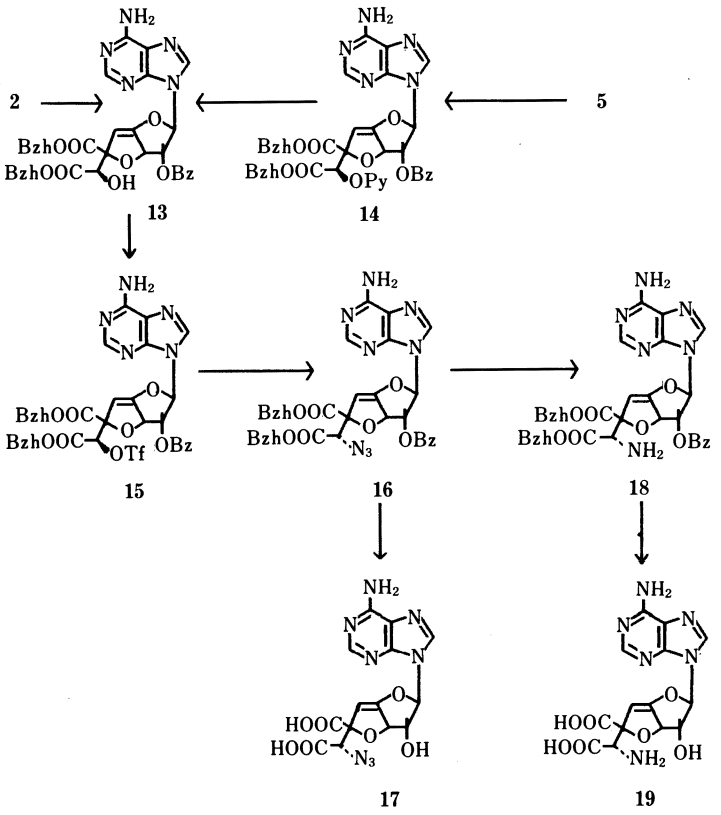

Fig. 2

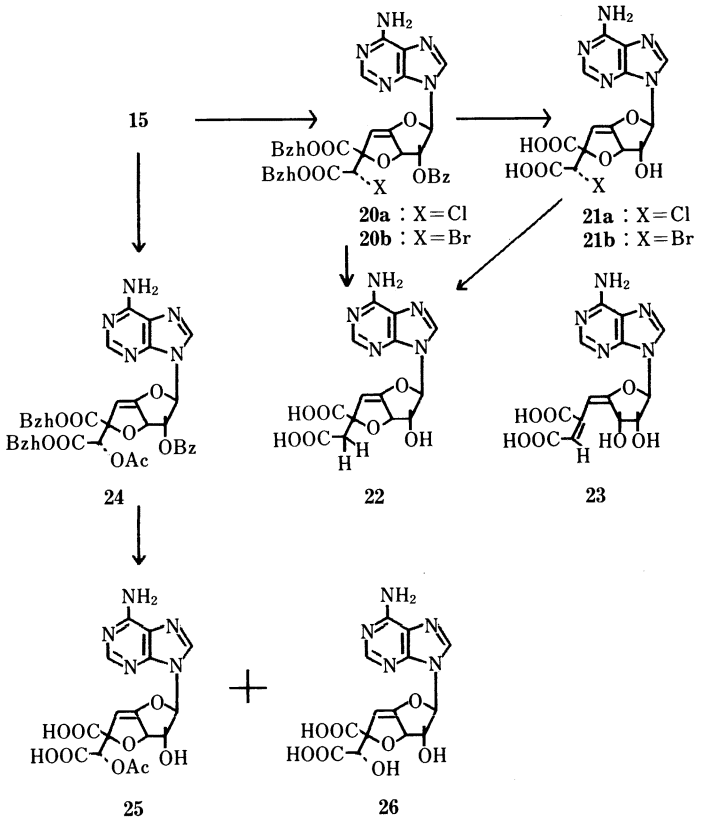

Fig. 3

determined by X-ray analysis, in all physical properties examined. Furthermore, compound **22** was also obtained by reducing **21b** with 80% aqueous acetic acid and zinc powder (16% yield).[15] This poor yield was a result of the generation of compound **23** as a by-product. The mechanism for the formation of **23** was thought to be that the furan ring was cleaved by the bromo atom in a β-elimination mechanism to generate the double bond between the 6′- and 7′-positions. Compound **24** was obtained by reacting **15** with well-dried sodium acetate which was dried by melting, in acetic acid, followed by protection with diphenyldiazomethane (68% yield). The stereoisomer of compound **1** (30%) was obtained by removing the benzhydryl groups of **24** with trifluoroacetic acid followed by treatment with a 20% methanolic solution of ammonia overnight. In this reaction, compound **25**, in which the acetyl group at the 7′-position was configurationally inverted, was also obtained in 18% yield.

PDE-Inhibitory Activity

On the whole, the PDE-inhibitory activities of the β-oriented derivatives at the 2′-position were almost equal to that of griseolic acid, and those of the substituted derivatives with inverted configuration at the 7′-position were a little lower than that of griseolic acid (Tables I and II).

The stereoisomer of griseolic acid (**26**) showed almost the same inhibitory activity against PDE as griseolic acid (**1**). These facts suggest that the configuration and the substituent at the 7′-position do not influence the affinity for the receptor site of PDE, and the PDE probably only recognizes the carboxy group of the sugar moiety of griseolic acid. Characteristic features were that the 2′-*epi*-bromo derivative (**9b**) showed a 1.7 times stronger activity than griseolic

TABLE I. PDE-Inhibitory Activity of 2′-Substituted Derivatives of Griseolic Acid

Compound No.		1	7	8	10a	10b	10c	11
2′-Substituent		OH	N_3	NH_2	I	Br	Cl	H
IC_{50} (μM)	cAMP	0.16	0.45	3.20	0.40	0.09	0.15	0.19
	cGMP	0.63	6.40	12.7	2.80	4.10	2.50	1.10
	cAMP/cGMP	0.25	0.07	0.25	0.14	0.02	0.06	0.17

TABLE II. PDE-Inhibitory Activity of 7′-Substituted Derivatives of Griseolic Acid

Compound No.		17	19	21a	21b	22	25	26
7′-Substituent		N_3	NH_2	Cl	Br	H	OAc	OH
IC_{50} (μM)	cAMP	4.40	2.00	1.50	3.40	0.16	0.76	0.22
	cGMP	13.0	4.20	14.8	19.0	0.63	2.80	1.05
	cAMP/cGMP	0.34	0.48	0.10	0.18	0.25	0.27	0.21

acid and the 2′-*epi*-amino-derivative (**8**) showed a 1/50 weaker activity. Accordingly, electron-withdrawing groups seemed to be better than electron-donating groups as the β-oriented substituent at the 2′-position (Tables I and II).

Conclusion

Considered as a whole, the inhibitory activities of the 2′β-substituted derivatives were almost equal to that of griseolic acid itself. However, the compound in which the substituent at the 2′-position was an amino group showed a weaker activity. Consequently, it was suggested that electron-withdrawing groups are more suitable for the 2′β-substituent than electron-donating groups for inhibitory activity. An electron-withdrawing 2′β-substituent may interact with the base, changing the conformation to one better suited for approaching the receptor site of PDE. On the other hand, substitution of 7′-OH with inverted configuration tended to reduce the inhibitory activity a little. Accordingly, it could be considered that the substituents with inverted configuration at the 7′-position show decreased affinity to the PDE because of the change in stereostructure around the 7′-position. From this result, it was suggested that the hydroxy groups in the sugar moiety do not play a significant role in interaction with the receptor site of PDE. This conclusion is consistent with that of Severin *et al.*[2]

Experimental

General——Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. NMR spectra were obtained with a Varian EM-390 spectrometer (90 MHz) and the chemical shifts are expressed in ppm from tetramethylsilane as internal standard: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; br d, broad doublet; br m, broad multiplet; br s, broad singlet. Ultraviolet (UV) spectra were obtained with a Hitachi 200-20 spectrophotometer. TLC was carried out on Merck Silica gel F_{254} precoated TLC plates with 0.25-mm layer thickness, and spots were visualized by UV irradiation or by spraying with 30% sulfuric acid followed by heating. Ordinary chromatography was performed by the rapid chromatography method[16] on Merck silica gel (Kieselgel 60, Art 9385).

Dimethyl *O*$^{2'}$-Benzoylgriseolate (3)——*O*$^{2'}$-Benzoylgriseolic acid (28.6 g, 59.2 mmol)[1] was suspended in 500 ml of methanol, and 41.2 ml (355 mmol) of benzoyl chloride was added dropwise over a period of about 15 min under ice-cooling and stirring. The mixture was stirred for an additional 1 h under the same conditions and then stirred for another 26 h at room temperature. The solvent was distilled off and the residue was dissolved in a mixture of ethyl acetate and 10% (w/v) aqueous sodium bicarbonate. The organic layer was separated from the aqueous layer, washed

with water, and dried over anhydrous magnesium sulfate, and the solvent was distilled off. The residue was dissolved in a mixture of ethyl acetate and ethanol and the solvents were distilled off, whereupon crystals were precipitated. These crystals were collected by filtration in a yield of 13.8 g (45.6%). Further, the mother liquor was concentrated to approximately 100 ml and poured into 1 l of hexane with stirring. The precipitated powder was collected by filtration and purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 12.5 g of **3**. UV $\lambda_{max}^{methanol}$ nm (ε): 229.5 (19300), 256.5 (17400). NMR (DMSO-d_6) δ: 8.41 (1H, s), 8.30 (1H, s), 7.02 (1H, s, 1′-H), 5.86 (1H, d, J=5.4 Hz, 2′-H), 6.40 (1H, dd, J=5.4, 2.4 Hz, 3′-H), 5.25 (1H, d, J=2.4 Hz, 5′-H), 4.65 (1H, s, 7′-H), 7.3—8.3 (7H, m, benzoyl+NH_2), 3.58 (3H, s, CH_3), 3.76 (3H, s, CH_3).

Dimethyl $O^{2'}$-Benzoyl-$O^{7'}$-(tetrahydropyran-2-yl)griseolate (4)——Compound **3** (25.6 g, 50 mmol) was suspended in 250 ml of dioxane and 10.5 g (55.2 mmol) of *p*-toluenesulfonic acid was added to the suspension to yield a clear yellow solution. 2,3-Dihydropyran (137 ml, 1.5 mol) was added to this clear solution and the mixture was stirred for 2.5 h at room temperature. Anhydrous potassium carbonate was added to the reaction mixture and the solvent was distilled off. The residue was dissolved in a mixture of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated from the aqueous layer, washed with water, and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, after which the solvent was distilled off. Hexane was added to the residue and the supernatant was removed. The remaining solution was purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 26.1 g (87.6%) of **4** as a colorless foam. UV $\lambda_{max}^{methanol}$ nm (ε): 230 (18100), 257 (17000). NMR (DMSO-d_6) δ: 8.40 (1H, s), 8.28 (1H, s), 7.07 (1H, s, 1′-H), 5.84 (1H, d, J=5.4 Hz, 2′-H), 6.41 (1H, dd, J=5.4, 2.4 Hz, 3′-H), 5.19, 5.36 (1H, d, J=2.4 Hz, 5′-H), 4.54, 4.81 (1H, s, 7′-H), 7.3—8.3 (7H, m, benzoyl+NH_2), 3.06 (3H, s, CH_3), 3.76 (3H, s, CH_3), 1.0—2.0 (9H, br m, tetrahydropyranyl).

Dibenzhydryl $O^{7'}$-(Tetrahydropyran-2-yl)griseolate (5)——Methanol (175 ml) and 175 ml of 1 N aqueous sodium hydroxide were added to 26.1 g (43.8 mmol) of compound **4**, the mixture was reacted for about 20 h with stirring at room temperature, and the pH of the solution was adjusted to about 7.0. The solvent was distilled off at a temperature below 30 °C and the residue was dissolved in 500 ml of acetone and 100 ml of water. Three equivalents of diphenyldiazomethane was added to this solution. The pH of the mixture was adjusted to 1.5 with 3 N hydrochloric acid and stirring was continued (the pH gradually rose to 3) while protecting the mixture from light. After 2.5—4 h, the solvent was distilled from the mixture. The residue was dissolved in a mixture of ethyl acetate and water. The organic layer was separated from the aqueous layer, washed with water, and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the residue was purified by silica gel chromatography (5% methanol–methylene chloride) to yield 16.0 g (46.0%) of **5** as a colorless foam. UV $\lambda_{max}^{methanol}$ nm (ε): 257 (17000). NMR (DMSO-d_6) δ: 8.36 (1H, s), 8.17 (1H, s), 4.67 (1H, d, J=5.4 Hz, 2′-H), 6.55 (1H, s, 1′-H), 6.3—6.5 (1H, br m, 3′-H), 5.20 , 5.30 (1H, d, J=2.4 Hz, 5′-H), 5.00, 4.83 (1H, s, 7′-H), 1.3—2.0 (9H, br m, tetrahydropyranyl), 7.2—7.6 (22H, m, benzhydryl+NH_2), 6.80 (2H, s, CH).

Dibenzhydryl $O^{7'}$-(Tetrahydropyran-2-yl)-$O^{2'}$-trifluoromethanesulfonylgriseolate (6)——Compound **5** (12.8 g, 16.1 mmol) and 5.9 g (48.3 mmol) of 4-(dimethylamino)pyridine were dissolved in 300 ml of dry methylene chloride. Trifluoromethanesulfonyl chloride (5.17 ml, 48.6 mmol) was added to the solution under a stream of nitrogen gas and under cooling with dry ice/acetone. The mixture was then stirred at room temperature for 2—2.5 h, after which ice-water was added to the reaction mixture. The organic layer was separated from the aqueous layer, washed successively with saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride, and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent was distilled off. The residue was purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 10.54 g (70.6%) of **6**. UV $\lambda_{max}^{methanol}$ nm (ε): 257.5 (17600). NMR (DMSO-d_6) δ: 8.40 (1H, s), 8.13 (1H, s), 7.1—7.8 (23H, m, 1′-H, overlapping with benzhydryl+NH_2), 6.10 (1H, d, J=5.4 Hz, 2′-H), 6.7—6.9 (3H, m, 3′-H, overlapping with CH), 5.29, 5.53 (1H, d, J=2.4 Hz, 5′-H), 4.90, 5.08 (1H, s, 7′-H), 1.0—2.0 (9H, br m, tetrahydropyranyl).

***S*-2′-Azido-2′-deoxygriseolic Acid (7)**——Compound **6** (2.0 g, 2.2 mmol) was dissolved in 8 ml of hexamethylphosphoric triamide. Sodium azide (0.28 g, 4.3 mmol) was added to the solution and the mixture was stirred for 3 h at room temperature. The reaction mixture was poured into ice-water containing sodium chloride. The precipitate was collected by filtration, washed with water, dissolved in ethyl acetate, and dried over anhydrous magnesium sulfate. The solvent was distilled off and the residue was purified by silica gel chromatography (3% methanol–methylene chloride) to yield 0.86 g (1.07 mmol) of dibenzhydryl *S*-2′-azido-2′-deoxy-$O^{7'}$-(tetrahydropyran-2-yl)griseolate. This compound (0.86 g, 1.07 mmol) and 0.13 g (0.52 mmol) of pyridine *p*-toluenesulfonate were dissolved in 1 ml of methylene chloride and 5 ml of ethanol and the mixture was heated at 50 °C for 12 h. A further 0.13 g (0.52 mmol) of pyridine *p*-toluenesulfonate was added and the mixture was allowed to react for a further 17 h at 60 °C. The solvent was distilled off and the residue was dissolved in methylene chloride. The resulting solution was washed with water and dried over anhydrous magnesium sulfate. The solvent was then distilled off and the residue was purified by silica gel chromatography (5% methanol–methylene chloride) to yield 0.36 g of dibenzhydryl *S*-2′-azido-2′-deoxygriseolate. This compound (0.36 g, 0.49 mmol) was dissolved in 3 ml of anisole and 3 ml of trifluoroacetic acid was added under ice-cooling. The mixture was left standing for 15 min. Dry toluene was then added and the solvent was distilled from the reaction mixture. Acetone and toluene were added to the residue and then distilled off. This process was repeated twice. The residue was dissolved in a small quantity of acetone and hexane was added to precipitate a powder. The

powder was collected by filtration and dissolved in saturated aqueous sodium bicarbonate. The pH of this solution was adjusted to 2.4 and the solution was purified by Rp-8 prepacked column (Merck) chromatography (5% (v/v) aqueous acetonitrile containing 0.02% (v/v) acetic acid) to yield 0.16 g (18.3%) of **7**. UV λ_{max} nm (ε): (H_2O) 257 (15800); (0.1 N NaOH) 258 (16300); (0.1 N HCl) 255.5 (16000). IR: 2125 cm^{-1} (N_3). NMR (DMSO-d_6) δ: 8.29 (1H, s), 8.20 (1H, s), 7.06 (1H, d, J=8.3 Hz, 1′-H), 4.8—5.2 (2H, m, 2′- and 5′-H), 6.08 (1H, dd, J=9.0, 2.4 Hz, 3′-H), 4.51 (1H, s, 7′-H), 7.69 (2H, br s, NH_2). $[\alpha]_D^{20}$ −53.8° (c=0.5, DMSO).

S-2′-Amino-2′-deoxygriseolic Acid (8)——Water (1.5 ml) and 6 ml of pyridine were added to compound **7** (0.14 g, 0.25 mmol). The air in the flask was replaced by nitrogen gas and then the solution was saturated with hydrogen sulfide at room temperature. The container was tightly stoppered and left standing at room temperature for 7—8 h and then at 5 °C overnight. The solvent was distilled off, and water was added to the residue, and then distilled off. This process was repeated and the residue was dissolved in 0.1 N hydrochloric acid. The insoluble matter was removed by filtration and the pH of the filtrate was adjusted to 2.3 with saturated aqueous sodium bicarbonate. The solution was purified by chromatography through an Rp-8 prepacked column (Merck) (3% (v/v) acetonitrile–0.02% (v/v) acetic acid–96.98% (v/v) water) to yield 93 mg (96%) of **8**. UV λ_{max} nm (ε): (H_2O) 256.5 (15600); (0.1 N NaOH) 258 (16300); (0.1 N HCl) 254 (16400). NMR (DMSO-d_6) δ: 8.24 (1H, s), 8.20 (1H, s), 6.76 (1H, br d, 1′-H), 3.7—4.44 (1H, br s, overlapping with H_2O, 2′-H), 5.86 (1H, br m, 3′-H), 4.95 (1H, d, J=2.4 Hz, 5′-H), 4.44 (1H, s, 7′-H), 7.34 (2H, br s, NH_2). $[\alpha]_D^{20}$ −73.6° (c=0.5, DMSO).

2′-Deoxy-S-2′-iodogriseolic Acid (10a)——Compound **6** (1.37 g, 1.48 mmol) was dissolved in 4 ml of hexamethylphosphoric triamide, and 0.8 g (6 mmol) of anhydrous lithium iodide was added. The mixture was left standing for 5—6 h at room temperature. A further 1 ml of hexamethylphosphoric triamide was added to the reaction mixture and the mixture was allowed to react for 6—7 h on an ultrasonic bath (up to 40 °C). The reaction mixture was then poured into ice-water. The precipitate was collected by filtration, washed with water, and dissolved in ethyl acetate; this solution was dried over anhydrous magnesium sulfate. The solvent was distilled off and the residue was purified by silica gel column chromatography (3% methanol–methylene chloride) to yield 0.5 g of dibenzhydryl 2′-deoxy-S-2′-iodo-$O^{7'}$-(tetrahydropyran-2-yl)griseolate. A 0.5 g (0.56 mmol) portion of this compound was dissolved in 1 ml of methylene chloride, and 0.14 g (0.56 mmol) of pyridine p-toluenesulfonate and 10 ml of ethanol were added to the solution. The mixture was heated at 60 °C for 8 h. The solvent was distilled from the reaction mixture and the residue was dissolved in a mixture of ethyl acetate and water. The organic layer was separated from the aqueous layer and dried over anhydrous magnesium sulfate. The solvent was then distilled off and the residue was purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 0.35 g of dibenzhydryl 2′-deoxy-S-2′-iodogriseolate. A 0.32 g (0.39 mmol) portion of this compound was dissolved in 3 ml of anisole, to which 3 ml of trifluoroacetic acid was then added under ice-cooling. The mixture was left standing for 30 min. Dry toluene was then added and the solvent was distilled from the reaction mixture. Acetone and toluene were added to the residue and distilled off. This process was repeated twice. The residue was suspended in a small quantity of acetone, to which hexane was added. The resulting precipitate was collected by filtration. This precipitate was dissolved in saturated aqueous sodium bicarbonate and the insoluble matter was removed. The pH of the residue was adjusted to 2.4 with 3 N hydrochloric acid and the precipitate was collected by filtration, washed with water and dried to yield 160 mg (22.1%) of **10a**. The mother liquor was purified by chromatography through an Rp-8 prepacked column (Merck) (5% (v/v) aqueous acetonitrile containing 0.02% (v/v) acetic acid) to yield a further 0.01 g (1.3%) of **10a**. UV λ_{max} nm (ε): (H_2O) 258 (15900); (0.1 N NaOH) 258 (17200); (0.1 N HCl) 257.5 (16200). NMR (DMSO-d_6) δ: 8.34 (1H, s), 8.22 (1H, s), 6.96 (1H, d, J=7.5 Hz, 1′-H), 5.00 (1H, dd, J=9.0, 7.5 Hz, 2′-H), 6.29 (1H, dd, J=9.0, 2.4 Hz, 3′-H), 5.18 (1H, d, J=2.4 Hz, 5′-H), 4.52 (1H, s, 7′-H), 7.40 (2H, br s, NH_2). $[\alpha]_D^{20}$ −56.8° (c=0.5, DMSO).

2′-Deoxy-S-2′-bromogriseolic Acid (10b)——Compound **6** (2.5 g, 2.69 mmol) was dissolved in 12 ml of hexamethylphosphoric triamide and 1.18 g (13.5 mmol) of anhydrous lithium bromide was added. The mixture was allowed to react for 4—5 h on an ultrasonic bath (up to 35 °C). The reaction product was stirred overnight at room temperature. It was then slowly poured into ice-water containing sodium chloride. The precipitate was collected by filtration, washed successively with ice-water and hexane, and dissolved in ethyl acetate; this solution was dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was evaporated to yield 2.26 g (2.69 mmol) of dibenzhydryl 2′-deoxy-S-2′-bromo-$O^{7'}$-(tetrahydropyran-2-yl)griseolate, which was dissolved in 5 ml of methylene chloride. Ethanol (20 ml) and 0.73 g (2.9 mmol) of pyridine p-toluenesulfonate were added to this solution and the mixture was allowed to react at 60 °C for 12 h. The solvent was distilled off and the residue was dissolved in methylene chloride. The solution was washed with water and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent was distilled off. The residue was purified by silica gel chromatography (5% methanol–methylene chloride) to yield 0.9 g (43%) of dibenzhydryl 2′-deoxy-S-2′-bromogriseolate. The procedures described for the synthesis of **10a** were repeated to yield 436 mg (36.5%) of **10b**. UV λ_{max} nm (ε): (H_2O) 257.5 (15400); (0.1 N NaOH) 257.5 (16200); (0.1 N HCl) 255 (15300). NMR (DMSO-d_6) δ: 8.36 (1H, s), 8.23 (1H, s), 7.10 (1H, d, J=7.5 Hz, 1′-H), 5.17 (1H, dd, J=9.0, 7.5 Hz, 2′-H), 6.30 (1H, dd, J=9.0, 2.4 Hz, 3′-H), 5.21 (1H, d, J=3.0 Hz, 5′-H), 4.52 (1H, s, 7′-H), 7.39 (2H, br s, NH_2). $[\alpha]_D^{20}$ −58° (c=0.5, DMSO).

S-2′-Chloro-2′-deoxygriseolic Acid (10c)——The procedures described for the synthesis of **10a** were repeated except that compound **6** (2 g, 2.16 mmol) was reacted with anhydrous lithium chloride (1.4 g, 33 mmol) in place of the

bromide and the reaction mixture was heated at 60 °C for 4 h to yield 0.18 g (21.0%) of **10c**. UV λ_{max} nm (ε): (H_2O) 257 (15000); (0.1 N NaOH) 257 (15200); (0.1 N HCl) 254.5 (14800). NMR (DMSO-d_6) δ: 8.36 (1H, s), 8.22 (1H, s), 7.14 (1H, d, J=7.5 Hz, 1′-H), 5.21 (1H, dd, J=9.0, 7.5 Hz, 2′-H), 6.25 (1H, dd, J=9.0, 2.4 Hz, 3′-H), 5.22 (1H, d, J=2.4 Hz, 5′-H), 4.54 (1H, s, 7′-H), 7.42 (2H, br s, NH_2).

2′-Deoxygriseolic Acid (11)——Compound **9a** (0.83 g, 1.07 mmol) and about 10 mg of α,α′-azobisisobutyronitrile were dissolved in 20 ml of benzene, and 0.7 ml (2.6 mmol) of tributyltin hydride was added under a stream of nitrogen gas. The mixture was refluxed for 45 min. The solvent was distilled off and the residue was purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 0.72 g (96.3%) of dibenzhydryl 2′-deoxygriseolate. This compound (0.72 g, 1.03 mmol) was dissolved in 5 ml of anisole, and 5 ml of trifluoroacetic acid was added under ice-cooling. The mixture was left standing for 15 min, after which dry toluene was added and the solvent was distilled off. Acetone and toluene were added to the residue and then distilled off. This process was repeated again, after which the residue was suspended in a small quantity of acetone. Hexane was added to the suspension to turn the solid matter into powder. This powder was collected by filtration and dissolved in saturated aqueous sodium bicarbonate. The pH of the solution was adjusted to 2.3 with 3 N hydrochloric acid and the solution was purified by chromatography through an Rp-8 prepacked column (Merck) (3% (v/v) aqueous acetonitrile containing 0.02% (v/v) acetic acid) to yield 0.269 g (69.2%) of **11**. UV λ_{max} nm (ε): (H_2O) 257.5 (15800); (0.1 N NaOH) 257.5 (15800); (0.1 N HCl) 255 (16700). NMR (DMSO-d_6) δ: 8.40 (1H, s), 8.24 (1H, s), 6.90 (1H, br m, 1′-H), 2.76 (2H, br m, 2′-H), 6.05 (1H, br m, 3′-H), 4.94 (1H, d, J=3.0 Hz, 5′-H), 4.50 (1H, s, 7′-H), 7.43 (2H, br s, NH_2). $[\alpha]_D^{20}$ −17.6° (c=0.5, DMSO).

Dibenzhydryl $O^{2'}$-Benzoyl-$O^{7'}$-trifluoromethanesulfonylgriseolate (15)——Compound **13** (7.99 g, 9.79 mmol) and 1.46 g (12.0 mmol) of 4-(dimethylamino)pyridine were introduced into a 100 ml three-necked round-bottomed flask and dried under reduced pressure in the presence of phosphorus pentoxide. Also in the presence of phosphorus pentoxide, 50 ml of methylene chloride was separately distilled and added to the flask. Trifluoromethanesulfonyl chloride (2.02 ml, 19.0 mmol) was then added under ice-cooling and protection from moisture, and the mixture was stirred for 2 h under the same conditions. After addition of 10 ml of water, the mixture was stirred for a further 15 min under ice-cooling. The reaction mixture was transferred to a separating funnel, to which 20 ml of 0.1 N hydrochloric acid was added to wash the organic layer. This layer was washed successively with saturated aqueous sodium chloride and saturated aqueous sodium bicarbonate, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (1% (v/v) methanol–methylene chloride). The fraction containing the main product was collected and concentrated to yield 7.38 g (79.5%) of **15** as a pale yellow foam. UV $\lambda_{max}^{methanol}$ nm (ε): 257 (17100). NMR (DMSO-d_6) δ: 8.33 (1H, s), 8.08 (1H, s), 7.1—8.2 (28H, m, 1′-H overlapping with benzhydryl, benzoyl and NH_2), 6.00 (1H, d, J=5.4 Hz, 2′-H), 6.93 (1H, dd, J=2.4, 5.4 Hz, 3′-H), 5.21 (1H, d, J=2.4 Hz, 5′-H), 6.05 (1H, s, 7′-H), 6.88 (2H, CH).

Dibenzhydryl *S*-7′-Azido-$O^{2'}$-benzoyl-7′-deoxygriseolate (16)——Compound **15** (935 mg, 0.99 mmol) was dissolved in 5 ml of well-dried hexamethylphosphoric triamide, to which 68.3 mg (1.05 mmol) of well-dried sodium azide (obtained by lyophilization from water) was added. The mixture was allowed to react at room temperature for 2 h under protection from moisture. The reaction mixture was poured into ice-water and the resulting insoluble matter was collected by filtration, washed with water and dried. The resulting solid material was purified by silica gel preparative TLC (1% methanol–methylene chloride). The main band was extracted with methylene chloride to yield 455 mg (54.9%) of **16** in the form of a pale yellow powder. The compound showed an extremely strong infrared (IR) spectral peak due to azide at 2100 cm^{-1}. UV $\lambda_{max}^{methanol}$ nm (ε): 257 (18900). NMR (DMSO-d_6) δ: 8.36 (1H, s), 8.11 (1H, s), 6.93 (1H, s, 1′-H), 5.85 (1H, d, J=5.4 Hz, 2′-H), 6.5—6.8 (1H, m, 3′-H), 5.34 (1H, d, J=2.4 Hz, 5′-H), 4.87 (1H, s, 7′-H), 7.1—8.2 (29H, m, NH_2, benzhydryl, benzoyl and 1/6 benzene), 6.58 (1H, s, CH), 6.71 (1H, s, CH).

***S*-7′-Azido-7′-deoxygriseolic Acid (17)**——Compound **16** (300 mg, 0.36 mmol) was dissolved in 5 ml of anisole. Trifluoroacetic acid (5 ml) was added to the resulting solution under ice-cooling and the mixture was left standing for 30 min in a tightly stoppered vessel. The solvent was distilled off under reduced pressure. The residue was dissolved in acetone, to which toluene was added and the solvent was distilled off. This process was repeated 3 times. The residue was dissolved in 10 ml of acetone and the solution was poured into 100 ml of hexane. The resulting precipitate was collected by filtration, thoroughly washed with hexane, and dried. The resulting pale yellow solid was dissolved in 20 ml of a 20% (v/v) solution of ammonia in methanol and the mixture was left standing overnight. The solvent was distilled off under reduced pressure and the resulting residue was dissolved in a small quantity of water. The pH of the resulting solution was adjusted to 2.0 and the solution was washed with a small quantity of ether and purified by Rp-8 (Merck) column chromatography (water containing 10% (v/v) acetonitrile). The main peaks were collected and the solvent was distilled off to yield 70 mg (48.5%) of **17** in the form of a pale yellow granular substance. The IR spectrum of this compound showed a strong peak at 2110 cm^{-1} which was ascribable to azide. UV $\lambda_{max}^{H_2O}$ nm (ε): 258 (15000). NMR (DMSO-d_6) δ: 8.36 (1H, s), 8.21 (1H, s), 6.50 (1H, s, 1′-H), 4.61 (1H, d, J=5.4 Hz, 2′-H), 6.08 (1H, dd, J=2.4, 5.4 Hz, 3′-H), 5.01 (1H, d, J=2.4 Hz, 5′-H), 4.27 (1H, s, 7′-H), 7.36 (2H, br s, NH_2). $[\alpha]_D^{20}$ −31.6° (c=0.25, DMSO).

Dibenzhydryl $O^{2'}$-Benzoyl-*S*-7′-amino-7′-deoxygriseolate (18)——Compound **16** (1.20 g, 1.43 mmol) was dissolved in 15 ml of pyridine, to which 5 ml of water was added. Nitrogen gas was passed through the mixture for approximately 5 min, and hydrogen sulfide gas was passed through it for a further 30 min under ice-cooling. The flask

was flushed with nitrogen gas and the reaction mixture was left standing at room temperature for 17 h in a tightly stoppered vessel. Nitrogen gas was passed through the reaction mixture for 1 h at room temperature to remove excess hydrogen sulfide gas. Acetic acid (10 ml) was added to the reaction product and the solvent was distilled off under reduced pressure. Ethanol (10 ml) was added to the residue and distilled off. This process was repeated twice. The residue was dissolved in 30 ml of ethyl acetate and 20 ml of water and subjected to fractionation of the organic and aqueous layers. The organic layer was washed successively with 20 ml each of 0.1 N hydrochloric acid, 5% (w/v) aqueous sodium bicarbonate, and saturated aqueous sodium chloride in that order, and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to yield a pale yellow residue. This residue was purified by prepacked silica gel column (Merck) chromatography (2% (v/v) methanol–methylene chloride) to yield 494 mg (42.7%) of **18** in the form of a pale yellow powder. UV $\lambda_{max}^{methanol}$ nm (ε): 260 (18300). NMR (DMSO-d_6) δ: 8.43 (1H, s), 8.15 (1H, s), 6.63 (1H, s, 1′-H), 5.90 (1H, d, J=6.8 Hz, 2′-H), 6.66, 6.63 (1H, dd, J=6.8, 2.4 Hz, 3′-H), 5.47 (1H, d, J=2.4 Hz, 5′-H), 4.01 (1H, s, 7′-H), 7.0—8.2 (28H, m, NH_2, benzhydryl, benzoyl and 1/6 benzene), 6.76 (1H, s, CH), 6.90 (1H, s, CH).

***S*-7′-Amino-7′-deoxygriseolic Acid (19)**——Compound **18** (434 mg, 0.53 mmol) was dissolved in 5 ml of anisole. Trifluoroacetic acid (5 ml) was added to the resulting solution under ice-cooling and the mixture was left standing for 30 min at room temperature in a tightly stoppered vessel. The solvent was distilled off under reduced pressure. Acetone (5 ml) and 5 ml of toluene were added to the residue and then distilled off. This process was repeated 3 times. The resulting residue was dissolved in 5 ml of ethanol and 5 ml of acetone, and the solution was slowly poured into 50 ml of a 1 : 1 (v/v) mixture of hexane and acetone with stirring. The resulting precipitate was collected by filtration, washed with hexane, and dried. The resulting ochre-colored powder was dissolved in 20 ml of 20% (v/v) methanolic ammonia and left standing for 17 h at room temperature in a tightly stoppered vessel. The solvent was distilled off under reduced pressure and 20 ml of water was added to the residue to dissolve it. The resulting solution was gradually acidified with 1 N hydrochloric acid, whereupon insoluble matter appeared but then dissolved when the pH reached 1. The solution was washed with 20 ml of ethyl acetate. The pH of the aqueous layer was adjusted to about 7.0 by adding sodium bicarbonate. The aqueous layer was then purified by chromatography through an Rp-8 prepacked column (Merck) (10% aqueous acetonitrile) to yield 133 mg (66.0%) of **19** in the form of a pale yellow powder. UV λ_{max} nm (ε): (H_2O) 257.5 (15300); (0.1 N NaOH) 259 (15600); (0.1 N HCl) 255.7 (15300).

Dibenzhydryl *O*$^{2'}$-Benzoyl-*S*-7′-chloro-7′-deoxygriseolate (20a)——Compound **15** (2.84 g, 3.0 mmol) was dissolved in 50 ml of dimethylformamide, and 1.27 g (30 mmol) of anhydrous lithium chloride was added. The mixture was stirred under heating at 100 °C for 1 h. The solvent was distilled off under reduced pressure. The resulting residue was dissolved in 30 ml of water and 50 ml of ethyl acetate and the organic layer was separated. This was washed with water and dried over anhydrous magnesium sulfate, and the solvent was evaporated off to yield a pale brown residue. The residue was separated and purified by silica gel column chromatography (5% (v/v) methanol–methylene chloride) to yield 1.74 g (69.5%) of **20a** as a pale yellow caramel-like substance. UV $\lambda_{max}^{methanol}$ nm (ε): 257 (16500). NMR (DMSO-d_6) δ: 8.39 (1H, s), 8.09 (1H, s), 6.64 (1H, s, 1′-H), 5.92 (1H, d, J=5.4 Hz, 2′-H), 6.72 (1H, dd, J=2.4, 5.4 Hz, 3′-H), 5.39 (1H, d, J=2.4 Hz, 5′-H), 5.21 (1H, s, 7′-H), 7.1—8.1 (27H, m, NH_2, benzhydryl and benzoyl), 6.88 (1H, s, CH), 6.97 (1H, s, CH).

***S*-7′-Chloro-7′-deoxygriseolic Acid (21a)**——Compound **20a** (1.34 g, 1.61 mmol) was dissolved in 10 ml of anisole. Trifluoroacetic acid (10 ml) was added to the resulting solution under ice-cooling, and the mixture was left standing for 30 min in a tightly stoppered vessel at room temperature. The solvent was distilled off under reduced pressure. The residue was dissolved in acetone, to which toluene was added, and the solvent was distilled off. This process was repeated 3 times. The residue was dissolved in a small quantity of acetone and the solution was slowly poured into 100 ml of hexane with stirring. The resulting white precipitate was collected by filtration, thoroughly washed with hexane, and then dried. The resulting pale yellow solid was dissolved in 20 ml of a 20% (v/v) methanolic ammonia, and the solution was left standing for 2 h at room temperature in a tightly stoppered vessel. The solvent was distilled off under reduced pressure and the resulting residue was dissolved in 30 ml of water. The water solution was washed twice with 20-ml portions of ether, and then its pH was adjusted to 2.0 using concentrated hydrochloric acid. The solution was purified by chromatography through an Rp-8 prepacked column (Merck) (10% aqueous acetonitrile) to yield 525 mg (82.0%) of **21a** in the form of a pale yellowish powder. UV λ_{max} nm (ε): (H_2O) 257 (15300); (0.1 N NaOH) 259.5 (15200); (0.1 N HCl) 257.5 (15100). NMR (DMSO-d_6) δ: 8.42 (1H, s), 8.31 (1H, s), 6.58 (1H, s, 1′-H), 4.70 (1H, d, J=5.4 Hz, 2′-H), 6.10 (1H, dd, J=2.4, 5.4 Hz, 3′-H), 5.25 (1H, d, J=2.4 Hz, 5′-H), 4.97 (1H, s, 7′-H). $[\alpha]_D^{20}$ −31.6° (c=0.25, DMSO).

Dibenzhydryl *O*$^{2'}$-Benzoyl-*S*-7′-bromo-7′-deoxygriseolate (20b)——Compound **15** (14.1 g, 14.87 mmol) was dissolved in 50 ml of dimethylformamide, and 13 g (149 mmol) of anhydrous lithium bromide was added. The mixture was stirred under heating at 95 °C for 22 min. The same procedure as described for the synthesis of compound **20a** was repeated to yield 6.9 g (52.8%) of **20b**. UV $\lambda_{max}^{methanol}$ nm (ε): 258 (17500). NMR (DMSO-d_6) δ: 8.40 (1H, s), 8.09 (1H, s), 6.67 (1H, s, 1′-H), 5.97 (1H, d, J=5.4 Hz, 2′-H), 6.75 (1H, dd, J=2.1, 5.4 Hz, 3′-H), 5.40 (1H, d, J=2.1 Hz, 5′-H), 5.13 (1H, s, 7′-H), 7.0—8.2 (28H, m, NH_2, benzhydryl, benzoyl and 1/6 benzene), 6.67 (1H, s, CH), 6.75 (1H, s, CH).

***S*-7′-Bromo-7′-deoxygriseolic Acid (21b)**——Compound **20b** (878 mg, 1 mmol) was dissolved in 10 ml of anisole.

The same procedure as described for the synthesis of compound **21a** was repeated to yield 250 mg (56.5%) of **21b**. UV $\lambda_{max}^{H_2O}$ nm (ε): 257 (16000). NMR (DMSO-d_6) δ: 8.47 (1H, s), 8.26 (1H, s), 6.80 (1H, s, 1'-H), 4.83 (1H, d, J=5.7 Hz, 2'-H), 6.52 (1H, dd, J=2.1, 5.7 Hz, 3'-H), 6.31 (1H, d, J=2.1 Hz, 5'-H), 5.13 (1H, s, 7'-H).

7'-Deoxygriseolic Acid (22)——**21a**→**22**: Compound **21a** (500 mg, 1.26 mmol) was dissolved in 30 ml of 80% aqueous acetic acid. Zinc powder (600 mg, 9.2 mol) was added in three approximately equal portions at intervals of 1 h with vigorous stirring. The stirring was continued under the same conditions. The insoluble matter was removed by filtration and the filtrate was evaporated to dryness. The pH of the resulting residue was adjusted to 2.0 with 1 N hydrochloric acid. The solution was purified on an Rp-8 prepacked column (Merck) (5% aqueous acetonitrile) to yield 250 mg (54.6%) of **22** in the form of a white powder. UV λ_{max} nm (ε): (H_2O) 257.5 (15600); (0.1 N NaOH) 258.4 (15700); (0.1 N HCl) 255.5 (15600). NMR (DMSO-d_6) δ: 8.40 (1H, s), 8.27 (1H, s), 6.51 (1H, s, 1'-H), 4.62 (1H, d, J=4.9 Hz, 2'-H), 6.03 (1H, dd, J=2.4, 4.9 Hz, 3'-H), 5.10 (1H, d, J=2.4 Hz, 5'-H), 2.92 (2H, dd, J=16.5, 27 Hz, 7'-H). $[\alpha]_D^{20}$ −3.8° (c=0.5, DMSO).

20b→**22**: Compound **20b** (3.51 g, 3.99 mmol) was dissolved in 40 ml of 80% aqueous acetic acid, and zinc powder (2.6 g, 39 mol) was added to the reaction solution with vigorous stirring at room temperature. The stirring was continued for 20 min. The insoluble matter was removed by filtration and the filtrate was evaporated to dryness. Addition and evaporation of toluene (30 ml) and acetone (30 ml) were repeated 3 times. The residue was dissolved in 140 ml of a 1 : 1 solution of ethyl acetate and 0.1 N hydrochloric acid. The organic layer was washed successively with 5% aqueous sodium bicarbonate (70 ml) and saturated aqueous sodium chloride (70 ml), and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to yield 3.18 g of a pale yellow caramel. This contained two main products and was purified by preparative silica gel TLC developed with methylene chloride containing 5% methanol. The more mobile material was deprotected by using the same procedure as described for the synthesis of **21a** to give 250 mg (16.4%) of **22**. The less mobile material was deprotected by using the same procedure as described for the synthesis of **21a** to give 150 mg (10.3%) of **23**.

23: NMR (DMSO-d_6) δ: 8.43 (1H, s), 8.23 (1H, s), 7.37 (2H, br s, NH_2), 6.18 (1H, d, J=6.6 Hz, 1'-H), 4.6—5.3 (2H, m), 2.3—2.8 (2H, m).

20b→**22**: Compound **20b** (5.5 g, 6.26 mmol) and 30 mg of α,α'-azobis-isobutyronitrile were dissolved in 100 ml of benzene, and 4.2 ml (15.6 mmol) of tributyltin hydride was added under a stream of nitrogen gas. The mixture was refluxed for 55 min. The solvent was distilled from the reaction mixture, and the residue was purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 4.8 g (98%) of dibenzhydryl $O^{2'}$-benzoyl-7'-deoxygriseolate. This compound (4.8 g, 6.00 mmol) was dissolved in 40 ml of anisole, and 5 ml of trifluoroacetic acid was added under ice-cooling. The mixture was left standing for 15 min, after which dry toluene was added and the solvent was distilled off. Acetone and toluene were added to the residue and distilled off. This process was repeated, after which the residue was dissolved in 50 ml of 0.5 N aqueous sodium hydroxide, and the mixture was left standing at room temperature for 3.5 h. The pH of the solution was adjusted to 2.3 with 3 N hydrochloric acid, and the solution was purified by chromatography through an Rp-8 prepacked column (Merck) (10% (v/v) aqueous acetonitrile containing 0.02% (v/v) acetic acid) to yield 1.84 g (82.4%) of **22**.

Dibenzhydryl $O^{2'}$-Benzoyl-*S*-7'-acetoxy-7'-deoxygriseolate (24)——Sodium acetate (which had previously been melted and dried) [3.2 g, 40 mmol] was dissolved in 50 ml of acetic acid with heating at 95 °C. Compound **15** (3.79 g, 4.0 mmol) was added and the mixture was stirred for 1 h at 95 °C under protection from moisture. The solvent was distilled off under reduced pressure and 10 ml each of acetone and toluene were added to the residue and distilled off. This process was repeated 3 times. The residue was dissolved in 90% (v/v) aqueous acetone. The pH of the solution was adjusted to a value no greater than 1 with 1 N hydrochloric acid, and diphenyldiazomethane was added to the mixture until the reddish color disappeared. The mixture was allowed to react at room temperature for approximately 1 h, and excess diphenyldiazomethane was decomposed with acetic acid. The solvent was distilled off under reduced pressure. The residue was dissolved in 50 ml of ethyl acetate and 50 ml of water and subjected to fractionation. The organic layer was washed successively with 5% (w/v) aqueous sodium bicarbonate and saturated aqueous sodium chloride, and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent was distilled off. The residue was purified by silica gel column chromatography (5% methanol–methylene chloride) to yield 2.33 g (67.9%) of **24** in the form of a pale yellow caramel-like substance. UV $\lambda_{max}^{methanol}$ nm (ε): 257 (18100). NMR (DMSO-d_6) δ: 8.38 (1H, s), 8.14 (1H, s), 7.18 (1H, s, 1'-H), 5.91 (1H, d, J=5.4 Hz, 2'-H), 6.79 (1H, dd, J=2.4, 5.4 Hz, 3'-H), 5.32 (1H, d, J=2.4 Hz, 5'-H), 6.00 (1H, s, 7'-H), 7.0—8.3 (27H, m, NH_2, benzhydryl and benzoyl), 6.79 (1H, s, CH), 6.91 (1H, s, CH), 1.88 (3H, s, CH_3).

***S*-7'-Hydroxy-7'-deoxygriseolic Acid (26)**——Compound **24** (2.23 g, 2.60 mmol) was dissolved in 20 ml of anisole and 20 ml of trifluoroacetic acid. The same procedure as described for the synthesis of compound **21b** was repeated to yield 300 mg (30.4%) of **26** in the form of a pale yellow powder. The NMR spectrum of the compound was identical with that of griseolic acid.[1] $[\alpha]_D^{20}$ −6.8° (c=0.5, DMSO) [*cf.* griseolic acid +6.9° (c=0.1, DMSO)].

***S*-7'-Acetoxy-7'-deoxygriseolic Acid (25)**——In the process of purification with an Rp-8 column described in connection with the synthesis of compound **26**, the second peak material eluted with water containing 10% (v/v) acetonitrile was collected to yield 200 mg (18.3%) of **25** in the form of a pale yellow powder. UV λ_{max} nm (ε): (H_2O) 257 (16100); (0.1 N NaOH) 258.2 (16500); (0.1 N HCl) 255 (16100). NMR (DMSO-d_6) δ: 8.38 (1H, s), 8.23 (1H, s),

6.56 (1H, s, 1′-H), 4.63 (1H, d, J=4.6 Hz, 2′-H), 6.07 (1H, q, J=1.9, 4.6 Hz, 3′-H), 5.13 (1H, d, J=1.9 Hz, 5′-H), 5.40 (1H, s, 7′-H), 7.42 (2H, br s, NH_2). $[\alpha]_D^{20}$ −22° (c=0.5, DMSO).

PDE Inhibitory Activity

The test was carried out essentially following the method of Pichard and Cheung.[17] Rat brains were homogenized in glass-glass or glass-Teflon homogenizers with four volumes of cold 0.17 M Tris–HCl buffer (pH 7.4), containing 5 mM aqueous $MgSO_4$. The homogenate was then centrifuged at 100000×g at 0 °C for 1 h. The clear supernatant solution was stored at −20 °C and used as a cAMP PDE preparation. Prior to use, this solution was diluted 100—150 times with 40 mM Tris–HCl buffer (pH 7.5). The reaction mixture (total volume, 0.1 ml), consisting of 40 mM Tris–HCl buffer (pH 7.5), 5 mM $MgSO_4$, 50 μM $CaCl_2$, 20 μM snake venom (*Crotalus atrox*, Sigma), 0.14 μM [^{14}C] cAMP, test material and enzyme solution, was incubated at 30 °C for 20 min. At the end of this time, the reaction mixture was treated with Amberlite IRP-58 resin, and the level of residual radioactivity of adenosine was determined. The experiment was carried out at a number of concentration levels of each active compound, and from the results, the 50% inhibition value (IC_{50}) was calculated.

A similar experiment was carried out with cGMP as the substrate instead of cAMP. The IC_{50} value toward cGMP PDE was also calculated. The results are summarized in Tables I and II.

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