Studies on Griseolic Acid Derivatives. IV. 1) Synthesis and Phosphodiesterase Inhibitory Activity of Acylated Derivatives of Griseolic Acid 2)

YOSHINOBU MUROFUSHI,a MISAKO KIMURA,a YASUTERU IJIMA,b MITSUO YAMAZAKIb and MASAKATSU KANEKO*,a

Chemical Research Laboratories and Biological Research Laboratories, Chemical Research Laboratories*a and Biological Research Laboratories,b Sankyo Co., Ltd., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan

(Received August 29, 1986)

Mono-, di- or triacylated griseolic acid derivatives were synthesized by selective acylation or by selective hydrolysis of the polyacylated derivatives. The inhibitory activities of these compounds against adenosine 3',5'-cyclic monophosphate or guanosine 3',5'-cyclic monophosphate phosphodiesterase were investigated to clarify the structure activity relationship. 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'-position had relatively little effect on the inhibitory activity.

Keywords-griseolic acid; selective partial acylation; adenosine 3', 5'-cyclic monophosphate; guanosine 3', 5'-cyclic monophosphate; phosphodiesterase; inhibition

Introduction

Griseolic acid is a new nucleoside type compound 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.4)

As illustrated, griseolic acid, which seems to be derived from adenosine and tartaric acid, has an adenine base, a bicyclic ring in its sugar moiety and two carboxylic acid groups. Thus, its structure is very similar to that of adenosine 3',5'-cyclic monophosphate (cAMP).

We have reported that griseolic acid is a strong competitive inhibitor of cAMP and guanosine 3',5'-cyclic monophosphate (cGMP) phosphodiesterases (PDE), and thus it increases the level of 3',5'-cyclic nucleotides in the tissues of treated animals.5) It seems to act as an antagonist for PDE, probably because its structure is very similar to that of a 3',5'- cyclic nucleotide. It is well-known that cAMP, which is widely distributed in animal tissues, functions as a second messenger for and mediates the effects of a large number of hormones; as a result, cAMP has a variety of important physiological and biochemical roles.

From this background, we have investigated the relationship between the structures of griseolic acid derivatives and their inhibitory activities against PDE. In this paper, we wish to report a synthetic method for acylated derivatives and an examination of their inhibitory activities against cyclic nucleotide PDE.

Synthesis

An interest in the structure–activity relationship of griseolic acid has prompted us to synthesize various partially acylated derivatives. Moreover, these compounds should be good intermediates for synthesizing other derivatives of griseolic acid, as will be reported elsewhere. We therefore developed methods for regioselective acylation of each functional group of
griseolic acid.

**N⁶,O²',O⁷'-Tribenzoylated Derivative (4)**—In order to synthesize the title compounds, the general method of acylation for nucleosides, that is, reaction with acid anhydride or acyl halide in pyridine, was applied to griseolic acid. However, the reaction mixture turned dark-brown and the starting material seemed to decompose. The desired compound was not formed. It seemed likely that this decomposition might be due to the double bond and carboxylic acid groups in the sugar moiety of griseolic acid.

Thus, we looked for a suitable protecting group, which could be removed under acidic conditions, for the two carboxylic acid groups. A benzhydryl group was found to be suitable for this purpose.

Dibenzhydryl griseolate (2) was obtained in good yield by reacting 1 with diphenyl-diazomethane in acetone containing water for 16 h at room temperature. This benzhydryl ester 2 was then benzoylated according to the general method to give the N⁶,N⁶,O²',O⁷'-tetrabenzoylated derivative (3a). Removal of the benzhydryl groups with trifluoroacetic acid in the presence of anisole gave the tribenzoyl compound (4). Under these reaction conditions, not only the carboxy-protecting groups, but also one of the N⁶-benzoyl groups were simultaneously removed quantitatively. Consequently, it is considered that this method is available for preparing an acylated adenine nucleoside-type compound which has only one acyl group at the N⁶-position. It has been well recognized that, in the proton nuclear magnetic resonance (¹H-NMR) spectrum, the signal of the hydrogen bound to the carbon atom carrying an acyloxy group of the nucleoside is usually observed at significantly lower field as compared to that of the original hydroxy nucleoside. The NMR signals of 2'-H and 7'-H of 4 were observed at about 1.3 ppm lower field than those of 1, as shown in Table I. Furthermore, the ultraviolet (UV) spectrum of 4 showed a peak at 277 nm which was very similar to that of
These results and the elemental analysis data support the structure of 4.

N^6-Benzooylated Derivative (5)—It has been well recognized that an acyl group attached to the amino group of adenosine is difficult to remove in alkaline conditions above pH 13, at which point the amido proton dissociates. On the other hand, the acyl group of the hydroxy group in the sugar moiety is easily hydrolyzed. Thus, the N^6-monobenzoyl derivative (5) was obtained in 75% yield by selective hydrolysis of the N^6,O^2',O^7'-tribenzoylated derivative 4. As shown in Table I, the NMR signals of 2'-H and 7'-H of 5 do not show any significant downfield shift as compared to those of 1. This fact, together with a UV spectrum whose λmax is almost the same as that of N^6-benzoyladenosine, and the elemental analysis data support the identification of 5 as N^6-benzooylgriseolic acid.

O^2'-Benzoylated Derivative (7)—Although selective acylation of the primary hydroxy group of a nucleoside is possible in some cases, it is generally difficult to selectively acylate one hydroxy group of a molecule having two secondary ones. In the case of p-toluene-sulfonylation, the authors have found that 2'-p-toluenesulfonyl-5'-adenylic acid can be obtained selectively by reacting 5'-adenylic acid with p-toluenesulfonyl chloride in the presence of sodium hydroxide in aqueous dioxane. We expected that the carboxylic acid moieties of 1 might play the same role as the phosphate group of 5'-AMP in an acylation and would give the O^2'-acyl derivative. As expected, O^2'-benzoylgriseolic acid 7 was obtained in good yield by reacting 1 with benzoyl chloride in a mixture of ethyl acetate and 5 M aqueous trisodium phosphate. A 1.3 ppm low-field shift of the NMR signal of 2'-H clearly supports the structure of 7.

O^2',O^7'-Diacylated Derivatives (9a and 9b)—Acylation of adenine nucleoside with acid anhydride usually gives a product acylated at hydroxyl groups in the sugar moiety as a main product. The benzhydryl ester 2 was treated with benzoic anhydride in acetone in the presence of sodium carbonate at refluxing temperature to give the dibenzoate 8a. Removal of the benzhydryl groups with trifluoroacetic acid in the presence of anisole gave di-O-benzooylgriseolic acid (9a). The structure of this compound was determined from the NMR and UV spectra and elemental analysis.

O^7'-Acylated Derivative (10)—Since all attempts of selective introduction of an acyl group at the O^7' position failed, we tried to remove the O^2'-benzoyl group of 9a. The O^2'benzoyl group of 9a was selectively removed by treatment with 20% methanolic ammonia in an ice bath for 2 h to give the O^7'-benzoyl derivative 10 in good yield. The structure of this compound was determined from the NMR and UV spectra and elemental analysis. It seems likely that the resistance of the O^7'-acyl group to hydrolysis is due to participation of the neighboring carboxylic acid group at the 9' position.

As shown in Fig. 2, the carboxy anion at the 9' position can attack the carbon atom of the benzoyl carbonyl group to form a dioxolane structure. This dioxolane anion may be stabilized by forming an ion pair with an ammonium cation. This type of dioxolane formation...
of acylated α-hydroxy carboxylic acid has been reported by Arcelli and Concilio.\textsuperscript{14})

$N\text{6, O7}'$-Dibenzoylated Derivative (6) —— Compound 4 was treated in the same manner as described for the synthesis of 10 to give the title compound 6 in low yield. This low yield may be due to partial removal of the $N\text{6}$-acyl group under these reaction conditions. The structure of this compound was determined from the NMR spectrum, in which the signal of $7'$-H is shifted downfield about 1.3 ppm compared to that of 1, and the UV spectrum, which is similar to that of 4, in addition to elemental analysis.

PDE Inhibitory Activity

As shown in Table II, the PDE inhibitory activity of griseolic acid seems to be weakened by acylation. Comparing the cAMP PDE inhibitory activity of the monobenzoylated derivative, the $N\text{6}$-benzoylated one 5 and the $O2'$-benzoylated one 7 show about 80 times and 40 times less activity, respectively, whereas the inhibitory activity of the $O7'$-benzoylated one (10) is decreased only two times. In addition, the $N\text{6, O7}'$-dibenzoylated derivative 6 shows about 200 times less activity, whereas the $O2', O7'$-dibenzoylated one (9a) shows only 20 times less activity. Further, the inhibitory activity of the $N\text{6}, O2', O7'$-tribenzoylated compound 4 is reduced about 460 times. A similar tendency was observed in the inhibitory activity on cGMP PDE. Thus, it seems likely that the amino group at the 6-position plays a very important role at the binding site of PDE. In contrast, the sugar hydroxy groups, especially that at the $7'$-position, do not play a significant role.

These findings are consistent with the conclusion of Severin et al.\textsuperscript{15}) that the amino group in the adenine moiety of cAMP was involved in binding with PDE. An investigation on the synthesis and PDE inhibitory activity of griseolic acid derivatives having various functional groups at the 6-position of the adenine moiety instead of the amino group is in progress in our laboratory to clarify more precisely the mode of action at the binding site of PDE.

Experimental

General —— Melting points were determined using 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. UV spectra were obtained using a Hitachi 200-20 spectrophotometer. The thin layer chromatography (TLC) was 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\textsuperscript{16}) using Merck silica gel (Kieselgel 60 Art. 9385).
Dibenzhydryl Griseolate (2)—Griseolic acid (10 g) was suspended in a mixture of 400 ml of acetone and 50 ml of water. To this was added a solution of 15.4 g of diphenylidiazomethane in 100 ml of acetone, and the mixture was stirred for 16 h at room temperature. The reaction product was then added dropwise to 21 of hexane. The resulting powdery substance was collected by filtration, washed with 500 ml of hexane, and dried at 55—65 °C for 10 h under a pressure of 1—2 mmHg to yield 17.95 g (95.5%) of 2 as a white powder. UV (methanol) λ_{max} nm (ε): 258 (11800).

NMR of 2 in water. To this was added a solution of 15.4 g of diphenyldiazomethane in 100 ml of acetone, and the mixture was cooled and left standing at 50 °C for 16 h. Methanol (20 ml) was added with ice-cooling, and the whole was stirred for 30 min. The solvent was then distilled off under reduced pressure. The residue was dissolved in a mixture of 100 ml of ethyl acetate and 50 ml of water, and the ethyl acetate layer was separated and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to leave a pale yellowish residue. This residue was dissolved in a small quantity of methylene chloride, to which ethanol was added. The solution was slowly distilled off under reduced pressure with an aspirator, leaving a yellowish powdery substance. This substance was collected by filtration, washed with ethanol and then dried to yield 26.4 g of 3a in the form of a yellow powder. A sample for analysis was obtained by silica gel column chromatography with a 10% (v/v) solution of acetone in benzene. UV (methanol) λ_{max} nm (ε): 273 (22000).

NMR (DMSO-d6) δ ppm: 8.89 (1H, s, 2 or 8-H), 8.63 (1H, s, 2 or 8-H), 6.04 (1H, d, J = 6.6 Hz, 2'-H), 6.63 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.63 (1H, d, J = 3.0 Hz, 5'-H), 5.13 (1H, s, 7'-H). Anal. Calcd for C_{40}H_{33}N_{5}O_{8}·1/2H_{2}O: C, 66.66; H, 4.75; N, 9.72. Found: C, 66.67; H, 4.60; N, 9.59.

Dibenzhydryl N^6,N^6,O^2',O^7-Tetrabenzyolgriseolate (3a)—Compound 2 (17.8 g) was dissolved in anhydrous pyridine. Then 18.5 ml of benzoyl chloride was added, with ice-cooling, and the mixture was kept standing at room temperature for 16 h, with protection from moisture. The reaction product was then ice-cooled, and 10 ml of water was added. The mixture was stirred at room temperature for 1 h. The solvent was then distilled off under reduced pressure. The residue was dissolved in a mixture of 100 ml of ethyl acetate and 50 ml of water, and the ethyl acetate layer was separated. This separated layer was washed with dilute hydrochloric acid, water, an aqueous solution of sodium bicarbonate, and a saturated aqueous solution of sodium chloride, in that order. The organic solution was separated and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to leave a pale yellowish residue. This residue was dissolved in a small quantity of methylene chloride, to which ethanol was added. The solution was slowly distilled off under reduced pressure with an aspirator, leaving a yellowish powdery substance. This substance was collected by filtration, washed with ethanol and then dried to yield 24.4 g of 3b in the form of a yellow powder. A sample for analysis was obtained by silica gel chromatography using methylene chloride containing 1% (v/v) methanol as the eluent. UV (methanol) λ_{max} nm (ε): 262 (15400), 280 sh (7900). NMR (DMSO-d6) δ ppm: 8.65 (1H, s, 2 or 8-H), 6.83 (1H, s, 2 or 8-H), 6.04 (1H, d, J = 6.6 Hz, 2'-H), 6.63 (1H, dd, J = 6.6, 3.0 Hz, 3'-H), 5.63 (1H, d, J = 3.0 Hz, 5'-H), 6.13 (1H, s, 7'-H). Anal. Calcd for C_{40}H_{33}N_{5}O_{11}: C, 72.13; H, 4.38; N, 6.21. Found: C, 72.13; H, 4.40; N, 6.30.

Dibenzhydryl N^6,N^6,O^2',O^7-Triacetylgriseolate (3b)—Compound 2 (1.06 g) was dissolved in 10 ml of anhydrous pyridine, then 1.13 ml of acetic anhydride was added, with ice-cooling. The mixture was stirred for 30 min under ice-cooling and left standing at 50 °C for 16 h. Methanol (20 ml) was added with ice-cooling, and the whole was stirred for 30 min. The solvent was distilled off under reduced pressure. Ethanol and water were added to the residue and the solvent was then distilled off. This process was repeated 4 times until the odor of pyridine could no longer be perceived. Subsequently, the product was dissolved in 15 ml of benzene, and the resulting solution was lyophilized to yield 1.21 g (96.6%) of 3b as a crude product. This yellowish powder can be used as such for subsequent reactions, but a sample for analysis was collected by silica gel chromatography using methylene chloride containing 1% (v/v) methanol as the eluent. UV (methanol) λ_{max} nm (ε): 262 (15400), 280 sh (7900). NMR (DMSO-d6) δ ppm: 8.65 (1H, s, 2 or 8-H), 8.59 (1H, s, 2 or 8-H), 7.50 (1H, d, J = 6.0 Hz, 2'-H), 6.49 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.35 (1H, d, J = 3.0 Hz, 5'-H), 5.99 (1H, s, 7'-H). Anal. Calcd for C_{40}H_{39}N_{5}O_{11}: C, 65.94; H, 4.69; N, 8.36. Found: C, 65.49; H, 4.46; N, 8.30.

Dibenzhydryl N^6,O^2',O^7-Tribenzyolgriseolic Acid (4)—Compound 3a (26.2 g) was dissolved in 52 ml of anisole. Trifluoroacetic acid (52 ml) was added, with ice-cooling, and the mixture was stirred for 4 h at room temperature. The solvent was distilled off under reduced pressure and the residue was dissolved in acetone. Toluene was added to the acetone solution and distilled off; this process was repeated 3 times. The residue was dissolved in 150 ml of ethylene carbonate and the solution was slowly poured into 25 ml of hexane with stirring. The resulting precipitate was collected by filtration, washed with hexane, and dried to yield 17 g of a white powder, which was dissolved in a mixture of 350 ml of ethyl acetate and 60 ml of water, and treated with activated carbon. Sodium bicarbonate (8 eq) dissolved in 90 ml of water was added to this solution with stirring, and the mixture was stirred for 2 h. The resulting solid was collected by filtration, to yield 17.7 g of 4 in an impure form. This was recrystallized from a 1:8 (v/v) mixture of water and acetone, using activated carbon, to yield 15.7 g (91.9%) of the diosodium salt of 4. UV (methanol) λ_{max} nm (ε): 228 (39800), 277 (24900). NMR (DMSO-d6) δ ppm: 8.85 (2H, s, 2 or 8-H), 6.98 (1H, s, 1'-H), 5.74 (1H, br d, J = 6.0 Hz, 2'-H), 6.48 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.25 (1H, d, J = 3.0 Hz, 5'-H), 6.47 (1H, s, 7'-H). Anal. Calcd for C_{35}H_{27}N_{5}O_{11}·H_{2}O: C, 55.78; H, 3.34; N, 9.29. Na, 6.10. Found: C, 55.43; H, 3.59; N, 9.23; Na, 6.21; mp 238—241 °C (dec.).

The diosodium salt of 4 (5.2 g) was suspended in a mixture of 300 ml of ethyl acetate and 108 ml of water, and the suspension was stirred until there was hardly and insoluble material left. The pH of the suspension was then adjusted to 1.3 by addition of 3N hydrochloric acid, under ice-cooling. The organic layer was separated, washed with a saturated aqueous solution of sodium chloride and dried over anhydrous magnesium sulfate. The solvent was then distilled off until the organic layer was concentrated to 270 ml. Hexane (270 ml) was added to the condensate, and the resulting white powdery substance was collected by filtration, washed with hexane and dried to yield 4.37 g (89.4%) of 4. UV (50% (v/v) aqueous methanol) λ_{max} nm (ε): 232 (41400), 278 (27700). NMR (DMSO-d6) δ ppm: 8.88 (1H, s, 2 or 8-H), 8.77 (1H, s, 2 or 8-H), 7.10—8.20 (16H, m, 1'-H), 5.92 (1H, d, J = 6.0 Hz, 2'-H), 6.47 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.43 (1H, d, J = 3.0 Hz, 5'-H), 5.79 (1H, s, 7'-H). Anal. Calcd for C_{35}H_{27}N_{5}O_{11}: C, 59.24; H, 3.84; N, 9.87. Found: C, 59.48; H, 3.65; N, 9.78.
N^6-Benzylogrisolic Acid (5)——The disodium salt of 4 (1.47 g) was dissolved in a 1 N aqueous solution of sodium hydroxide, and the solution was left standing for 15 h at room temperature. Ethyl acetate (30 ml) was then added and the pH of the solution was adjusted to 2.0 by addition of 2 N hydrochloric acid, under ice-cooling. Insoluble material formed. The mixture was stirred for a further 20 min, and the precipitate was collected by filtration and recrystallized from aqueous acetone to yield 725 mg (75.0%) of 5 as pale yellow crystals. UV (methanol) \( \lambda_{\text{max}} \) nm (c): 278 (25400). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.87 (1H, s, 2 or 8-H), 8.74 (1H, s, 2 or 8-H), 6.67 (1H, m, 1'-H), 4.72 (1H, br d, J = 6.0 Hz, 2'-H), 6.08 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.18 (1H, d, J = 3.0 Hz, 5'-H), 4.56 (1H, s, 7'-H). Anal. Calcd for C_{27}H_{25}N_5O_{10}·1/2H_2O: C, 51.22; H, 3.68; N, 14.22. Found: C, 51.63; H, 3.74; N, 14.36.

N^6,O^6-Dibenzylogrisolic Acid (6)——The disodium salt of 4 (1.7 g) was dissolved with ice-cooling in 17 ml of a 0.5 N aqueous solution of sodium hydroxide, and the mixture was stirred for 1 h. The pH of the solution was then adjusted to 1 by addition of 3 N hydrochloric acid. Acetone and a solution of 1.5 g of diphenylidiazomethane in 10 ml of acetone were added, and the mixture was stirred for 50 min at room temperature. The reaction mixture was treated in the conventional manner to yield 0.92 g of dibenzhydryl N^6,O^6-dibenzylgrisolate. This compound was dissolved in 8 ml of anisole. Trifluoroacetic acid (8 ml) was then added, under ice-cooling, and the mixture was left standing for 30 min at room temperature. The reaction mixture was treated in the same manner as described for 9b to yield 515 mg (37.9%) of 6. UV (50% (v/v) aqueous methanol) \( \lambda_{\text{max}} \) nm (c): 279 (23200). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.83 (1H, s, 2 or 8-H), 8.73 (1H, s, 2 or 8-H), 7.39 (2H, s, protons of benzene), 6.69 (1H, m, 1'-H), 4.76 (1H, d, J = 6.0 Hz, 2'-H), 6.08 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.20 (1H, d, J = 3.0 Hz, 5'-H), 5.82 (1H, s, 7'-H). Anal. Calcd for C_{28}H_{21}N_5O_{10}·2/3H_2O·1/3C_6H_6: C, 57.60; H, 3.89; N, 11.20. Found: C, 57.56; H, 3.90; N, 10.96.

O^6-Benzylgrisolic Acid (7)——Grisolic acid (6.81 g) was dissolved in 120 ml of a 0.5 M aqueous solution of trisodium phosphate. Ethyl acetate (120 ml) was then added. The mixture was stirred and ice-cooled whilst 18 ml of benzoyl chloride was added, and the whole was then stirred for a further 3 h. The reaction product was transferred into a separating funnel, the aqueous layer was separated, and the organic layer was washed with 20 ml of water. The aqueous layer and the washings were combined and washed with 50 ml of ethyl acetate. A further 100 ml of ethyl acetate was added to the aqueous layer, whose pH was then adjusted to 2.0 by adding concentrated hydrochloric acid, under ice-cooling. A solid substance formed, but the mixture was left standing overnight in a refrigerator. The solid substance was collected by filtration, washed with a small quantity of water, and then dried to yield 7.60 g (87.5%) of 7. UV (methanol) \( \lambda_{\text{max}} \) nm (c): 230 (17600), 257 (16000). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.43 (1H, s, 2 or 8-H), 8.33 (1H, s, 2 or 8-H), 7.48 (2H, s, NH$_2$), 7.27 (1H, s, 1'-H), 5.90 (1H, d, J = 6.0 Hz, 2'-H), 6.41 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.28 (1H, d, J = 3.0 Hz, 5'-H), 4.55 (1H, s, 7'-H). Anal. Calcd for C_{27}H_{24}N_5O_9·1/2H_2O·C: 51.52; H, 3.68; N, 7.50. Found: C, 51.03; H, 3.43; N, 14.25.

Dibenzyhydryl O^6, O^6-Dibenzylogrisolate (8a)——Compound 3 (7.11 g) was dissolved in 200 ml of acetone. Anhydrous benzoic anhydride (22.6 g) and anhydrous sodium carbonate (27.6 g) were then added, and the mixture was refluxed for 7 h. Insoluble inorganic substances were filtered off, and the solvent was distilled from the filtrate under reduced pressure to yield 0.92 g of dibenzhydryl N^6,O^6-dibenzylgrisolate. This compound was dissolved in 8 ml of anisole. Trifluoroacetic acid (8 ml) was then added, under ice-cooling, and the mixture was left standing for 50 min at room temperature. The reaction mixture was purified by preparative thin layer chromatography using methylene chloride containing 1% (v/v) methanol as the eluent to yield 4.3 g (46.7%) of 8a. UV (methanol) \( \lambda_{\text{max}} \) nm (c): 257 (19500), 280 sh (5500). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.43 (1H, s, 2 or 8-H), 8.17 (1H, s, 2 or 8-H), 7.09 (2H, s, NH$_2$), 5.93 (1H, d, J = 6.0 Hz, 2'-H), 6.85 (1H, dd, J = 6.0, 3.0 Hz, 3'-H), 5.57 (1H, d, J = 3.0 Hz, 5'-H), 6.16 (1H, s, 7'-H). Anal. Calcd for C_{31}H_{24}N_5O_{10}·1/2H_2O·O: 69.81; H, 4.56; N, 7.54. Found: C, 69.93; H, 4.46; N, 7.50.

Dibenzyhydryl O^6, O^6-Diacetylgrisolate (8b)——Compound 2 (1.37 g) was suspended in 20 ml of anhydrous pyridine, and 0.94 ml of acetic anhydride was added, with ice-cooling. The mixture was then stirred whilst protecting it from moisture. Ethanol was added to the reaction product, whilst ice-cooling, and the mixture was stirred for 30 min. The residue obtained by distilling the solvent from the reaction product under reduced pressure was dissolved in 30 ml of chloroform, and the solution was washed with water. The organic layer was separated and the solvent was distilled off under reduced pressure. The resulting residue was purified by preparative thin layer chromatography using benzene containing 10% (v/v) methanol as the developing solvent to yield 936 mg (61.3%) of 8b as a white solid. UV (methanol) \( \lambda_{\text{max}} \) nm (c): 257 (15400). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.35 (1H, s, 2 or 8-H), 8.13 (1H, s, 2 or 8-H), 7.2—8.8 (22H, m, protons of NH$_2$ and benzhydryl groups), 6.92 (1H, s, 1'-H), 5.70 (1H, d, J = 6.0 Hz, 2'-H), 6.60 (1H dd, J = 6.0, 3.0 Hz, 3'-H), 5.28 (1H, d, J = 3.0 Hz, 5'-H), 5.98 (1H, s, 7'-H). Anal. Calcd for C_{44}H_{37}N_5O_{10}·1/2H_2O·C: 65.66; H, 4.76; N, 8.70. Found: C, 65.61; H, 4.46; N, 8.17.

O^2, O^7-Dibenzylogrisolic Acid (9a)——Compound 8a (2.80 g) was dissolved in 10 ml of anisole. Trifluoroacetic acid (10 ml) was then added, under ice-cooling, and the mixture was left standing at room temperature for 1 h. The residue obtained by distilling off the solvent under reduced pressure was dissolved in acetone, toluene was added, and then the solvent was distilled off. This process was repeated 3 times. The resulting yellowish-caramel-like substance was dissolved in 20 ml of acetone, and this solution was slowly poured into 200 ml of hexane, with stirring. The mixture was then stirred for 30 min, and the precipitate was collected by filtration. The precipitate was suspended in 20 ml of a 5% (w/v) aqueous solution of sodium bicarbonate and 20 ml of water. Ethyl acetate (50 ml) was added and the pH of the mixture was adjusted to 0.5—1.0 by the addition of concentrated hydrochloric acid so as to completely dissolve the precipitate. The pH of this solution was then adjusted to 2.0 by addition of sodium bicarbonate, and the
resulting white crystalline substance was collected by filtration, washed with 100 ml of water and 100 ml of hexane, and then dried to yield 1.75 g (98.9\%) of 9a as a white powder. UV (methanol) \( \lambda_{\text{max}} \) nm (c): 230 (28000), 256 (17700). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.48 (1H, s, 2 or 8-H), 8.32 (1H, s, 2 or 8-H), 7.2–7.8 (12H, m, protons of NH\(_2\) and benzoyl groups), 7.15 (1H, s, 1'-H), 5.87 (1H, d, \( J = 6.0 \) Hz, 2'-H), 6.51 (1H dd, \( J = 6.0, 3.0 \) Hz, 3'-H), 5.40 (1H, d, \( J = 3.0 \) Hz, 5'-H), 5.80 (1H, s, 7'-H). Anal. Calcd for \( C_{28}H_{21}N_5O_{10} \cdot 1/2H_2O \): C, 56.38; H, 3.85; N, 11.74. Found: C, 56.06; H, 3.94; N, 11.26.

O\(^2-\)O\(^-\)-Diacetylgriseolic Acid (9b)——— Compound 8b (1.0 g) was dissolved in 10 ml of anisole. Trifluoroacetic acid (10 ml) was then added, under ice-cooling, and the mixture was left standing at room temperature for 30 min. The solvent was distilled from the product under reduced pressure. The resulting residue was dissolved in acetone. Toluene was added to the solution for extraction, and then distilled off under reduced pressure; this process was repeated 3 times to leave a pale yellowish residue. The extract was dissolved in 10 ml of acetone, and this solution was slowly poured into 250 ml of hexane with stirring. The resulting white precipitate was collected by filtration, washed with hexane, and dried. The precipitate was dissolved in 10 ml of saturated aqueous solution of sodium bicarbonate, with ice-cooling. The mixture was acidified by addition of 3 N hydrochloric acid, and a white precipitate was formed. On further addition of hydrochloric acid to pH 0.5–1.0, the precipitate dissolved again to yield a clear solution. This clear solution was subjected to reverse phase column chromatography using a prepacked column RP-8 (Merck), which was washed with water and then eluted with a 10% (v/v) aqueous solution of acetonitrile. The main peaks of the eluate were collected to leave 412 mg (71.1\%) of 9b as a pale yellowish powder. UV (methanol) \( \lambda_{\text{max}} \) nm (\( \varepsilon \)): 257 (15400). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.37 (1H, s, 2 or 8-H), 8.23 (1H, s, 2 or 8-H), 7.41 (2H, s, NH\(_2\)), 6.85 (1H, s, 1'-H), 5.64 (1H, d, \( J = 6.0 \) Hz, 2'-H), 6.24 (1H dd, \( J = 6.0, 3.0 \) Hz, 3'-H), 5.13 (1H, d, \( J = 3.0 \) Hz, 5'-H), 5.64 (1H, s, 7'-H). Anal. Calcd for \( C_{18}H_{17}N_5O_{10} \cdot 2H_2O \): C, 43.46; H, 3.85; N, 14.08. Found: C, 43.70; H, 3.99; N, 14.92.

O\(^-\)-Benzy1griseolic Acid (10)——— Compound 9a (1.174 g) was dissolved in a 20\% (w/v) aqueous solution of acetonitrile. The main peaks of the eluate were collected to leave 412 mg (71.1\%) of 9a as a pale yellowish powder. UV (methanol) \( \lambda_{\text{max}} \) nm (\( \varepsilon \)): 230 (28000), 256 (17700). NMR (DMSO-\( d_6 \)) \( \delta \) ppm: 8.42 (1H, s, 2 or 8-H), 8.26 (1H, s, 2 or 8-H), 7.2–7.8 (12H, m, protons of NH, and benzoyl groups), 7.15 (1H, s, 1'-H), 5.87 (1H, d, \( J = 6.0 \) Hz, 2'-H), 6.51 (1H dd, \( J = 6.0, 3.0 \) Hz, 3'-H), 5.40 (1H, d, \( J = 3.0 \) Hz, 5'-H), 5.80 (1H, s, 7'-H). Anal. Calcd for \( C_{28}H_{21}N_5O_{10} \cdot 1/2H_2O \): C, 56.38; H, 3.85; N, 11.74. Found: C, 56.06; H, 3.94; N, 11.26.

PDE Inhibitory Activity

The test was carried out following essentially the method of Pichard and Cheung.\(^{17}\) The rat brains were homogenized using glass-glass or glass–Teflon homogenizers with four volumes of cold 0.17 M Tris–HCl buffer, pH 7.4, containing 5 mM MgSO\(_4\). The homogenate was then centrifuged at 100000 \( \times \) g at 0°C for 1 h. The clear supernatant solution was stored at \(-20^\circ\)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 \( \mu \)M CaCl\(_2\), 20 \( \mu \)M of snake venom (Crotalus atrox, Sigma), 0.14 \( \mu \)M \(^{3}H\) 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.

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

1) The previous paper of this series was published in \textit{Nucleic Acid Research Symposium Series}, No. 17, 45 (1986).
2) This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University in March, 1986.


