GRISEOLIC ACID, AN INHIBITOR OF CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE PHOSPHODIESTERASE

II. THE STRUCTURE OF GRISEOLIC ACID

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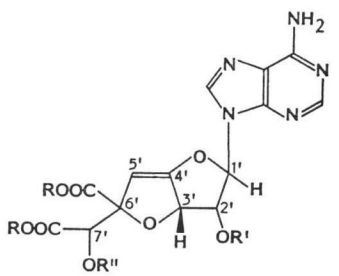
Griseolic acid, a potent inhibitor of cyclic adenosine 3′,5′-monophosphate phosphodiesterase, was isolated from the fermentation broth of *Streptomyces griseoaurantiacus* SANK 63479. Treatment of griseolic acid with HCl - MeOH gave adenine and pseudo-sugar. The structure of griseolic acid, adenine nucleoside type structure, was elucidated by chemical degradation and X-ray analysis, and was shown to be structure **1**.

In the previous paper[1], we reported the isolation, characterization and biological activities of griseolic acid produced by *Streptomyces griseoaurantiacus* SANK 63479. In this paper, we report the structural elucidation of griseolic acid by chemical degradation. The complete structure and the absolute configuration were determined by means of X-ray crystallography of 2′-methoxygriseolic acid (**4**), obtained by hydrolysis of diester (**3**) with KOH solution (*vide infra*).

Griseolic acid (**1**), $C_{14}H_{13}N_5O_8$, FAB-MS: *m/z* 380 (MH^+), mp 220°C, was shown to have an N-9 substituted adenine moiety as evidenced by the UV absorption maxima at 256 nm in 0.1 N HCl, and 260 nm in 0.1 N NaOH, and ^1H and ^{13}C NMR spectra. Signals at 8.89 and 8.76 ppm of the ^1H NMR spectrum and 159.2 (s), 148.4 (s), 145.7 (d), 143.2 (d) and 119.7 (s) ppm of the ^{13}C NMR spectrum were assigned to the above mentioned adenine structure, which was actually obtained from **1** by acid hydrolysis. The ^{13}C NMR spectrum of **1** also showed signals at 171.5 and 171.2 ppm assignable to two carboxy carbons. Treatment of **1** with excess diazomethane in DMSO - MeOH mixture for 10 minutes gave diester (**2**), $C_{16}H_{17}N_5O_8$, mp 130~132°C, *m/z* 407 (M^+), and diester monomethoxide (**3**), $C_{17}H_{19}N_5O_8$, *m/z* 421 (M^+). In the ^1H NMR spectrum of **2**, methyl ester signals appeared at 3.60 and 3.78 ppm, on the other hand, corresponding methyl signals appeared at 3.66 and 3.72 ppm in the spectrum of **3**. Therefore, a methyl signal at 3.52 ppm in **3** was assigned to the methyl ether group.

Scheme 1.



	R	R′	R″
1	H	H	H
2	CH_3	H	H
3	CH_3	CH_3	H
4	H	CH_3	H
5	CH_3	Ac	Ac
6	CH_3	CH_3	Ac

Table 1. ^{1}H NMR spectral data (ppm).

	H-1′	H-2′	H-3′	H-5′	H-7′	Solvent
1	6.67 (s)	4.77 (d)	5.79 (q)	5.33 (d)	(4.7)	D_2O
2	6.58 (s)	4.75 (d)	6.25 (q)	5.08 (d)	4.73 (s)	DMSO
3	6.58 (s)	4.50 (d)	6.15 (q)	5.04 (d)	4.63 (s)	DMSO
4	6.65 (s)	4.34 (d)	5.66 (q)	5.21 (d)	4.46 (s)	D_2O
5	6.58 (s)	5.68 (d)	6.20 (q)	5.09 (d)	5.88 (s)	$CDCl_3$
6	6.40 (s)	4.40 (d)	6.10 (q)	5.10 (d)	5.87 (s)	$CDCl_3$

( ): Overlapping, s: singlet, d: doublet, q: quartet.

This structural information was confirmed by the fact that hydrolysis of **3** with KOH solution gave monomethoxy derivative (**4**). Acetylation of **2** and **3** with acetic anhydride in pyridine at room temperature overnight gave diacetate (**5**), $C_{20}H_{21}N_5O_{10}$, *m/z* 491 (M^+), and monoacetate (**6**), $C_{19}H_{21}N_5O_9$, respectively. These observations showed the presence of two carboxylic acids, two hydroxyl groups, and two ethereal oxygens in the structure; moreover one double bond with a bicyclic ring system as **1** could be deduced, with the concept of an unsaturation number of 11. Assignments of signals of the ^{1}H NMR spectrum of griseolic acid together with those of **2**, **3**, **4**, **5** and **6** are shown in Table 1. From a comparison of the spectral data of **2** and **5**, and **3** and **6**, it was shown that acetylation shifted the H-2′ and H-7′ protons to a low field in the former, while shifting only the H-7′ proton to a low field in the latter. Therefore, the methylation with diazomethane of **1** was deduced to attack the hydroxyl at the C-2′ position.

Methanolysis of **1** with 5% HCl in absolute methanol gave two UV positive compounds **7** and **8**, and one UV negative compound **9**. Compound **7** was shown to be identical to adenine by comparisons of IR, UV and mass spectra. In the ^{1}H NMR spectrum of **8**, $C_{16}H_{18}N_5O_8Cl$, *m/z* 443 (M^+), the methyl signal appeared at 3.85 and 3.83 ppm and also the signal of the isolated methylene appeared as an AB quartet at 3.2 ppm. Therefore, these observations strongly suggested that hydrogen chloride was introduced into the double bond. The third compound **9**, $C_{13}H_{20}O_{10}$, *m/z* 366 (M^+), was an anomeric

Scheme 2.

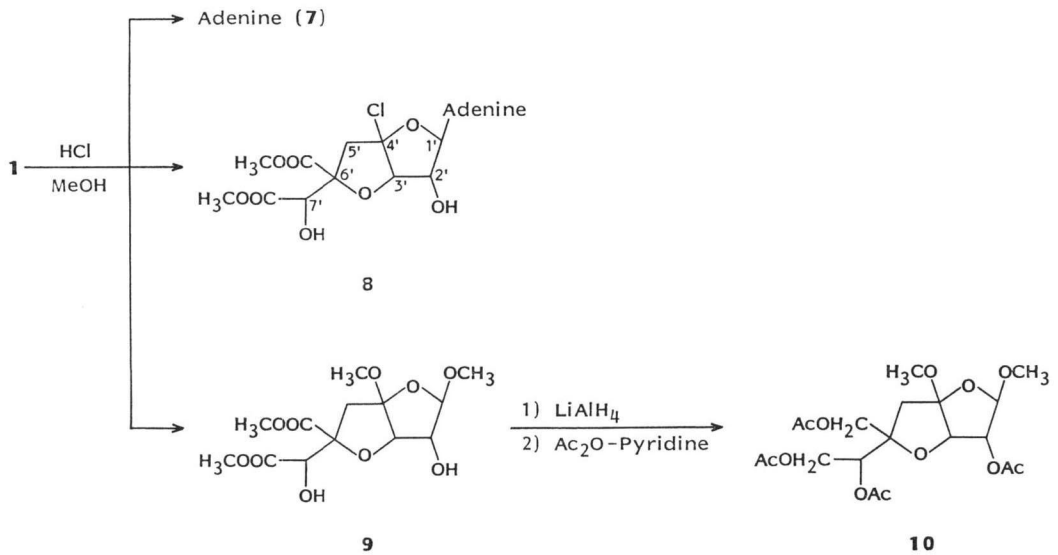
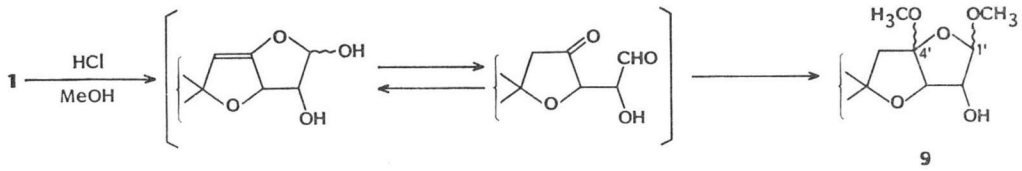


Fig. 1.



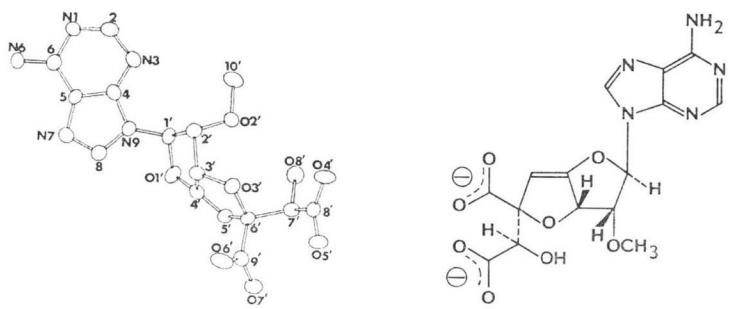
mixture. The ^{1}H NMR spectrum of **9** showed the presence of two methyl ester and two methoxy groups. One of the methoxy groups was at the anomeric position at C-1′ in the sugar type structure (pseudo-sugar), and the other was assigned to the C-4′ position with the mechanistic consideration for its formation *via* a keto-aldehyde intermediate as shown in Fig. 1.

Reduction of **9** with $LiAlH_4$ in THF followed by acetylation with acetic anhydride in pyridine afforded tetraacetate **10**, an anomeric mixture, $C_{19}H_{28}O_{12}$, *m/z* 448. The ^{1}H NMR spectrum of **10** showed the signal of two protons of an AB quartet at 4.06 and 4.22 (J_{AB}=12.0) and three protons of an ABX type at 4.12, 4.42 (J_{AB}=12.0, J_{AX}=8.0 and J_{BX}=3.0) coupled with a signal at 5.41 ppm (X part). This suggested the presence of two primary hydroxyl groups derived from the carboxylic acid by the above reduction.

Based on all the above evidence and X-ray analysis, the structure of griseolic acid was shown to be structure **1**.

X-Ray crystallographical data of **4** are as follows: $(C_{15}H_{13}N_5O_8)^{-2}$. $2K^+ \cdot 3H_2O$, MW=523.6, orthorhombic, $P2_12_12_1$, a=35.749 (8), b=8.315 (3), c=6.945 (1) Å, U=2064.4 Å^3, Z=4, Dx=1.68 g·cm^{-3}, $\mu(CuK_\alpha)$=4.8 mm^{-1}. Intensity data to θ=64° were recorded on a Rigaku AFC-5 apparatus equipped with a rotating-anode X-ray generator (graphite-monochromated CuK_α radiation). A total of 2,024 independent reflections were corrected for Lorentz and polarization factors but not for absorption. Structure **4** was solved by MULTAN[2] and refined by block-diagonal least-squares methods. Hydrogen atoms were located from difference syntheses. The final least-squares refinement with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms lowered R-values by 0.056 (Rw=0.066, 1923 observed reflections (Fo $\geqq$2.0 σ Fo)). Anomalous dispersion corrections in these calculations were applied to the scattering factor of the potassium atom, and the enantiomeric structure was also refined separately. It converged to R=0.073 (Rw=0.079) and could be rejected at a significance level much lower than 0.005[3]. Fig. 2 shows the conformation and the absolute configuration of 2′-methoxygriseolic acid (**4**): its absolute configura-

Fig. 2.



tion at the C-1′ reference center is consistent with that of adenosine[4]. 2′-Methoxygriseolic acid (**4**) is dinegatively charged with the two carboxylate groups attached to the C-6′ and C-7′ atoms each carrying a negative charge, and this net negative charge is neutralized by the two K^+ ions. The bond lengths and bond angles in the adenine ring are similar to the values found in other neutral adenine derivatives[4]. The adenine base is anti with respect to the pseudo-sugar moiety, with a glycosidic torsion angle χ_{CN} of 6.8°. The self-pairing of the adenine bases is formed by a hydrogen bonding network of NH···N bonds of length 2.895 and 3.143 Å linking each NH_2 group with the N-1 and N-7 atoms. This adenine pairing scheme involving both Watson-Crick (N-6 and N-1) and Hoogsteen (N-6 and N-7) has been observed in several other neutral adenine bases[5]. All available H-atoms involving those of water molecules participate in the hydrogen bonds.

Experimental

The UV spectra were run on a Hitachi 124 recording spectrophotometer. The NMR spectra were measured with Varian HA-100 and Hitachi R-24 spectrometers. The mass spectra were measured on Jeol JMS-01SG and Jeol JMS-100 spectrometers. Preparative TLC was performed by use of TLC-plates Kieselgel 60 F_{254} (Merck). For column chromatography, silica gel (CC-7, Mallinckrodt) was used.

Treatment of Griseolic Acid (1) with Diazomethane

To a stirred solution of 1.0 g of griseolic acid (**1**) in 15 ml of dimethylsulfoxide and 50 ml of MeOH was added an excess amount of diazomethane in ether solution for 10 minutes in an ice-bath. After evaporation of the solvent, the residue was charged on a silica gel column (100 g) and developed with $CHCl_3$ and then EtOAc, yielding 100 mg of diester monomethoxide (**3**) and 600 mg of diester (**2**).

Acetylation of 2

To a solution of 40 mg of diester (**2**) in 0.5 ml of pyridine, 15 μl of acetic anhydride was added. The mixture was allowed to stand overnight at room temp. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC on silica gel using $CHCl_3$ - MeOH (9: 1) to give 30 mg of acetate (**5**).

Acetylation of 3

To a solution of 30 mg of diester monomethoxide (**3**) in 0.5 ml of pyridine, 20 μl of acetic anhydride was added. The mixture was allowed to stand overnight at room temp. The reaction mixture was worked up as above and purified by preparative TLC on silica gel developed with $CHCl_3$ - MeOH (9: 1) to give 25 mg of acetate (**6**).

Hydrolysis of 3

To a stirred solution of 150 mg of diester monomethoxide (**3**) in 10 ml of MeOH and 2 ml of H_2O was added dropwise 1 ml of 5% KOH. After keeping at room temp overnight, the reaction mixture was nuetralized with HCl and concd to a small volume. The residue was chromatographed on Sephadex LH-20 (40 ml) and developed with H_2O to give 85 mg of 2′-methoxygriseolic acid (**4**).

Methanolysis of Griseolic Acid (1)

A solution of 200 mg of griseolic acid in 5 ml of 5% HCl - MeOH was heated at 60°C for 18 hours in a sealed tube. After removal of the solvent under reduced pressure, the residue was chromatographed on silica gel (5 g) and eluted with $CHCl_3$ - MeOH (9: 1) to give 20 mg of adenine (**7**), 70 mg of **8** and 44 mg of **9**.

Reduction and Acetylation of 9

To a stirred solution of 40 mg of **9** in 5 ml of THF was added 50 mg of lithium aluminium hydride. After refluxing for 5 hours, 1 ml of H_2O was added to the reaction mixture and the resulting mixture

was removed under reduced pressure. The product was purified by column chromatography on silica gel (5 g) and eluted with $CHCl_3$ - MeOH (9: 1) to give crude alcohol. This alcohol was dissolved in 1 ml of anhydride pyridine and 0.3 ml of acetic anhydride, and allowed to stand at room temp overnight. After removal of the solvent under reduced pressure, the residue was charged on a silica gel column (5 g) and developed with benzene - EtOAc (10: 2), yielding 20 mg of acetate (**10**).

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