NEW ANTIBIOTICS, GRISEUSINS A AND B

ISOLATION AND CHARACTERIZATION

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New antibiotics, griseusins A and B were isolated from a strain of *Streptomyces griseus*. Both antibiotics have a 5-hydroxy-1, 4-naphthoquinone chromophore and their molecular formulae were determined to be $C_{22}H_{20}O_{10}$ and $C_{22}H_{22}O_{10}$, respectively. The griseusins are active against gram-positive bacteria.

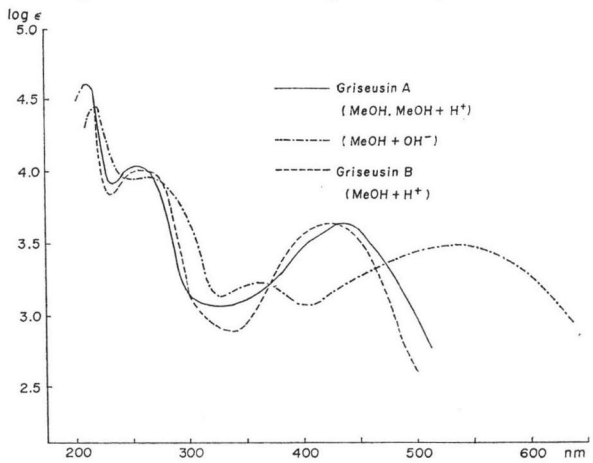
A strain of *Streptomyces griseus*, K-63, found in a soil sample collected in Peru, produces antibiotic substances active against gram-positive bacteria. The active principles, griseusins A and B, were extracted from the fermentation broth with ethyl acetate under the conditions of neutral and acidic pH, respectively. Each component was purified by chromatography on silicic acid and successive recrystallization from methanol as orange crystals.

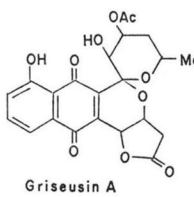
The less polar component, griseusin A, is soluble in chloroform, acetone and ethyl acetate, sparingly soluble in methanol, and insoluble in petroleum ether and water. The more polar component, griseusin B, is soluble in dioxane but hardly soluble in most organic solvents and water.

The UV and visible spectra, shown in Fig. 1, suggest that griseusins are derivatives of 5-hydroxy-1,4-naphthoquinone (juglone). In fact, addition of alkali resulted in a remarkable bathochromic shift and the griseusins showed positive color reaction to magnesium acetate. Furthermore, the IR spectra (Fig. 2) of the griseusins exhibit the chelated quinone bands (A: 1646 cm^{-1}, B: 1640 cm^{-1}) and the non-chelated quinone bands (A: 1669 cm^{-1}, B: 1667 cm^{-1}).

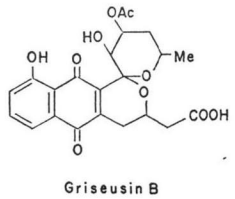
From elementary analyses, and PMR and ^{13}C-NMR spectra, the molecular formulae of griseusins A and B were determined to be $C_{22}H_{20}O_{10}$ and $C_{22}H_{22}O_{10}$, respectively.

Fig. 1. UV and visible spectra of griseusins



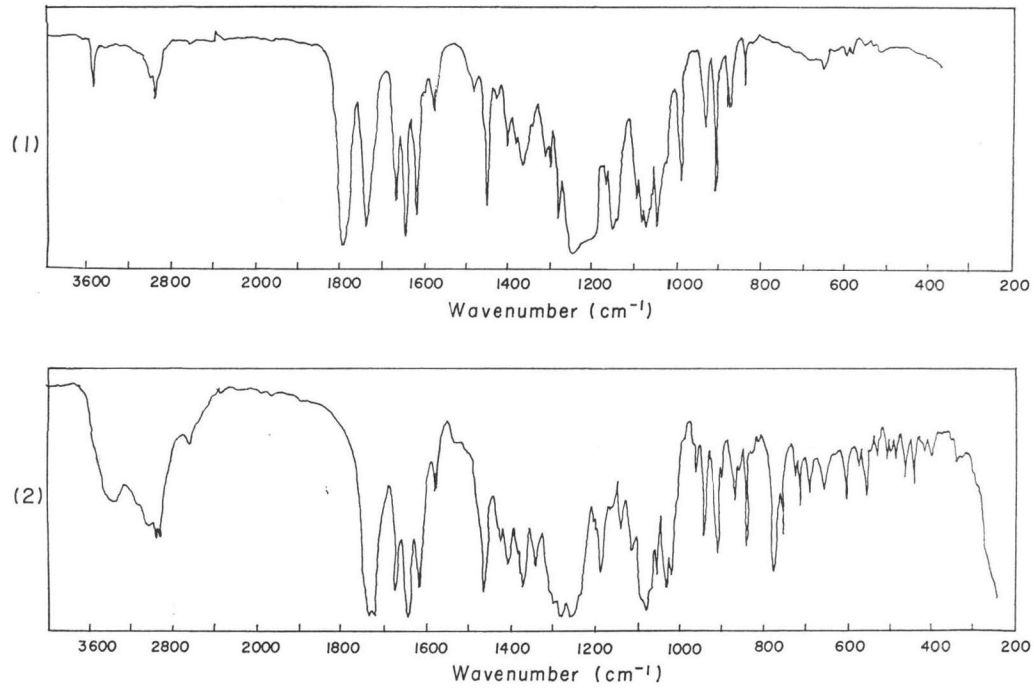


Griseusin A



Griseusin B

Fig. 2. IR spectra of griseusins. (1) Griseusin A in $CHCl_3$. (2) Griseusin B in KBr tablet.



Other antibiotics which contain the 5-hydroxy-1, 4-naphthoquinone chromophore have been reported, however, kalafungin[1] ($C_{16}H_{12}O_6$), juglomycins[2] ($C_{14}H_{10}O_6$) and nanaomycin A[3] ($C_{16}H_{14}O_6$) are distinguished from griseusins in respect to the number of carbon atoms.

The structures of the griseusins were determined to be those shown above (preceding page) and the details will be reported elsewhere.

Table 1. Antibacterial activity of griseusins (agar dilution method, using modified MUELLER-HINTON medium 'Nissan')

	MIC (mcg/ml)	
	A	B
Bacillus subtilis PCI 219	3.13	1.56
Bacillus anthracis	1.56	1.56
Staphylococcus aureus FDA 209 P JC-1	3.13	1.56
Staphylococcus aureus 80257*	1.56	1.56
Staphylococcus aureus Smith	3.13	1.56
Diplococcus pneumoniae type I	1.56	1.56
Streptococcus pyogenes C-203	0.78	0.78
Escherichia coli NIHJ JC-2	>50	>50
Escherichia coli 80750*	>50	>50
Klebsiella pneumoniae	>50	>50
Salmonella typhimurium	>50	>50
Pseudomonas aeruginosa	>50	>50

* Resistant to sulfonamides and antibiotics.

Griseusins are active against gram-positive bacteria *in vitro* as summarized in Table 1, but showed no survival effect in mice infected with *Streptococcus pyogenes* and *Diplococcus pneumoniae*. The LD_{50} of griseusins A and B, were 5.31 mg/kg and 50~100 mg/kg (in mice, i.p.), respectively.

Experimental

Fermentation and isolation of griseusins

The culture media for the fermentation of the *Streptomyces griseus*, K-63 were as follows:

Seed medium: 2.0 % soluble starch, 0.5 % glycerin, 0.3 % glucose, 1.0 % Soytone (Difco), 0.5 % corn steep liquor, 0.35 % NaCl and 0.5 % $CaCO_3$. The pH was adjusted to 7.0 before sterilization.

Production medium: 1.5 % soluble starch, 0.5 % glycerin, 1.0 % Soytone, 0.5 % corn

steep liquor, 0.3 % NaCl and 0.5 % $CaCO_3$. The pH was adjusted to 7.0 with NaOH.

The strain was inoculated into 800 ml of the seed medium in a 2-liter Erlenmeyer flask, and the medium was cultured at 28°C for 24 hours on a rotary shaker (190 r.p.m.). The culture was transferred to a 30-liter jar fermenter containing 20 liters of the production medium. Fermentation was carried out at 28°C for 48 hours under aeration of 20 liters/min and agitation of 550 r.p.m.

About 125 liters of the cultured broth was filtered. The filtrate (pH 6.9) was extracted with 72 liters of ethyl acetate. The ethyl acetate layer was washed with 10 liters of water and condensed *in vacuo* to 200 ml. To the solution was added one liter of *n*-hexane to precipitate 7.1 g of crude griseusin A.

The water layer which removed griseusin A was acidified (pH 3.0) with HCl and extracted with 36 liters of ethyl acetate. The ethyl acetate layer was treated as above to give 22 g of precipitates containing griseusin B.

Purification of griseusin A

The crude griseusin A (7.1 g) was chromatographed on a column of Mallinckrodt Silicic AR CC-7 (140 g), and eluted with chloroform - methanol (methanol 0~5 %) and recrystallization from methanol gave 2.2 g of griseusin A as orange prisms, mp 165~167°C, $[\alpha]_D^{23.5}$ −147.8° (±1.9°) (*c* 0.997, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε): 212.5 (4.59), 253 (4.05), 433 (3.61); $\lambda_{max}^{MeOH+NaOH}$ nm (log ε): 213 (4.44), 255 (3.95), 360 (3.21), 536 (3.49). ^{13}C NMR: 22 carbon signals (in $CDCl_3$ and in d_6-acetone).

Anal. Calcd. for $C_{22}H_{20}O_{10}\cdot 3/2H_2O$: C, 56.05; H, 4.92; H_2O, 5.73 %. M.W. 471.40.
Found: C, 56.15; H, 5.12; H_2O, 5.37 %. M.W. 490.7.
(osmometry in $CHCl_3$)

Purification of griseusin B

The crude griseusin B (22 g) was chromatographed on a column of silicic acid (440 g) with chloroform - methanol (methanol 0~5 %). The griseusin B fractions eluted with chloroform - methanol (97 : 3) were combined and evaporation of the solution gave 3.28 g of almost pure antibiotic. Recrystallization from methanol afforded pure griseusin B as orange prisms, mp 210°C (dec.), $[\alpha]_D^{24.5}$ −190.2° (±4.7°) (*c* 0.5, DMF), UV λ_{max}^{MeOH} nm (log ε): 212 (4.60), 254 (4.00), 269 sh (3.98), 427 (3.62).

Anal. Calcd. for $C_{22}H_{22}O_{10}$: C, 59.19; H, 4.97; O, 35.85.
Found: C, 59.27; H, 5.03; O, 35.85.

Acknowledgement

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