8-METHOXYGRISEORHODIN C, A NEW MEMBER OF GRISEORHODIN ANTIBIOTIC

Sir:

In the screening course for new antibiotics, a new griseorhodin-type antibiotic, 8-methoxygriseorhodin C (**1**) was isolated from the culture broth of a *Streptomyces* strain, SIPI-A_5-0044. The organism was isolated from a soil sample collected in Jiangsu Province, People's Republic of China. In this communication, we would like to report the production, isolation and structural elucidation of 8-methoxygriseorhodin C.

The producing strain was inoculated in a 500-ml baffled Erlenmeyer flask containing 110 ml medium consisting of glycerol 2.0%, soybean meal 1.5%, $CoCl_3 \cdot 6H_2O$ 0.0005% and K_2HPO_4 0.1% (pH was adjusted to 6.2 before sterilization with 1 M KH_2PO_4) and shaked on a rotary shaker at 28°C for 53 hours. The seed culture (each 2%) was transferred into 46 flasks containing the same medium and cultivation was carried out for 96 hours under the same condition described above. Bioactivity was monitored by disc assay against *Bacillus subtilis* PCI 219.

The harvested broth thus obtained was adjusted to pH 2 and filtrated. The antibiotic in the filtrate (4,960 ml) was extracted with EtOAc (2,500 ml × 2). The antibiotic in the mycelium cake was extracted with MeOH (1,000 ml) and filtrated. The MeOH solution was concentrated and the active substance was extracted with EtOAc. This EtOAc layer was combined with the extract from the broth filtrate and dried over anhydrous Na_2SO_4, then concentrated *in vacuo* to give a dark red powder (2.4 g). This red powder was dissolved in acidic MeOH (pH 2) and soluble portion was chromatographed on a Sephadex LH-20 column and eluted with acidic MeOH. The bioactive fractions were collected and concentrated to give 417.9 mg of red powder, which was purified by a centrifugal partition chromatography (CPC). The chromatography was performed using a CPC apparatus model NMF (Sanki Engineering Limited) with a solvent system of $CHCl_3$-MeOH-H_2O (7:13:8). The bioactive fraction of CPC (67.6 mg) thus obtained was further purified on a Sephadex LH-20 column and preparative TLC; $CHCl_3$-MeOH-AcOH (50:2:1). The bands (Rf 0.23) on TLC plates were cut off, and extracted with acidic MeOH and chromatographed on a Sephadex LH-20 column again to offer 6.6 mg of 8-methoxygriseorhodin C.

8-Methoxygriseorhodin C (**1**) is soluble in DMSO and insoluble in water, hexane and petroleum ether. It is also soluble in MeOH, acetone and $CHCl_3$ under acidic conditions, but slightly soluble in these solvents under alkaline conditions. Other physico-chemical properties are summarized in Table 1.

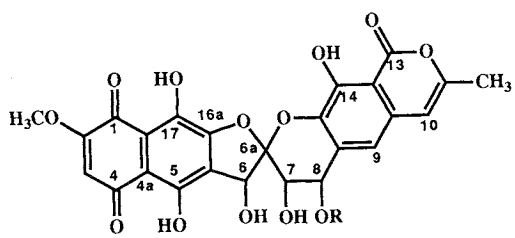
The MW and formula of **1** were determined to be 540 and $C_{26}H_{20}O_{13}$ by means of HRFAB-MS (negative ion, M^-, found *m/z* 540.0905; calcd for $C_{26}H_{20}O_{13}$, 540.0904), ^1H and ^{13}C NMR spectroscopy. The IR and UV spectra of **1** were similar to those of griseorhodins[1]. The NMR spectra of **1** exhibited 14 resonances of proton and 26 resonances of ^{13}C. They are very similar to those of griseorhodin C (**2**)[2,3] (Fig. 1) with all the signals exception of C-8 (Table 2). The C-8 of **2** connects with a OH group (δ_H 5.76 d), but C-8 of **1** connects with a OCH_3 group (δ_H 3.56 s, δ_C 57.70). The

Table 1. Physico-chemical properties of 8-methoxygriseorhodin C.

Appearance	Red powder
MP (°C, dec)	194~195
FD-MS (*m/z*)	540 (M^+)
FAB-MS (*m/z*)	540 (M^-)
Formula	$C_{26}H_{20}O_{13}$
UV λ_{max} nm (log ε)	
in MeOH	231 (4.58), 260 (sh), 275 (sh), 310 (3.71), 357 (3.61), 534 (3.69), 572 (3.62)
in 1 N HCl-MeOH (1:9)	230 (4.55), 260 (sh), 275 (sh), 312 (3.74), 358 (3.60), 486 (3.57)
in 1 N NaOH-MeOH (1:9)	271 (3.97), 298 (sh), 391 (3.71), 541 (3.74), 585 (3.72)
IR (KBr) cm^{-1}	3450, 1690, 1650, 1610, 1460, 1250, 1160, 980, 940
Rf value[a]	0.23

[a] Silica gel plate: E. Merck, Art. No. 5715, solvent: $CHCl_3$-MeOH-AcOH (50:2:1).

Fig. 1. Chemical structure of 8-methoxygriseorhodin C.



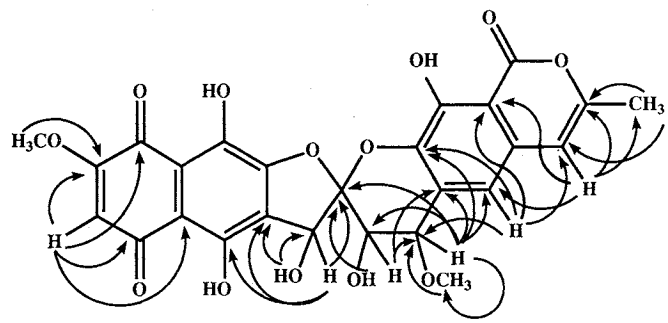
8-Methoxygriseorhodin C (**1**) R=CH_3
Griseorhodin C (**2**) R=H

Table 2. NMR data of 8-methoxygriseorhodin C and griseorhodin C.

Position	8-Methoxygriseorhodin C[a]		Griseorhodin C	
	δ_C (ppm)	δ_H (ppm, J in Hz)	δ_C (ppm)[2]	δ_H (ppm, J in Hz)[5]
1-C	180.45		179.6	
2-C	160.42		160.4	
2-OCH_3	57.07	3.88 s	57.0	3.90 s
3-C	110.26	6.40 s	110.0	6.40 s
4-C	185.92		185.5	
4a-C	106.69		106.8	
5-C	154.83	13.25 s[c], (5-OH)	157.3	13.30 s
5a-C	124.66		130.4[d]	
6-C	73.66	5.20 d (8.0), 6.39 d (8.0), (6-OH)	73.7	5.23 d (8), 6.32 d (9)
6a-C	111.38		105.0[d]	
7-C	62.11	4.56 dd (5.0, 1.8), 5.89 d (5.0), (7-OH)	66.5	4.40 dd (5, 2), 5.76 d (5)
8-C	76.53	4.25 d (1.8)	67.3	4.60 dd (5, 2), 5.76 d (5), (8-OH)
8-OCH_3	57.70	3.56 s		
8a-C	130.32[b]		122.6[d]	
9-C	117.02	7.10 s	116.8	7.10 s
9a-C	130.69[b,c]		136.8	
10-C	103.81	6.57 br s	103.9	6.58 q (1)
11-C	152.16		111.8[d]	
11-CH_3	18.74	2.22 br s	18.6	2.25 d (1)
13-C	165.69		165.8	
13a-C	105.38		132.0[d]	
14-C	148.85[c]	10.78 s[c], (14-OH)	152.1	10.82 s
14a-C	137.05		146.5[d]	
16a-C	146.65[c]		148.8	
17-C	156.53[c]	11.80 br s[c], (17-OH)	154.8	11.77 br s
17a-C	114.14[c]		114.2	

[a] ^1H NMR was measured in 400 MHz, ^{13}C NMR was measured in 100 MHz; solvents were DMSO-d_6.
[b] The assignments could be exchangeable.
[c] The assignments were completed by means of comparison with NMR data of griseorhodins A, C and other analogues[1,2,5~7].
[d] These assignments might be reassigned.

Fig. 2. HMBC correlations of 8-methoxygriseorhodin C.



assignment of 8-OCH_3 in **1** was confirmed by heteronuclear multiple-bond correlation (HMBC)[4] spectrum: the cross peaks between the methyl protons of 8-OCH_3 and C-8, 8-H and the methoxy carbon were observed. Downfield shift of C-8 signal (from δ_C 67.3 in **2** to δ_C 76.53 in **1**) and coupling pattern of 8-H signal (from doublet (J=1.8 Hz) in **1** to doublet of doublets (J=2 and 5 Hz) in **2**)

Table 3. Antimicrobial activity of 8-methoxygriseorhodin C[a].

Organism	MIC (μg/ml)	Organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	0.78	*E. coli* BE1121	25
S. aureus Smith	0.78	*E. coli* BE1186	50
S. aureus MS9610	1.56	*Shigella dysenteriae* JS11910	25
S. aureus No. 5 (MRSA)	0.78	*S. flexneri* 4b JS11811	100
S. aureus No. 17 (MRSA)	0.78	*S. sonnei* JS11746	100
Micrococcus luteus FDA 16	3.12	*Salmonella typhi* T-63	100
M. luteus IFO 3333	1.56	*S. enteritidis* 1891	>50
M. luteus PCI 1001	3.12	*Proteus vulgaris* OX19	>50
Bacillus anthracis	1.56	*P. mirabilis* IFM OM-9	100
B. subtilis NRRL B-558	3.12	*P. rettgeri* GN311	>50
B. subtilis PCI 219	3.12	*P. rettgeri* GN466	>50
B. cereus ATCC 10702	0.78	*Serratia marcescens*	50
Corynebacterium bovis 1810	3.12	*Pseudomonas aeruginosa* A3	>50
Escherichia coli NIHJ	3.12	*P. aeruginosa* GN315	>50
E. coli K-12	>100	*Klebsiella pneumoniae* PCI 602	25
E. coli K-12 ML1629	>100	*Mycobacterium smegmatis* ATCC 607	>100
E. coli BEM11	100	*Candida albicans* 3147	>50

[a] Agar dilution method (Mueller-Hinton agar, 37°C, 18 hours).

were consistent with the presence of an OCH_3 group at the C-8 position. Other HMBC correlations of **1** are shown in Fig. 2. Therefore, **1** is a new member of griseorhodin C obtained from the fermentation broth. The ^1H NMR data of the 8-methoxy compound which was derived from griseorhodin A by ECKARDT *et al.*[5] also supported this conclusion.

8-Methoxygriseorhodin C (**1**) showed strong inhibitory activity to Gram-positive bacteria but weak inhibitory activity to Gram-negative bacteria and fungi. The antimicrobial activity against various microorganisms is summarized in Table 3.

The strain, SIPI-A_5-0044 co-produced griseorhodin A[6] and two unidentified minor components.

Additional studies on other biological properties and the stereochemistry of **1** are in progress and will be reported later.

JIANXIN YANG
SHAOXIA FAN
HONGSHENG PEI
BAOQUAN ZHU
WENSI XU

Shanghai Institute of Pharmaceutical Industry,
1320 Beijing Xi Lu, Shanghai 200040, PR. China

HIROSHI NAGANAWA
MASA HAMADA
TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

(Received June 12, 1991)

References

1) ECKARDT, K.; H. THRUM, G. BRADLER & R. FUEGNER: Griseorhodins, a new group of *Actinomycete* dyes acting as antibiotics. Antibiotiki 10: 603~612, 1965
2) ECKARDT, K.; D. TRESSELT & W. IHN: The structure of the antibiotic griseorhodin C. J. Antibiotics 31: 970~973, 1978
3) STROSHANE, R. M.; J. A. CHAN, E. A. RUBALCABA, A. L. GARRETSON, A. A. ASZALOS & P. P. ROLLER: Isolation and structure elucidation of a novel griseorhodin. J. Antibiotics 32: 197~204, 1979
4) BAX, A. & M. F. SUMMERS: ^1H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986
5) ECKARDT, K.; D. TRESSELT & B. SCHÖNECKER: Antibiotika aus Actinomyceten. Zur Stereochemie der Griseorhodine. Tetrahedron 35: 1621~1624, 1979
6) TRESSELT, D.; K. ECKARDT & W. IHN: Antibiotika aus Actinomyceten. Zur chemischen Konstitution des Antibiotikums Griseorhodin A. II. Ableitung der Struktur. Tetrahedron 34: 2693~2699, 1978
7) SUETSUNA, K. & Y. OSAJIMA: Isolation of structure of dideoxygriseorhodin C produced by a *Streptomyces* sp. Agric. Biol. Chem. 53: 241~242, 1989