ISOLATION AND STRUCTURE ELUCIDATION OF A NOVEL GRISEORHODIN[1]

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Three antibiotics possessing cytotoxic properties were isolated from a strain of *Streptomyces griseus* (FCRC-57). One was found to be identical with griseorhodin A.[2~6] A second, FCRC-57-U, was found to be identical to griseorhodin C[7]. FCRC-57-G is a new antibiotic structurally related to griseorhodins A and C, and is active against KB cells *in vitro*. The structure of this new antibiotic was determined using mass spectrometry, proton and carbon nuclear magnetic resonance spectroscopy and synthesis.

In the course of a continuing search for antitumor substances of microbial origin, a broth from a *Streptomyces* species isolated from a local bean field was found to show significant activity *in vitro* against cell derived from human carcinoma of the nasopharynx (KB)*, as well as against some microorganisms. It is the purpose of this paper to present in detail the isolation and structure elucidation of the components of this fermentation responsible for the biological activity: griseorhodin A (**1**) and FCRC-57-U (**2**) and FCRC-57-G (**3**).

Fermentations were carried out in 2-liter baffled shake flasks containing 400 ml complex medium which were agitated at 200 rpm on a rotary shaker at 28°C for 40 hours. Fractionation of the broth, followed by KB cytotoxicity and by antimicrobial testing against *Bacillus subtilis, Staphylococcus aureus*, and *Penicillium notatum*, revealed that the KB inhibitory activity was concentrated in the mycelial cake and could be extracted from the acetone-washed cake by ethyl acetate subsequent to acidification. The red ethyl acetate fraction, following concentration, was column-chromatographed on LH-20 with dimethylformamide elution. The material separated cleanly into three bands: red, brown, and purple. The antimicrobial and anti-KB activities were observed in the red band, which was concentrated, dissolved in acetone, and precipitated with petroleum ether. This crude red product was submitted to further column chromatography on silica gel with methanol, chloroform, and a trace of acetic acid as eluent, yielding **1**, **2**, and **3** as well as two minor components. All three major components demonstrated quite similar activity against *Bacillus subtilis, Staphylococcus aureus*, and *Penicillium notatum*, as well as against KB cells *in vitro*. In addition, ultraviolet spectroscopy in methanol indicated a common chromophoric system in all three compounds. Compound **1** was identified as griseorhodin A based on the following arguments:

The mass spectrum of **1** showed a molecular ion at *m/e* 508, and diagnostically important fragment

* *In vitro* studies with KB cells (Dr. D. PERLMAN, University of Wisconsin) revealed an ED_{50} against KB for FCRC-57-G of 24 μg/ml, for FCRC-57-U of 30 μg/ml.

Fig. 1. Mass spectral fragmentation pattern of griseorhodin A and FCRC-57-U.

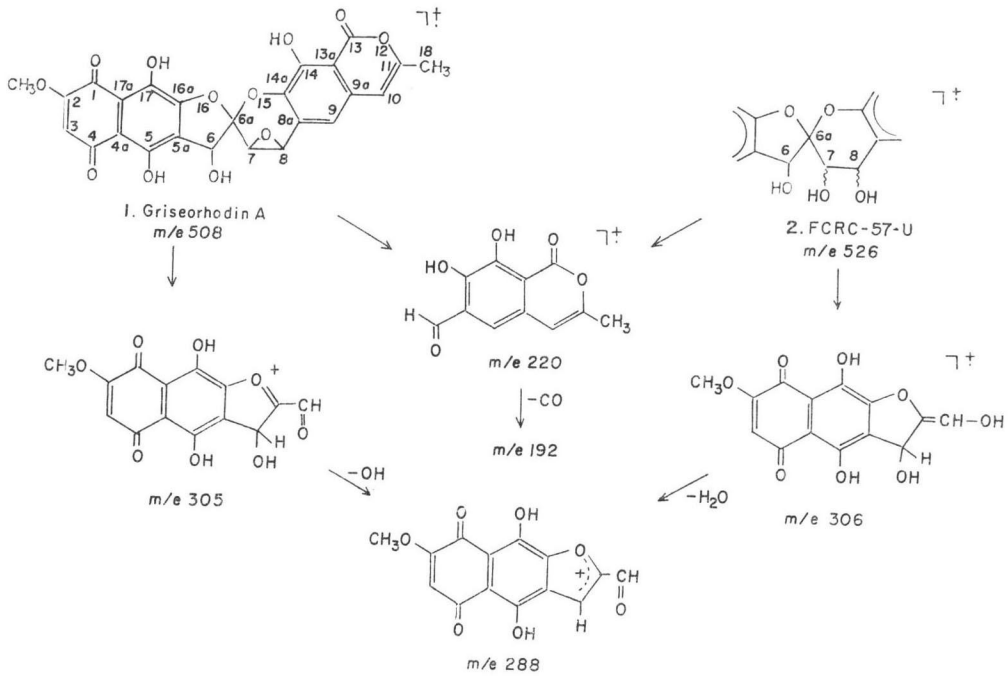


Table 1. ^{1}H-NMR Assignments of griseorhodin A (**1**), FCRC-57-U (**2**), FCRC-57-G (**3**) and 8-deutero-FCRC-57-G (**4**).

Compound	Chemical shift[a]											
	3-H	6-H	7-H	8-H	9-H	10-H	18-H	2-OCH_3	5-OH	17-OH	6-OH	14-OH
1[b]	6.40	5.70	4.26 (d)	4.42 (d)	7.26	6.54	2.23	3.88	13.22	11.74	6.90	10.70
2[c]	6.59	6.01	5.17 (d)	5.26 (d)	7.28	6.59	2.35	4.02				
3[c]	6.53	6.01	5.06 (d)	3.36 (dd) 3.80 (dd)	7.20	6.62	2.36	4.03				
4[c]	6.54	6.01	5.06 (d)	3.46 (d)	7.20	6.61	2.37	4.06				

[a] In ppm downfield from internal Me_4Si.
[b] In $(CD_3)_2SO$.
[c] In CF_3CO_2D.
[d] Irradiation at 5.06 ppm sharpens the dd at 3.36 and 3.80 ppm to d.

ions at *m/e* 288 and 220 (the base peak). These ions arise from the naphthazarin and isocoumarin portions of **1**, respectively, (Fig. 1), and compare well to the fragmentation pattern observed for a similar type compound, purpuromycin.[8] The ^{1}H-NMR spectrum of **1** (Table 1) reveals the presence of a methoxyl (δ 3.88), a methyl (δ 2.23), three phenolic hydroxyls (δ 10.70, 11.74, 13.22) and a benzylic hydroxyl (δ 6.90). In addition to these, singlets were observed at δ 6.40, 5.70, 6.54 and 7.26, and doublets at δ 4.26 and 4.42 ppm. Consideration of the mass spectrum and ^{1}H-NMR spectrum suggested that **1** is the previously reported compound griseorhodin A.[2~6] This was confirmed by comparison of the IR-spectrum, HPLC retention time and antimicrobial spectrum of this compound with an authentic sample (kindly provided by Dr. JOHN DOUROS of NCI) of griseorhodin A.

Structure assignments of the two other components, **2** and **3** were made based on experiments detailed below. Since the ^{13}C-NMR data have not been reported for griseorhodin A, we felt it necessary to assign the ^{13}C-NMR absorptions of this compound as an aid to the structure elucidation of the other members of this series of compounds. The ^{13}C-NMR signals of **1** were assigned (Table 2) using standard arguments for chemical shifts, specific proton decoupling, and comparison to published spectra of similar compounds.[8~13] The resonances in the ^{13}C-NMR spectra of **1**, **2**, and **3** were quite similar throughout the range of chemical shifts, with the exception of the region between 30 and 75 ppm. The mass spectrum of **2** showed a molecular ion at *m/e* 526, which is 18 amu higher than that observed for **1**. Otherwise the mass spectra of compounds **1** and **2** were very similar. One significant difference appeared to be the presence of ion *m/e* 305 for griseorhodin A and of ion *m/e* 306 for compound **2**. The formation of these ions can be rationalized as shown in Fig. 1. The ^{1}H-NMR spectrum of **2** (Table 1) exhibited two doublets at δ 5.26 (8-H) and 5.17 (7-H) ppm, $J_{7,8}=2$ Hz. The remainder of the spectrum is quite similar to the spectrum of **1**. Comparison of the ^{13}C-NMR of **2** (Table 2) with that of griseorhodin A (**1**) shows a downfield shift of the methine carbons, C-7 and C-8, from 48.7 and 54.6 ppm to 66.5 and 67.3 ppm, respectively. Other chemical shifts remained unchanged.

Table 2. ^{13}C-NMR Chemical shifts of griseorhodin A, FCRC-57-U and FCRC-57-G.*

Carbon	Griseorhodin A (**1**)	FCRC-57-U (**2**)	FCRC-57-G (**3**)
1	179.6	179.6	180.2
2	160.5	160.4	160.4
3	109.5(d)	110.0(d)	110.2(d)
4	185.5	185.5	185.8
4a	106.8	106.8	106.7
5	157.3	157.3	156.4
5a	130.2*	130.4*	130.3*
6	75.0(d)	73.7(d)	72.6(d)
6a	106.6	105.0	104.1
7	48.7(d)	66.5(d)	59.3(d)
8	54.6(d)	67.3(d)	30.7(t)
8a	123.7	122.6	124.9
9	115.8(d)	116.8	115.7(d)
9a	135.7*	136.8*	137.0*
10	103.6(d)	103.9(d)	103.9(d)
11	111.2	111.8	111.8
13	165.8	165.8	165.9
13a	132.0*	132.0*	129.4*
14	153.3	152.1	151.8
14a	147.5	146.5	146.2
16a	148.8	148.8	148.7
17	154.5	154.8	154.6
17a	114.2	114.2	114.2
18	18.6(q)	18.6(q)	18.6(q)
2-OCH_3	56.9(q)	57.0(q)	57.0(q)

* Assignment were made on the basis of comparisons with other naphthoquinones and compounds derived from them, internal comparisons, values derived from similar compounds in the literature, off resonance decoupling, specific proton decoupling and theoretical considerations. Values given are in parts per million downfield from internal Me_4Si.

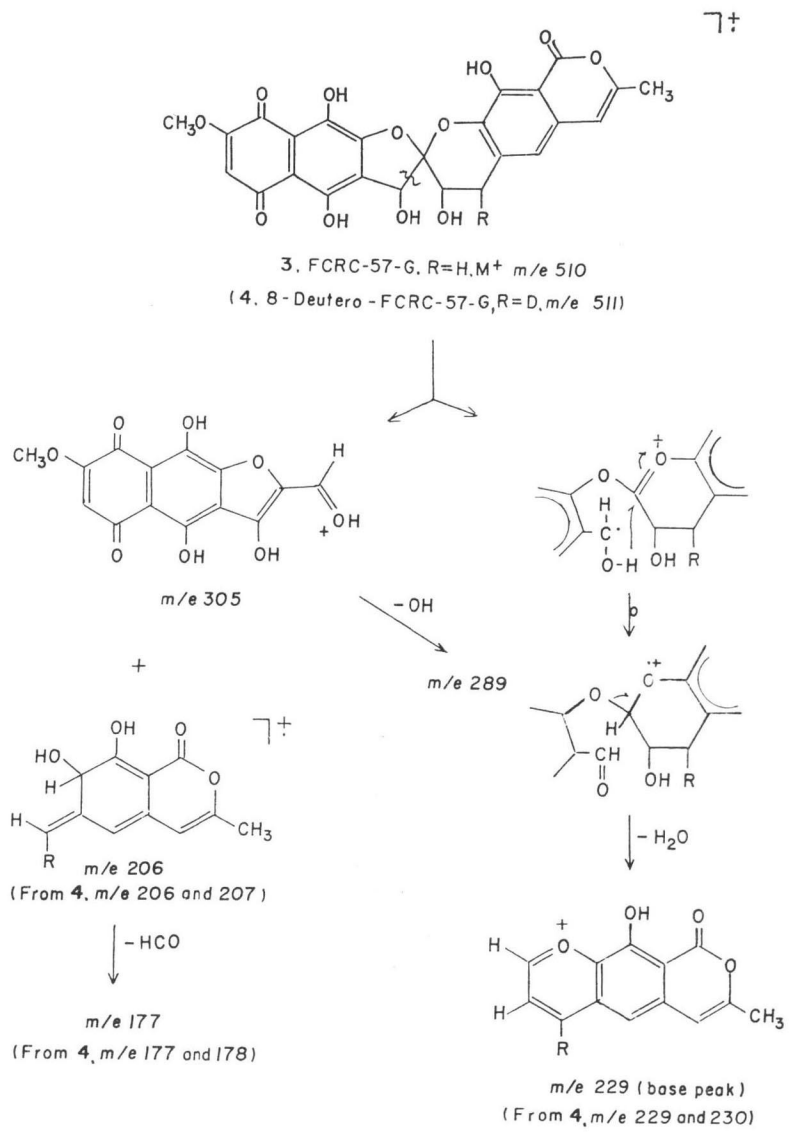
Consideration of the above results suggested that **2** is probably a C-7, C-8 dihydroxy compound. This structure assignment was confirmed by synthesis of **2** from **1** by epoxide opening using trifluoroacetic acid and water. The synthetic product was shown to be identical to the natural one by IR, UV, ^{1}H-NMR, mass spectrometry, HPLC and antimicrobial activity.

After purification to homogeneity by high pressure liquid chromatography, assignment of the structure **3** was made to the third component on the basis of its mass spectral fragmentation pattern and its ^{1}H-NMR spectrum. The molecular ion in the mass spectrum appeared at *m/e* 510 and several of the diagnostic ions are rationalized in Fig. 2 and supported by high resolution measurements.*

* ECKARDT[6] reports the molecular weight of griseorhodin B as 510.

Comparison of this fragmentation pattern to that observed for **1** and **2** (Fig. 1), leads to the conclusion that the reduction site is at C-8 rather than at C-7. Reduction at C-7 should give rise to an important ion at *m/e* 220 as observed in the mass spectrum of **1** and **2**, instead of the significant fragment observed at *m/e* 206. The base peak at *m/e* 229 can be rationalized (Fig. 2) as incorporating the isocoumarin portion of the molecule.

Fig. 2. Mass spectral fragmentation pattern of FCRC-57-G and 8-deutero-FCRC-57-G.



The ^{1}H-NMR spectral assignment of **3** and the deuterio derivative of **3** are shown in Table 1. These spectra again confirmed the assignment of **3** because the two doublets of doublets at δ 3.36 and 3.80 ppm (assigned to the protons on C-8) collapse into a doublet upon replacement of a hydrogen with deuterium. In addition, irradiation of the 7-H (5.06 ppm) sharpens the 2dd system into a 2d system. The assignments for the protons of C-3, C-9, and C-10 were based on the proton assignments of purpuromycin.[8]

The ^{13}C-NMR of **3**, when compared to **1** and **2** exhibited a large upfield shift for the peak assigned to C-8, which is a triplet in the off-resonance decoupled spectrum. A smaller upfield shift in the peak attributed to C-7 was observed in **3**. The remaining peaks of the spectrum of **3** do not shift with respect to those of **1** and **2**.

This structure assignment was confirmed by synthesizing **3** from **1** using sodium borohydride in isopropanol-dioxane. The synthetic product showed all the peaks in the mass spectrum of **3**. A similar mode of reduction on epoxide at the benzylic position has precedent in the benzo[a]pyrene system.[14,15] Reduction using sodium borodeuteride instead of sodium borohydride gave the deuterio

derivative of **3**. The mass spectrum of this labeled compound confirms the assignment of **3**.

We propose the name griseorhodin G for **3**.*

Experimental

General

Melting points were determined on a Thomas-Hoover Capillary Melting Point apparatus and are corrected. Ultraviolet absorption spectra were determined on a GCA-McPherson Model 700 recording spectrophotometer. Infrared spectra were determined on a Perkin-Elmer Model 180 recording spectrophotometer.

Nuclear magnetic resonance spectra were obtained on a Varian XL-100 spectrometer or a JEOL/FX-60/FT-NMR spectrometer with tetramethylsilane as an internal reference standard. Mass spectra were determined on a JEOL Model JMS-01SG-2 double focusing mass spectrometer at an ionizing potential of 70 eV, trap current of 200 μA, and accelerating voltage of 10 KV. Samples were introduced on a solid probe and spectra were taken at 250~300°C. High resolution data were collected of Ilford Q-2 photoplate at approximately 20,000 resolution. Plates were read by a JEOL Model JMA-1C-0 data system. Microanalyses were carried out by Galbraith Laboratories, Inc., Knoxville, Tennessee. Petroleum ether refers to the fraction of b.p. 60~68°C. All thin-layer chromatography was carried out on prepared plates (E. Merck, Silica Gel 60, F-254, 250 microns). Visualization of TLC was effected with visible and short-wavelength UV detection. High pressure liquid chromatography was carried out on a Waters Associates ALC/GPC 200 series instrument, a Waters Associates μBondapak C_{18} Column (3.9 mm × 30 cm) and a Schoeffel SF 770 Spectroflow monitor set at 254 nm (0.04 AUFS) for detection. Methanol - water - acetic acid (60: 40: 1) was used as eluent at 2 ml/min. flow. Centrifugation was carried out on a Sorvall R2-B refrigerated centrifuge.

Production, Extraction, and Preliminary Fractionation of the Metabolites of *S. griseus*, FCRC-57

A strain of *Streptomyces griseus* (FCRC-57)** was isolated from a crop soil at Fort Detrick, Maryland. The culture was maintained on agar slants. Seed and production media consisted of dextrose (15 g), soy flour (10 g), peptone (5 g), meat extract (5 g), sodium chloride (5 g), potassium phosphate dibasic (0.5 g), and tap water (1 liter). Seed medium (200 ml) contained in a 1-liter baffled flask was inoculated with an agar slice and incubated at 28°C on a rotary shaker at 200 rpm. After two days, a 5% (v/v) inoculum was added to inoculate production medium (400 ml) contained in 2-liter baffled shake flasks. Incubation continued as above. The fermentation broth (12.5 liters) from a 48-hour culture was centrifuged at 6,000 × g for 20 minutes. The pellet was washed with 50% aqueous acetone (3 liters), centrifuged as above and decanted. The washed pellet was transferred to a Waring Commercial blender to which water (25 ml), 6 N hydrochloric acid (60 ml), and ethyl acetate (800 ml) was added. Acidification changed the pellet color from purple to red. The blender was run at full speed for 2 minutes. The red organic solution was decanted and the cake was extracted a second time with ethyl acetate (600 ml). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, then concentrated to dryness on a rotary evaporator. The resulting dried extract was dissolved in dimethylformamide (30 ml) and chromatographed in six portions on a Sephadex LH-20–100 column (700 ml, 4 cm × 40 cm). Elution with dimethylformamide gave three bands: red, purple, and brown. The red band was collected and concentrated to dryness on a rotary evaporator. The resulting red solid was dissolved in a small amount of acetone and precipitated by addition of petroleum ether.

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* ECKARDT[2~6] reports griseorhodins B, C, K, and L in addition to A. UMEZAWA "Index of Antibiotics from Actinomycetes" (Univ. of Tokyo Press, Tokyo, 1967), lists griseorhodins A_2, B, C, C_2, and K. Only the structure of griseorhodin A had been previously described at the time of our initial report in April, 1978. The structure of FCRC-57-U appears to be identical with griseorhodin C as reported recently by ECKARDT *et al.*[7] These independent contemporary studies give accordant results confirming the assigned structure.

** Identified by Dr. RUTH E. GORDON, Waksman Institute of Microbiology, Rutgers, The State University, Piscataway, New Jersey 08854.

The precipitate was collected by centrifugation and dried overnight *in vacuo* to give 650 mg of crude product. The crude product (500 mg) was further chromatographed on Silicar CC-7 (Mallinckrodt, 1 lb, 5.5 cm × 60 cm) with initial elution using chloroform containing 0.2% acetic acid. A step-wise gradient was then employed using successively 1 liter of 1%, 2%, 3%, and 4% methanol in chloroform containing 0.2% acetic acid. Fractions of 6 ml were collected at a flow rate of 1 ml/min. after reaching 4% methanol concentration.

Isolation of Griseorhodin A (**1**)

Tubes 50~149 were pooled based upon their TLC homogeneity (one spot in chloroform - methanol - acetic acid, (96: 3: 1, Rf 0.36). Concentration to dryness, trituration with petroleum ether, and overnight drying *in vacuo*, gave 238 mg of **1**: mp 280~282°C (dec); UV (MeOH) λ_{max} (log ϵ) 231 (4.84), 255 (infl), 280 (4.18), 312 (3.77), 360 (3.71), 490 (infl), 510 (3.68), 540 (3.58), 570 (3.33); IR (KBr) 3500~3220, 1688, 1649, 1600, 1450, 1310, 1290, 1240, 1225 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.23 (3H, s, 18-H), 3.88 (3H, s, 2-OCH_3), 4.26 (1H, d, J=4 Hz, 7-H), 4.42 (1H, d, J=4 Hz, 8-H), 5.70 (1-H, s, 6-H), 10.70 (1H, s, 14-OH), 11.74 (1H, broad, 17-OH), 13.22 (1H, s, 5-OH); mass spectrum (probe 280°C) *m/e* 508 (60%, M^+), 490 (26), 462 (10), 305 (8), 289 (38), 288 (40), 245 (32), 229 (42), 220 (100), 204 (30), 192 (48), 174 (52); HPLC K′=3.13.

Anal. Calcd. for $C_{25}H_{16}O_{12}\cdot H_2O$: C, 57.03; H, 3.42.
Found: C, 56.82; H, 3.62.

Isolation of FCRC-57-U (**2**)

Tubes 211~250 were pooled based upon their TLC homogeneity (one spot, Rf 0.09 in chloroform - methanol - acetic acid, 96: 3: 1). Concentration to dryness, trituration with petroleum ether, and overnight drying *in vacuo* gave 27 mg of **2**: mp 220~225°C (dec); UV (MeOH) λ_{max} (log ϵ) 230 (4.77), 260 (infl), 275 (4.08), 310 (3.87), 360 (3.78), 480 (infl), 505 (3.79), 540 (3.60), 580 (infl); IR (KBr) 3460~3360, 1680, 1640, 1600, 1440, 1390, 1310, 1240, 1230 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 235 (3H, s, 18-H), 4.02 (3H, s, 2-OCH_3), 5.17 (1H, d, J=2 Hz, 7-H), 5.26 (1H, d, J=2 Hz, 8-H), 6.01 (1H, s, 6-H), 6.59 (2H, s, 3-H, 10-H), 7.38 (1H, s, 9-H); mass spectrum (probe 280°C) *m/e* 526 (9%, M^+), 508 (5), 492 (20), 306 (23), 288 (85), 260 (35), 220 (100), 204 (15), 192 (55) and 174 (67); HPLC K′=1.25.

Anal. Calcd. for $C_{25}H_{18}O_{13}\cdot H_2O$: C, 55.15; H, 3.68.
Found: C, 54.97; H, 3.85.

Isolation of FCRC-57-G (**3**)

Tubes 150~210 were pooled based upon their TLC homogeneity (one spot, Rf 0.29, chloroform - methanol - acetic acid, 96: 3: 1). Concentration to dryness, trituration with petroleum ether, and overnight drying *in vacuo* gave 107 mg of **3**. Final purification was effected using HPLC preparatively. Compound **3** (5 mg) was dissolved in 0.5 ml of glacial acetic acid and filtered through a Millipore fluoropore filter. Forty μl injections using the conditions described above gave two major peaks: K′=2.36 (76%), K′ Impurity=1.94 (24%), α=1.36. The peak at K′=2.36 was collected and concentrated to dryness on a rotary evaporator to give 3 mg of **3**. mp 270~274°C (dec); UV (MeOH) λ_{max} (log ϵ) 233 (4.78), 260 (infl), 315 (3.95), 355 (3.84), 475 (infl), 505 (3.87), 545 (3.71); IR (KBr) 3480~3400, 1680, 1640, 1600, 1450, 1320, 1280, 1240, 1225 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 2.36 (3H, s, 18-H), 3.36, 3.80 (2H, dd, J_{gem}=18 Hz, $J_{7,8}$=2 Hz, 8-H), 4.03 (3H, s, 2-OCH_3), 5.06 (1H, d, $J_{7,8}$=2 Hz, 7-H), 6.01 (1H, s, 6-H), 6.53 (1H, s, 3-H), 6.62 (1H, s, 10-H), 7.20 (1H, s, 9-H); mass spectrum (probe 270°C) *m/e* 510 (32%, M^+, $C_{25}H_{18}O_{12}$, obs. 510.0795, calcd. 510. 0797), 492 (14), 464 (15), 306 (14), 305 (30,$C_{14}H_9O_8$), 289 (16, $C_{14}H_9O_7$), 288 (21, $C_{14}H_9O_7$), 288 (21, $C_{14}H_8O_7$), 229 (100, $C_{13}H_9O_4$), 206 (65, $C_{11}H_{10}O_4$), 177 (24, $C_{10}H_9O_3$) and 176 (23); HPLC K′=2.36.

Synthesis of FCRC-57-U (**2**)

Griseorhodin A (**1**, 10 mg, 0.02 mmol) was dissolved in 4 ml of trifluoroacetic acid and allowed to stand at room temperature in the dark. After 16 hours, water was added to give a red precipitate. The solid was removed by centrifugation and purified by chromatography on Silica gel (CC-7 neutralized silica gel, 2.5 cm × 25 cm) using chloroform - methanol - acetic acid (98: 2: 1) as eluant. The major red band was collected and concentrated to dryness. Ether trituration and overnight

drying *in vacuo* gave 8 mg (77%) of pure **2**: mp 220~225°C (dec); IR (KBr) 3460~3360, 1680, 1640, 1600, 1440, 1390, 1310, 1240, 1230 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 2.35 (3H, s, 18-H), 4.02 (3H, s, 2-OCH_3), 5.17 (1H, d, J=2 Hz, 7-H), 5.26 (1H, d, J=2 Hz, 8-H), 6.01 (1H, s, 6-H), 6.59 (2H, s, 3-H, 10-H), 7.38 (1H, s, 9-H); mass spectrum (probe 250°C) *m/e* 526 (11%), 508 (3), 492 (1), 306 (28), 288 (35), 260 (16), 220 (100), 204 (13), 192 (63) and 174 (75); HPLC K′=1.25.

Anal. Calcd. for $C_{25}H_{18}O_{13}\cdot H_2O$: C, 55.14; H, 3.67.
Found: C, 55.09; H, 3.64.

The stereochemistry of the synthetic compounds, FCRC-57-U and G, in relation to the natural ones, is not known. However, all measured spectral data and biological activity were found to be identical.

Synthesis of FCRC-57-G (**3**)

Griseorhodin A (**1**, 240 mg, 0.47 mmol) was dissolved in 60 ml of dioxane and 24 ml of 2-propanol. Sodium borohydride (600 mg, 15.8 mmol) was added over a period of 5 hours. The solution was heated at 50°C for 16 hours, then evaporated to dryness. The residue was dissolved in 80 ml of water, adjusted to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate (40 ml, 3×). The ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was purified by column chromatography on Silicar CC-7 neutralized silica gel (3.5 cm×40 cm) and eluted with chloroform - methanol - acetic acid (98: 2: 1). Ten ml samples were collected and tubes 73~120 were pooled and concentrated to dryness. The residue was dissolved in trifluoroacetic acid (2 ml) and precipitated with water. The precipitate was isolated by centrifugation, then dried overnight *in vacuo* to give **3** (17 mg, 7%): mp 270~273°C (dec); IR (KBr) 3480~3400, 1680, 1640, 1600, 1450, 1320, 1280, 1240, 1225 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 2.36 (3H, s, 18-H), 3.36, 3.80 (2H, dd, J_{gem}=18 Hz, $J_{7,8}$=2 Hz, 8-H), 4.03 (3H, s, 2-OCH_3), 5.06 (1H, d, $J_{7,8}$=2 Hz, 7-H), 6.01 (1H, s, 6-H), 6.53 (1H, s, 3-H), 6.62 (1H, s, 10-H), 7.20 (1H, s, 9-H); mass spectrum (probe 255°C) *m/e* 510 (11%), 492 (12), 464 (11), 306 (2), 305 (8), 289 (14), 288 (6), 229 (78), 206 (100), 177 (40) and 176 (24); HPLC K′=2.36.

Anal. Calcd. for $C_{25}H_{18}O_{12}\cdot H_2O$: C, 56.82; H, 3.79.
Found: C, 56.84; H, 3.65.

Synthesis of 8-Deutero-FCRC-57-G (**4**)

Griseorhodin A (**1**, 40 mg, 0.08 mmol) was dissolved in 10 ml of dioxane and 4 ml of 2-propanol. Sodium borodeuteride (100 mg, 7.6 mmol) was added over a period of 5 hours. The mixture was heated at 50°C for 16 hours. Work-up and purification were as described for the synthesis of **3**. Four mg (10%) of the deutero isomer was obtained: mp 270~274°C (dec); IR (KBr) 3480~3400, 1680, 1640, 1600, 1450, 1320, 1280, 1240, 1225 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 2.37 (3H, s, 18-H), 3.46 (1H, d, J=2 Hz), 4.06 (3H, s, 2-OCH_3), 5.06 (1H, d, J=2 Hz, 7-H), 6.01 (1H, s, 6-H), 6.54 (1H, s, 3-H), 6.61 (1H, s, 10-H), 7.20 (1H, s, 9-H); mass spectrum (probe 270°C) *m/e* 511 (18%, d_1-M^+), 493 (16), 492 (20), 465 (15), 464 (15), 306 (6), 305 (13), 289 (38), 288 (13), 230 (92), 229 (75), 207 (100), 206 (51), 178 (14), 177 (25) and 176 (26).

Anal. Calcd. for $C_{25}H_{17}O_{12}D\cdot H_2O$: C, 56.71; H, 3.59.
Found: C, 57.02; H, 3.81.

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