

## THE STRUCTURE OF THE ANTIBIOTIC GRISEORHODIN C

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A combination of chemical reactions and NMR data has established the structure of griseorhodin C to be **II**.

Griseorhodins are hydroxyquinone antibiotics produced by *Streptomyces californicus* strain JA 2640 and strain ATCC 3312<sup>1,2)</sup>. Recently we reported on the chemical structure of the main component griseorhodin A (**I**)<sup>3,4)</sup>. From the antibiotic complex griseorhodin C was separated as a red substance by means of column chromatography using cellulose powder or buffered silica gel. The substance is closely related to griseorhodin A and its general physico-chemical properties are similar to those of A. This paper describes the studies on the chemical structure of griseorhodin C.

Table 1. Proton magnetic resonance parameters of compounds **I**~**IV**

	Chemical shift and coupling constants J			
	<b>I</b>	<b>II</b>	<b>IIIa</b>	<b>IV</b>
4'-OH*	13.22 s	13.30 s	13.24 s	OAc: $\left\{ \begin{array}{l} 2.00 \text{ s} \\ 2.04 \text{ s} \\ 2.20 \text{ s} \\ 2.22 \text{ s} \\ 2.31 \text{ s} \\ 2.33 \text{ s} \end{array} \right.$
9'-OH*	11.76 s b	11.77 s b	11.85 s b	
10-OH*	10.80 s	10.82 s	10.87 s	
3'-OH*	6.90 (A) d	6.32 (A) d	6.39 (A) d	
3'-H	5.32 (X) d** $J_{AX}=8 \text{ Hz}$	5.23 (X) d** $J_{AX}=8 \text{ Hz}$	5.26 (X) d** $J_{AX}=8 \text{ Hz}$	6.20 s
3-H	4.30 (A) d	4.40 (A) dd***	4.51 (A) dd***	5.62 (A) d
3-OH*	—	5.76 (M) d	6.29 (M) d	—
4-OH*	—	5.76 (R) d	OAc: 2.12 s	—
4-H	4.40 (B) d $J_{AB}=4 \text{ Hz}$	4.60 (X) dd*** $J_{AX}=2 \text{ Hz}$ $J_{AM}=5 \text{ Hz}$ $J_{RX}=5 \text{ Hz}$	5.76 (X) d $J_{AX}=2 \text{ Hz}$ $J_{AM}=5 \text{ Hz}$	6.11 (X) d $J_{AX}=2 \text{ Hz}$
6-H	6.44 (A) q	6.58 (A) q	6.53 (A) q	6.59 (A) q
7-CH <sub>3</sub>	2.23 (X) d $J_{AX} \sim 1 \text{ Hz}$	2.25 (X) d $J_{AX} \sim 1 \text{ Hz}$	2.23 (X) d $J_{AX} \sim 1 \text{ Hz}$	2.14 (X) d $J_{AX} \sim 1 \text{ Hz}$
6'-H	6.37 s	6.40 s	6.39 s	6.56 s
5-H	7.10 s	7.10 s	7.05 s	7.57 s
7'-OCH <sub>3</sub>	3.92 s	3.90 s	3.89 s	3.82 s

Notes: Measured on 100 MHz spectrometers in DMSO-d<sub>6</sub> solutions.

$\delta$  in ppm downfield from internal TMS.

\* All OH-signals disappear by addition of D<sub>2</sub>O.

\*\* Singlet after addition of D<sub>2</sub>O.

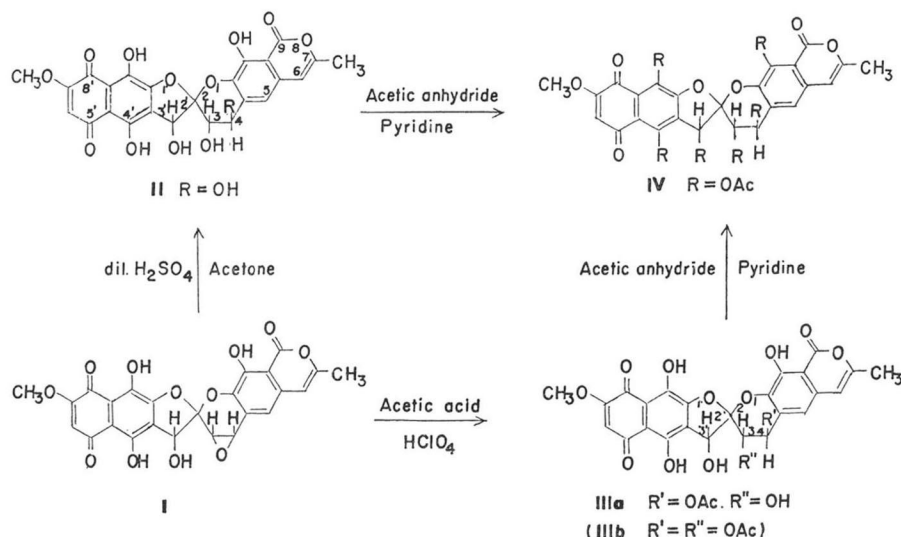
\*\*\* Only one doublet ( $J_{AX}$ ) after addition of D<sub>2</sub>O.

Abbreviations: s, singlet; d, doublet; dd, doublet of doublets; q, quartet; b, broad.

The microanalytical and molecular weight data for griseorhodin C indicate the molecular formula  $C_{25}H_{18}O_{18}$ . Since griseorhodin C was also obtained from griseorhodin A by means of chemical reactions<sup>6)</sup> both antibiotics were expected to have a similar basic constitution. The NMR spectra in dimethyl sulfoxide are shown in Table 1. All protons of griseorhodin A (I) are also detectable in the spectrum of griseorhodin C (II). Differences could be observed regarding the coupling constants of 3-H and 4-H. Furthermore, a signal at  $\delta$  5.76 ppm (2 protons,  $D_2O$  exchangeable) which is not observed in the spectrum of griseorhodin A confirms the presence of two additional OH groups in the molecule of griseorhodin C.  $D_2O$  also converts the signals at  $\delta$  4.40 ppm and  $\delta$  4.60 ppm to sharp doublets. Therefore, the two additional OH groups should be secondary alcohols located at C-3 and C-4. (Analogous behaviour was observed with the signals of the third CHOH group located at ring D of both griseorhodins A and C).

In accordance to these findings treatment of griseorhodin C with acetic anhydride in pyridine gave a hexaacetate ( $C_{37}H_{30}O_{19}$ ). In the  $^1H$ -NMR spectrum of griseorhodin C hexaacetate (substance IV in Table 1) a significant downfield shift of the proton signals at  $\delta$  4.40 ppm,  $\delta$  4.60 ppm and  $\delta$  5.23 ppm ( $\Delta\delta$  1.22 ppm,  $\Delta\delta$  1.51 ppm,  $\Delta\delta$  0.97 ppm, respectively) was detected which clearly proves the presence of the three secondary OH groups at the positions C-3, C-4 and C-3' in the molecule of griseorhodin C. These observations suggested structure II for the antibiotic which was verified in the following ways.

Fig. 1. Griseorhodins A (I) and C (II) and their derivatives.



Treatment of griseorhodin A with  $HClO_4$  in acetic acid gave IIIa and IIIb. In IIIa the epoxide ring is converted to a  $CHOH-CHOCOCH_3$  group. From the NMR spectrum (Table 1) the presence of two secondary hydroxyls is evident at C-3 and C-3' proved by addition of  $D_2O$  and downfield shift after acetylation. The acetate of IIIa obtained by treatment with acetic anhydride in pyridine was found to be identical in all respects with the hexaacetate IV obtained by acetylation of griseorhodin C. Finally, griseorhodin C was formed when griseorhodin A was treated with dil.  $H_2SO_4$  in acetone<sup>5,6)</sup>. Generally, under those conditions ring opening of epoxides fused to a six-membered ring gives *trans*-

diols. The identity of the constitution formula and the stereochemistry of this reaction product with those of griseorhodin C isolated from the natural griseorhodin mixture was confirmed by TLC, IR, NMR, CD and mass spectrum. The stereochemistry of griseorhodin C is deduced to be 3' (R), 2'/2 (S), 3 (R), 4 (S) or the enantiomer 3' (S), 2'/2 (R), 3 (S), 4 (R)<sup>5</sup>.

Griseorhodin C is active against a variety of Gram-positive and Gram-negative bacteria<sup>6</sup>.

### Experimental

Melting points are uncorrected. <sup>1</sup>H-NMR spectra were recorded on a 100 MHz spectrometer from the Central Institute for the Construction of Scientific Instruments, Academy of Sciences of the German Democratic Republic. Mass spectra were recorded on a JEOL JMS-D 100 spectrometer at 75 eV, direct inlet system, heated at 220~270°C. Exact mass measurements were performed using the peak matching technique (PFK as standard).

Thin-Layer Chromatography (TLC): The isolation of the antibiotics and the derivatives **IIIa** and **IIIb** was followed by TLC. Samples were applied to layers of silica gel (Eastman Chromagram Sheet, buffered with 0.5 N KH<sub>2</sub>PO<sub>4</sub><sup>8</sup>) air-dried, circular and ascending technique). Development was with chloroform - methanol (95 : 5 by volume) for separation of griseorhodins. For chromatography of **IIIa** and **IIIb** ethyl acetate was used.

#### Griseorhodin C (II)

Separation of griseorhodin C from natural griseorhodin mixture was performed as described<sup>4,6</sup>. Mp. 252~254°C, red powder. UV maxima in chloroform, 316, 361, 510 nm ( $\epsilon$  8200, 6250 and 6500, respectively).

Anal. Calcd. for C<sub>25</sub>H<sub>18</sub>O<sub>13</sub> (526): C, 57.03; H, 3.42

Found: C, 56.98; H, 3.51

Mass spectrum ( $m/e$ ): M<sup>+</sup> 526; (M-H<sub>2</sub>O)<sup>+</sup> 508.0707, calcd. for C<sub>25</sub>H<sub>16</sub>O<sub>12</sub> 508.0641.

Preparation from griseorhodin A: Griseorhodin A (500 mg) was suspended in acetone (60 ml). After addition of 10% H<sub>2</sub>SO<sub>4</sub> (5 ml) the mixture was refluxed for 4 hours. The reaction mixture was precipitated by addition of water (100 ml) and isolated in the usual manner. The crude product was purified by column chromatography from chloroform - methanol (95 : 5 by volume) on KH<sub>2</sub>PO<sub>4</sub>-buffered silica gel. The fractions of the main red zones of several columns were combined and evaporated to 50 ml. After cooling, the precipitate was collected, washed, and dried. The substance was identical in all respects with the natural material.

#### Reaction of griseorhodin A with acetic acid and HClO<sub>4</sub>

HClO<sub>4</sub> (70%, 3 ml) was added to a solution of griseorhodin A (500 mg) in glacial acetic acid (250 ml). The reaction mixture was kept at room temperature for 17 hours, then diluted with water (250 ml). The mixture was extracted with ethyl acetate (250 ml, the organic layer could be separated after saturation with sodium chloride). The ethyl acetate extract was washed with water (5 × 200 ml) and dried over sodium sulfate. The extract was then evaporated and applied to columns of silica gel (KH<sub>2</sub>PO<sub>4</sub>-buffered silica gel according to CORONELLI *et al.*<sup>7</sup>) and BARDONE *et al.*<sup>8</sup>), Merck 0.05~0.2 mm, 2.5 × 50 cm) packed in ethyl acetate. Elution with the same solvent. **IIIa** was isolated from the third zone, **IIIb** from the second red zone.

**IIIa**: Fine red needles (ethyl acetate), mp. 255~257°C; IR (CO): 1600, 1648, 1685, 1728 cm<sup>-1</sup> (KBr).

Anal. Calcd. for C<sub>27</sub>H<sub>20</sub>O<sub>14</sub> (568): C, 57.04; H, 3.52

Found: C, 57.13; H, 3.63

Mass spectrum ( $m/e$ ): M<sup>+</sup> 568.0863, calcd. for C<sub>27</sub>H<sub>20</sub>O<sub>14</sub>: 568.0852; (M-H<sub>2</sub>O)<sup>+</sup> 550.

**IIIb**: Red needles (ethyl acetate), mp. 150~152°C; IR (CO): 1605, 1648, 1685, 1742 cm<sup>-1</sup> (KBr).

Anal. Calcd. for C<sub>29</sub>H<sub>22</sub>O<sub>15</sub> (610): C, 57.05; H, 3.61

Found: C, 56.93; H, 3.92

Mass spectrum ( $m/e$ ): M<sup>+</sup> 610; (M-CH<sub>3</sub>COOH)<sup>+</sup> 550.

$^1\text{H}$  NMR spectrum ( $\text{DMSO}-d_6$ ): 3-H: 5.60D, 4-H: 5.97D,  $J_{3\text{-H}, 4\text{-H}}=2\text{ Hz}$ , 2 OAc,  $\text{CH}_3$ : 2.26, 2.19, 2.12.

Hexaacetate of griseorhodin C (IV):

Acetic anhydride (0.5 ml) was added to a solution of griseorhodin C (20 mg) in pyridine (0.5 ml). The reaction mixture was kept at room temperature for 6 hours, then the acetate was precipitated by addition of water. The crude acetate was dissolved in chloroform and precipitated by addition of cyclohexane to yield IV. The substance was identical with the acetate of **IIIa**, proved by comparison of IR, mass and NMR spectra.

Pentaacetate of **IIIa**:

Acetylation of 50 mg of **IIIa** by employing the same procedure as described for the acetylation of griseorhodin C. The crude product (45 mg) was recrystallized from ethanol to obtain the yellow pentaacetate of **IIIa**. Mp.  $210\sim 220^\circ\text{C}$ .

Anal. Calcd. for  $\text{C}_{37}\text{H}_{36}\text{O}_{19}$  (778): C, 57.07; H, 3.89

Found: C, 57.32; H, 4.06

Mass spectrum ( $m/e$ ):  $\text{M}^+$  778;  $(\text{M}-\text{CH}_2\text{CO})^+$  736.1252, calcd. for  $\text{C}_{35}\text{H}_{28}\text{O}_{18}$  736.1275.

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