

Isolation and Identification of Griseorhodin C Produced by a *Streptomyces* sp. No. 76^{*1}

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The fraction of crude red pigment was extracted from the mycelia of an actinomycete, *Streptomyces* sp. No. 76 derived from soil. The fraction was purified by using silica gel column chromatography and was furthermore divided into two subfractions containing one and other pigments by using Sephadex LH-20 column chromatography. Among their pigments, one pigment was purified by using high pressure liquid chromatography (HPLC). The pigment specimen purified was identified as a novel griseorhodin C reported previously on the basis of the comparative ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectra of the pigment and an authentic specimen.

In the course of our search for non-toxic red pigment of microbial origin for use as food colors, an actinomycete was isolated from a soil sample collected. In the case of growth in shake culture on a number of liquid media, this organism produced a water-soluble and red pigment complex. The UV spectrum of red pigment is similar to those of griseorhodin¹⁻⁸⁾ and purpuromyacin⁹⁾. In the present paper, we deal with the production, isolation and structural elucidation of compound 1 (Fig. 1) as griseorhodin C.

Material and Methods

Microorganisms

Streptomyces sp. No. 76 which had been isolated from a soil sample collected in Fukuoka was used throughout this experiment.

Growth and Cultivation

The strain was incubated for 7 days at 28 °C on a slant medium consisting of 1 % glucose, 0.1 % meat extract, 0.1 % yeast extract, 0.2 % polypeptone and 2 % agar (pH 7.5). Thereafter, agar slant cultures were inoculated into 500 ml Erlenmeyer flasks containing 100 ml of a medium consisting of 2.5 % soluble starch, 0.8 % yeast extract, 0.5 % polypeptone and 0.5 % NaCl (pH 7.2). The cultivation was conducted on a rotary shaker for 3 days at 28 °C.

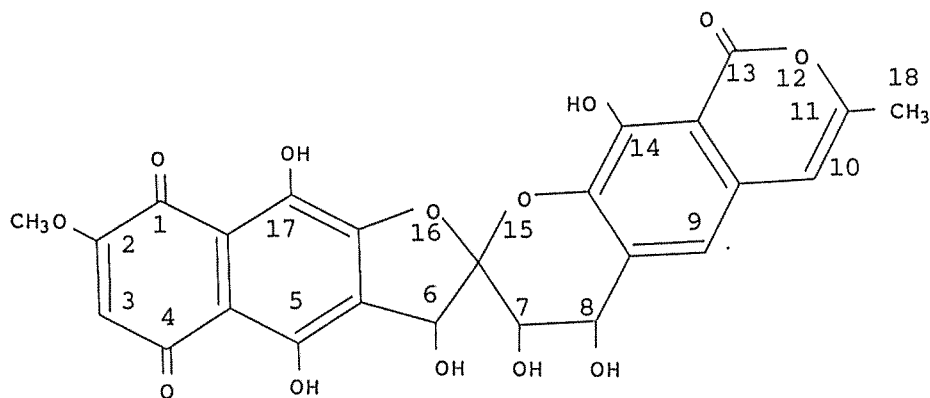
Methods of Analysis

Various spectra of pigment purified were measured on the following apparatus: Shimadzu UV-300 spectrophotometer for UV spectra, Shimadzu FTIR-4000 spectrophotometer for IR spectrum, JEOL DX-300 spectrometer for EI-MS and FD-MS, JEOL GX-500 (500 MHz) and

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Compound 1

Fig. 1. Structure of griseorhodin C.

JEOL GX-270 for C-H COSY NMR. Chemical shifts are given in ppm (in δ) relative to TMS (0 ppm) as an internal standard.

Results and Discussion

Fermentative Production and Purification

An example of the fermentation kinetics was shown in Fig. 2. The pigment production was maximally reached after 2 days when the culture broth become reddish purple, indicating the accumulation pigments. The pigment was produced in both the mycelia and the broth. The mycelia (450 g, wet weight) obtained from 10 liters of the culture broth were extracted with 5 liters of ethyl acetate at pH 3. After washing with saturated sodium bicarbonate, the solvent layer was evaporated to dryness. The residue (5.2 g) was dissolved in a small amount of dimethyl sulfoxide (DMSO) and subjected to silica gel column chromatography. The eluent with benzene-methanol-water (10:2:1) fraction containing griseorhodins was concentrated give a crude dark red powder. The powder was dissolved in a small amount of dimethylformamide (DMFA) and subjected to Sephadex LH-20 column chromatography. Development

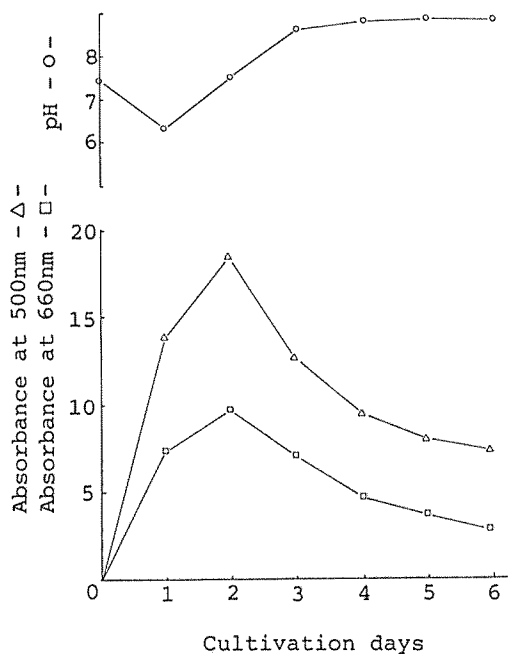


Fig. 2. The time course of pigment production.

of the column with DMFA gave two red bands which could be separated. The first eluted band was collected and concentrated to dryness to give red-colored powder (880 mg). The second eluted band, possible one of griseorhodin group, has not identified yet. For further purification the powder was dissolved in a minimum volume of DMSO and then subjected to preparative HPLC on a column (20 i. d. \times 250 cm) of Develosil ODS-10 with isocratic elution with acetone-0.05N acetic acid (55:45). The red-colored fraction was collected and concentrated to obtain a red amorphous powder (580 mg) in a pure form as compound 1.

Physico-chemical Properties and Structural Elucidation

1 melted at 260-265 $^{\circ}\text{C}$ with composition. It was soluble in alkaline water, and slightly soluble in chloroform, acetone and methanol. It turned to red from orange in acidic solution and purple from orange in alkaline solution. It

gave a reddish purple color with magnesium acetate in methanol. Fehling, Molisch, Sakaguchi and Ninhydrin reactions were negative. No optical rotation was observed.

The molecular formula was determined on FD-MS and EI-MS, which were shown in Figs 3 and 4, and on elemental analysis (Calcd. for $\text{C}_{25}\text{H}_{18}\text{O}_{13}$: C, 57.03, H, 3.42 %. Found: C, 56.77, H, 3.35 %). The UV spectrum of 1 was shown in Fig. 5. The UV spectrum was characteristic of griseorhodin and suggested the presence of similar chromophore in the molecule of 1. The IR spectrum of 1 (Fig. 6) showed characteristic absorption bands at 1650 and 1610 cm^{-1} (quinone CO). The $^1\text{H-NMR}$ spectrum of 1 (Fig. 7) revealed the presence of methoxy (δ 3.92), a methyl (δ 2.25), three phenolic hydroxyls (δ 10.81, 11.79, 13.19) and benzylic hydroxyl (δ 6.30). Furthermore, singlets were observed at δ 5.25, 6.42, 6.56 and 7.17 and doublets at δ 4.65, 4.70, and 5.29. The $^{13}\text{C-NMR}$ spectrum of 1 (Fig. 8) showed the presence of 25 carbons.

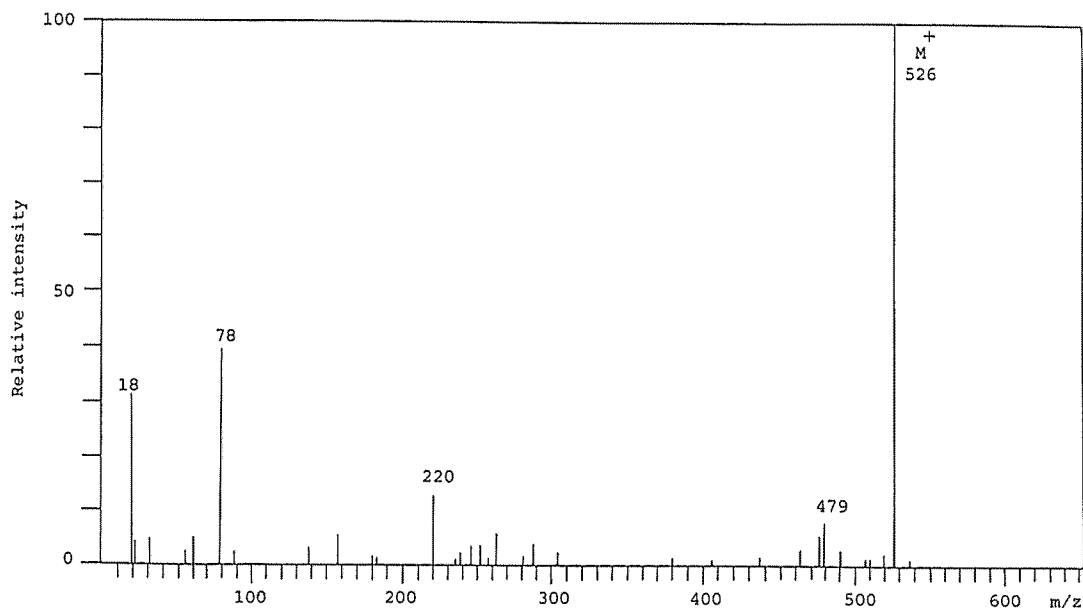


Fig. 3. FD-MS spectrum of 1.

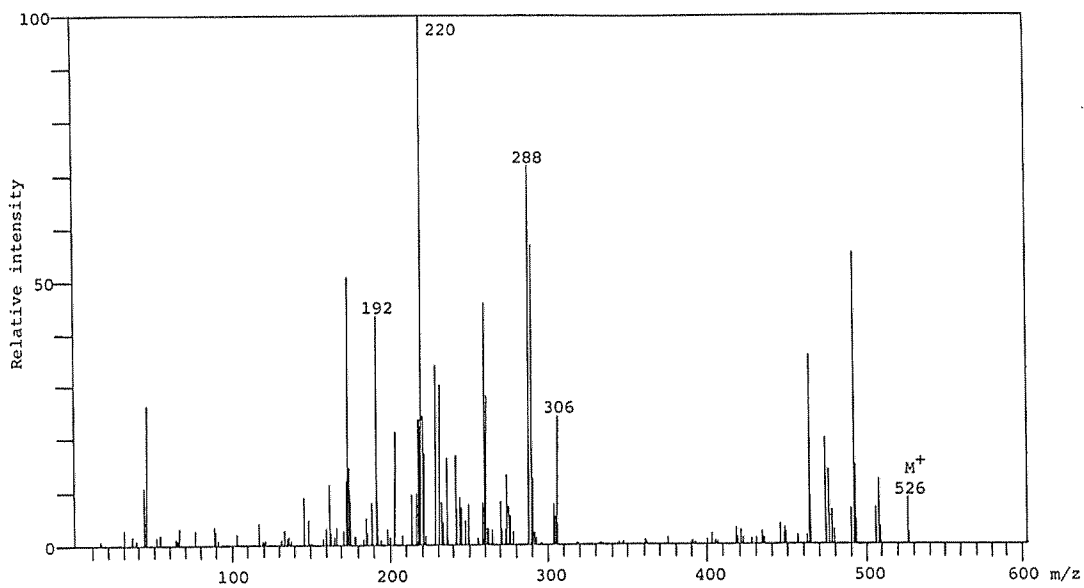


Fig. 4. EI-MS spectrum of 1.

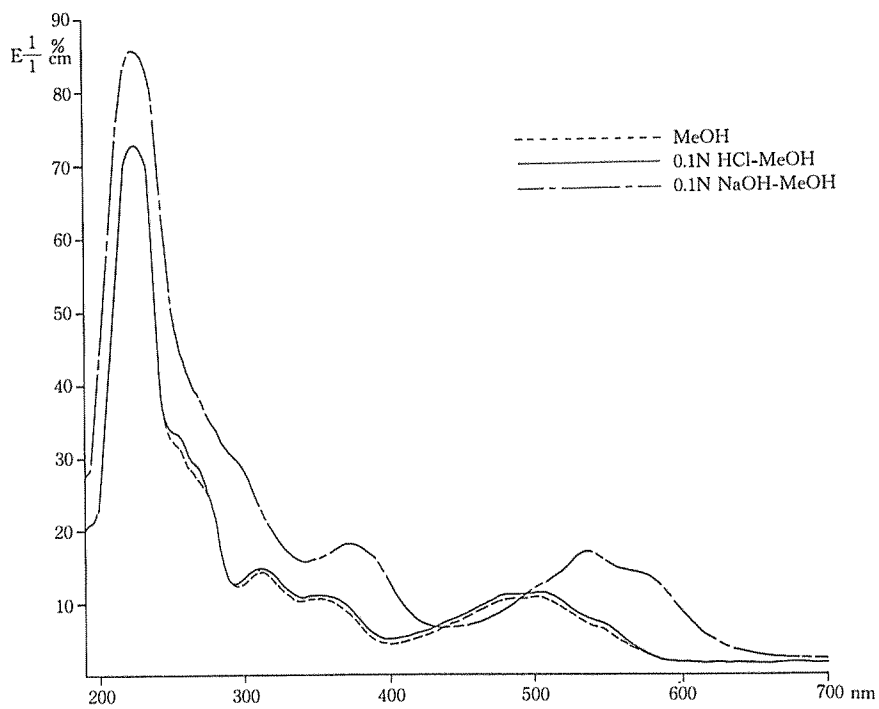


Fig. 5. UV spectra of 1.

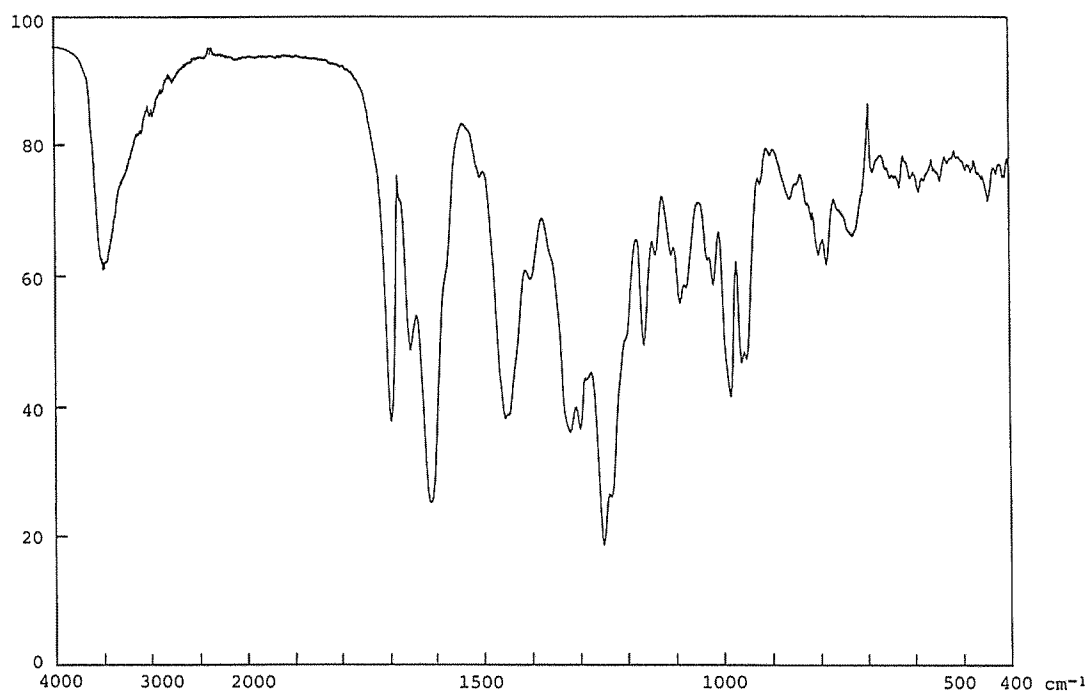
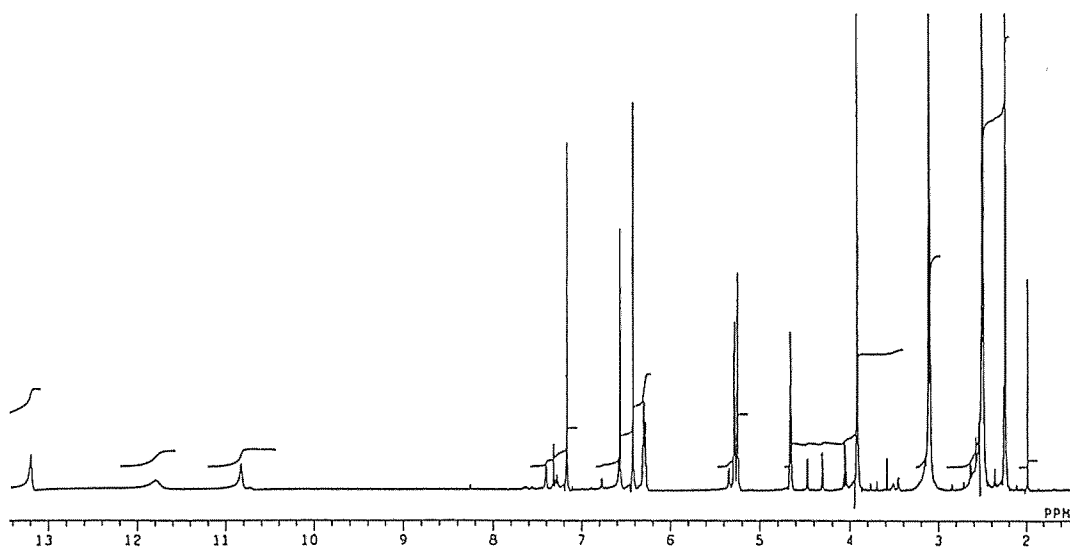


Fig. 6. IR spectrum of 1 (KBr).

Fig. 7. ¹H-NMR spectrum of 1 (DMSO-*d*₆).

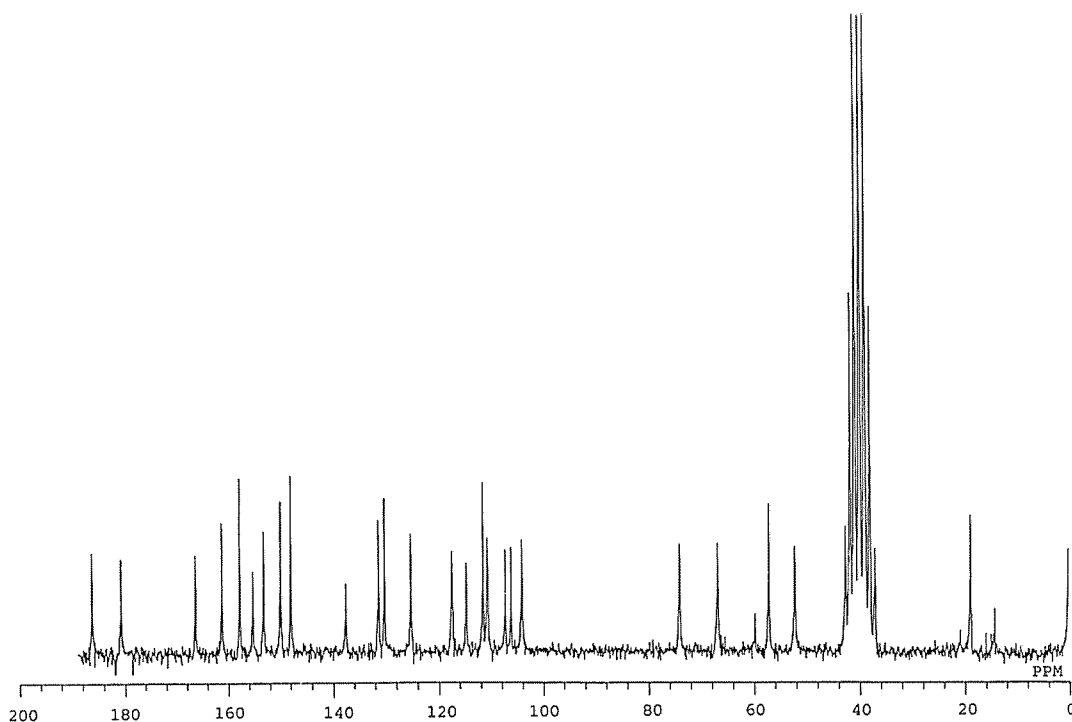


Fig. 8. ^{13}C -NMR spectrum of **1** (DMSO-d_6).

The C-H COSY NMR spectrum (Fig. 9) elucidated the mutual connection of those carbons and protons. The chemical shift of C-9, C-3, C-10, C-6, C-7, C-8 and 2-OCH₃ correlated with each protons. ^1H - and ^{13}C -NMR assignments of **1** and an authentic griseorhodin C^{1,2)} are summarized in Table 1. The mass spectrum of **1** showed diagnostically important fragment ions at m/z 288 and 220 as the base peak. These ions arise from the fusarubin¹⁰⁾ portions of **1**, respectively, (Fig. 10), and accord with fragmentation pattern observed for a similar type compound, purpuromycin.⁹⁾

In the study on the pigment produced by *Streptomyces* sp. No. 76, it was the most important point to decide the fundamental chromophore. The UV spectrum of **1** was

similar to those of the griseorhodin group and suggested the presence of similar chromophore in the molecule of **1**. In consideration of the mass spectrum and the 500 MHz ^1H -NMR spectrum, it supports that **1** is the previously reported compound griseorhodin C.¹⁾ This will be confirmed by comparison of the IR-spectrum, HPLC retention time and antimicrobial spectrum of this compound with an authentic griseorhodin C

Acknowledgements

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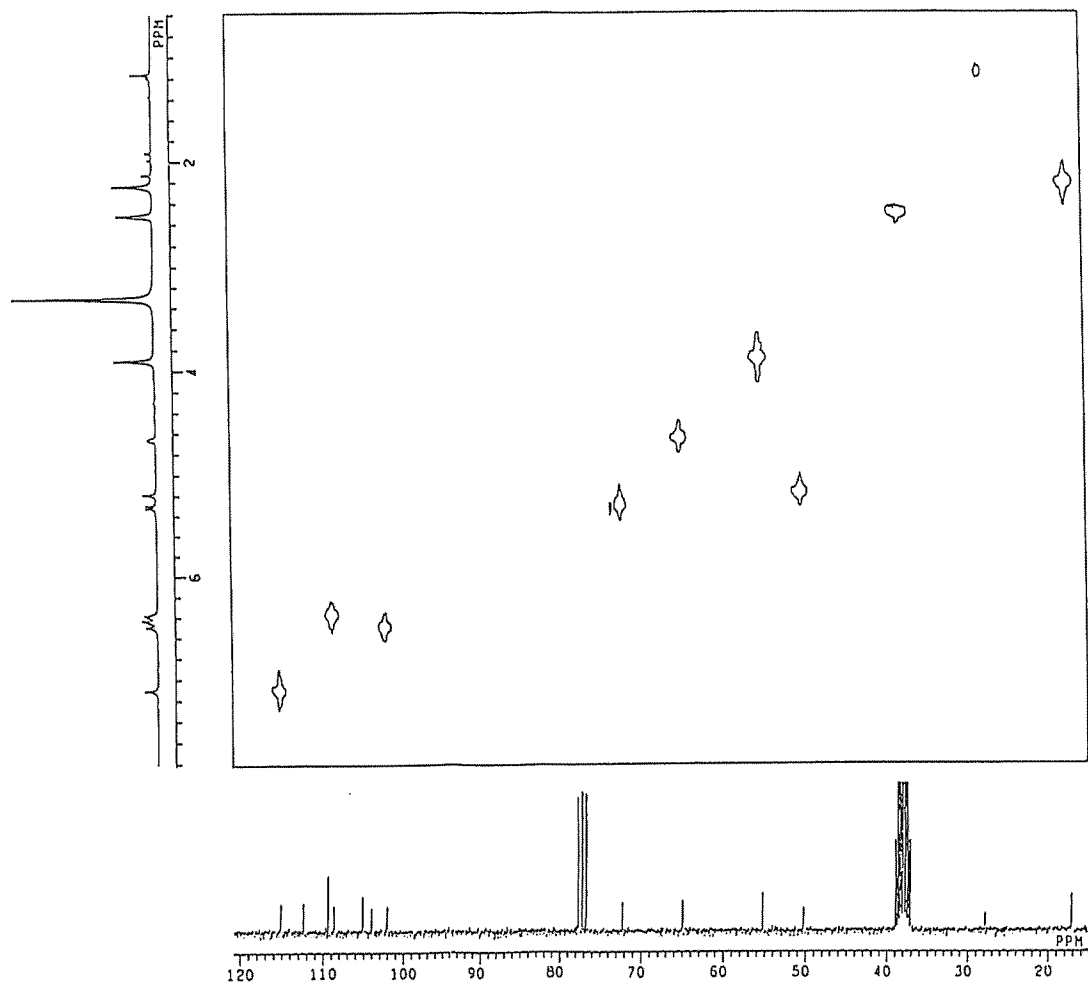


Fig. 9. C-H COSY NMR spectrum of 1 (DMSO- d_6).

Eckardt of Akademie der Wissenschaften der DDR for providing us with an authentic griseorhodin C.

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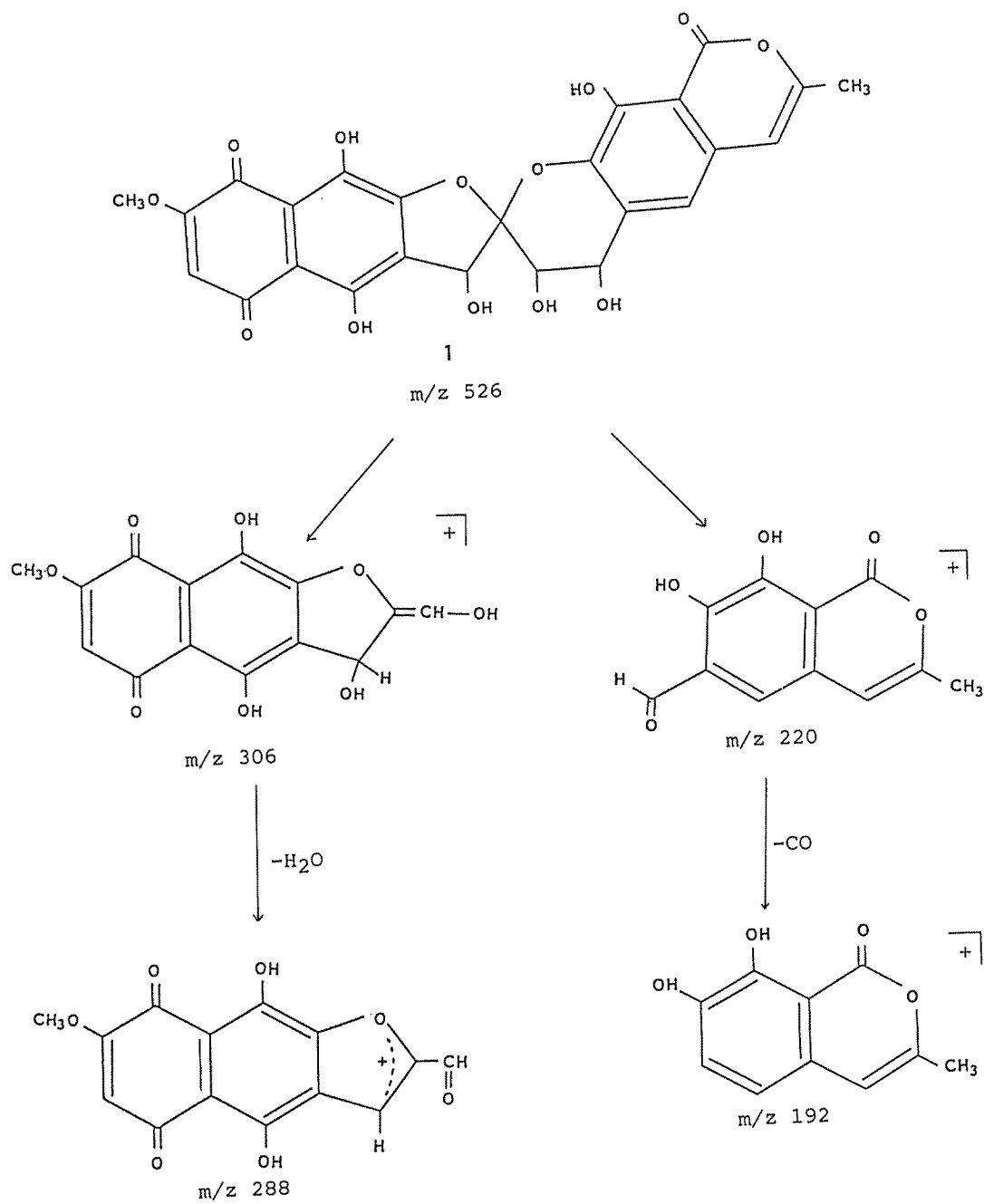


Fig. 10. Mass spectral fragmentation pattern of 1.

Table 1. ^1H - and ^{13}C -NMR assignments of **1** and authentic griseorhodin C in $\text{DMSO}-d_6$

Position	1		Authentic griseorhodin C	
	Proton	Carbon	Proton	Carbon
1		180.6		179.6
2		161.2		160.4
3	6.42	110.6	6.42	110.0
4		186.2		185.5
4a		107.2		106.8
5		157.8		157.3
5a		130.2		130.4
6	5.25	74.1	5.25	73.7
6a		106.2		105.0
7	4.65	67.3	4.48	66.5
8	4.70	67.3	4.67	67.3
8a		125.3		122.6
9	7.17	117.4	7.17	116.8
9a		137.6		136.8
10	6.56	104.0	6.57	103.9
11		111.6		111.8
13		166.4		165.8
13a		131.2		132.0
14		153.2		152.1
14a		148.0		146.5
16a		149.9		148.8
17		155.2		154.8
17a		114.7		114.2
18	2.25	18.8	2.25	18.6
2-OCH ₃	3.92	57.0	3.92	57.0
7-OH	5.29		5.35	
8-OH	5.29		5.35	
5-OH	13.19		13.21	
17-OH	11.79		11.79	
6-OH	6.30		6.30	
14-OH	10.81		10.83	

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放線菌No.76の生産するグリセオロジン C の単離と同定

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放線菌の生産する赤色色素探索過程で土壌から分離した *Streptomyces* sp. No. 76 が二種類の赤色色素を生産することを見出した。シリカゲルとセファデックス LH-20 カラムクロマトグラフィー及び逆相 HPLC により、二種類の色素を単離精製した。二種類の色素のうちの一つは、FD-MS 及び元素分析値より分子式は $C_{25}H_{18}O_{13}$ であった。又、UV, IR, 1H -NMR, ^{13}C -NMR 及び、2D-COSY NMR スペクトルより、既知物質グリセオロジン C と同定した。