NOTE

Dechlororoseophilin: a new cytotoxic metabolite from *Streptomyces griseoviridis*

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The Journal of Antibiotics (2009) 62, 531–532; doi:10.1038/ja.2009.59; published online 17 July 2009**Keywords:** cytotoxic metabolite; dechlororoseophilin; roseophilin; *Streptomyces griseoviridis*

Roseophilin,¹ a cytotoxic pigment produced by *Streptomyces griseoviridis* 2464-S5, has a unique structure containing two pyrrole and one furan rings as shown in Figure 1. The strain also produces a structurally related tripyrrole antibiotic, prodigiosin R1.² Recently, we identified the *rph* gene cluster for prodigiosin biosynthesis in *S. griseoviridis*.³ The *rph* cluster, however, does not contain genes characteristic of roseophilin biosynthesis, although roseophilin is considered to be biosynthesized partially with the same pathway as prodigiosin R1. We attempted to search the biosynthetic intermediates of roseophilin for further biosynthetic study. Color-guided fractionation of the culture extract resulted in the isolation of a new metabolite, dechlororoseophilin (**1**, Figure 1). We report herein the fermentation, isolation, structure elucidation and biological activity of **1**.

The producing organism was cultivated in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 4.0%, soybean meal 1.5%, molasses 1% and calcium carbonate 0.4% (pH 6.8) on a rotary shaker at 27 °C for 5 days. The fermentation broth (2.0 l) was centrifuged and the mycelium was extracted with acetone. After evaporation, the aqueous concentrate was extracted with ethyl acetate. The extract was applied to a silica gel column, which was washed with chloroform–methanol (10:1) and eluted with chloroform–methanol–29% ammonia water (200:20:1). The eluted pigment was subjected to reversed-phase HPLC (XBridge C₁₈, Waters Corporation, Milford, MA, USA) using 88% methanol with 0.2% triethylamine. A pigment fraction was concentrated and dissolved in ethyl acetate. After the solution was washed with 0.1 M hydrochloric acid and water, the organic layer was concentrated to dryness to give a red powder of **1** (8.2 mg). The physico-chemical properties of **1** are summarized as follows: m.p. 81–86 °C; high-resolution FAB-MS *m/z* 419.2697 (MH⁺, calcd for C₂₇H₃₅N₂O₂, 419.2701); UV λ_{max} (ε) 536 nm (142 000) in methanol, 536 nm (143 000) in 0.01 M HCl–methanol, 300 nm (43 400), 485 nm (42 100) in 0.01 M NaOH–methanol; IR (KBr) ν_{max} 3450, 2910, 1590, 1290 cm^{−1}.

The molecular formula of **1** was established as C₂₇H₃₄N₂O₂ by high-resolution FAB-MS. ¹³C and ¹H NMR data for **1** are summarized in Table 1. All one-bond ¹H–¹³C connectivities were confirmed by the heteronuclear multiple-quantum coherence (HMQC)⁴ spectrum. COSY and heteronuclear multiple-bond correlation (HMBC)⁵ experiments identified three partial structures as shown in Figure 2. Three aromatic protons (1-H–3-H) were coupled with each other and an exchangeable proton (1-NH) was coupled with 1-H. A pyrrole moiety was constructed by ¹H–¹³C long-range correlations from 1-H to C-2, C-3 and C-4, from 2-H to C-1 and C-4, and from 3-H to C-1 and C-4. NOESY correlations on 1-H, 2-H and 3-H confirmed the structure of a 2-pyrrolyl group and their assignments. ¹H–¹³C long-range couplings were observed from 6-H to C-5, C-7 and C-8, and from a methoxy proton (27-H₃) to C-7. A high-field chemical shift for C-6 (δ 94.8) and a low-field chemical shift for C-5 (δ 162.0) showed that a 2,5-disubstituted 3-methoxyfuran moiety consisted of C-5 to C-8 and C-27. A small vicinal coupling (<1 Hz) was observed between 22-H and 23-H in the COSY spectrum. Both the protons displayed long-range correlations to three sp² carbons (C-9, C-10 and C-11), indicating that a five-membered ring was composed of C-9, C-10, C-11, C-22 and C-23. Long-range couplings from 24-H to C-11 and from 23-H to C-12 established a sequence of C-24/C-23/C-11/C-12. This substructure was expanded to include the second pyrrole ring due to long-range correlations from 12-H to C-10, C-11 and C-13 and chemical shifts for C-10 to C-13. Proton spin networks from 14-H₂ to 26-H₃ and HMBC data constructed a branched alkyl chain (Figure 2), which was connected to C-13 by a long-range correlation from 14-H (δ 2.83) to C-13. The remaining exchangeable proton (δ 13.46) was assigned to 10-NH in the pyrrolinium ion. The partial structures were joined to establish the structure of **1** from NOEs between 3-H and 6-H, between 6-H and 7-OCH₃, and between 7-OCH₃ and 22-H (Figure 2). The absolute stereochemistry of roseophilin has been identified as 22*R*,23*R* by synthetic studies.^{6,7} Roseophilin and **1**

Figure 1 Structures of dechlororoseophilin (**1**) and related compounds produced by *Streptomyces griseoviridis* 2464-S5.

Table 1 ^{13}C and ^1H NMR data for dechlororoseophilin in CDCl_3

No.	δ_{C}	δ_{H} (J=Hz)
1	128.8	7.36 br s
2	112.1	6.32 dd (4.0, 2.5)
3	116.9	6.91 dd (4.0, 1.0)
4	120.5	
5	162.0	
6	94.8	6.43 s
7	166.7	
8	133.5	
9	144.0	
10	135.3	
11	159.0	
12	110.4	6.16 s
13	164.3	
14	28.2	3.52 m, 2.83 ddd (13.5, 6.0, 3.5)
15	28.0	2.07 m, 1.30 m
16	24.9	1.31 m, 1.15 m
17	28.3	0.99 m, 0.42 m
18	27.0	0.88 m, 0.77 m
19	27.5	0.75 m, 0.40 m
20	24.5	0.98 m, 0.90 m
21	34.1	1.99 m, 1.78 m
22	55.0	3.77 dd (4.5, 3.0)
23	51.6	2.69 d (6.5)
24	33.1	1.79 m
25	21.4	1.00 3H d (6.5)
26	19.6	0.78 3H d (6.5)
27	59.9	4.10 3H s
1-NH		13.80 br
10-NH		13.46 br

exhibited similar ^{13}C chemical shifts (± 0.4 p.p.m.)¹ in the alkyl region and were required to have the same relative stereochemistry. Dechlororoseophilin is a candidate intermediate for roseophilin biosynthesis. It is expected that the compound can be used for the identification of a gene involved in the chlorination process.

Figure 2 NMR analyses of **1**. Bold lines indicate ^1H spin networks, arrows show ^1H - ^{13}C long-range correlations and dashed curves display NOE correlations.

The cytotoxic activities of **1** and roseophilin were examined by using four tumor cell lines. The cells were plated and incubated for 3 days with various concentrations of samples. After the cells were treated with 0.5 mg ml^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 4 h at 37°C , cell growth was measured as absorbance at 570 nm. The IC_{50} values for dechlororoseophilin and roseophilin were 2.6 and $1.0 \mu\text{M}$ against HT-1080 human fibrosarcoma cells, 2.1 and $0.72 \mu\text{M}$ against C6 rat glioma cells, 2.7 and $2.1 \mu\text{M}$ against MCF7 human breast cancer cells, and 2.0 and $1.9 \mu\text{M}$ against T-47D human breast cancer cells, respectively.

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