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NOTE

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A Quinoline Antibiotic from *Rhodococcus erythropolis* JCM 6824

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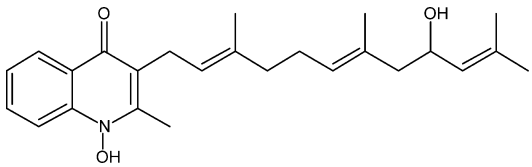
Abstract A new quinoline antibiotic, aurachin RE, was isolated and identified from a culture broth of *Rhodococcus erythropolis* JCM 6824. The aurachin RE structure was determined based on NMR and mass spectrometric analysis. The structure is similar to that of aurachin C antibiotics that have been identified from *Stigmatella aurantiaca*. Compared to aurachin C, however, aurachin RE exhibits a wide and strong antimicrobial spectrum against both high- and low-GC Gram-positive bacteria.

Keywords *Actinobacteria*, antibiotic, aurachin, quinoline, *Rhodococcus*

Large numbers of antibiotics have been isolated from the *Actinobacteria*, including those from the genera, *Streptomyces* and *Nocardia*. Recently, we have reported the genus *Rhodococcus* is also a prolific antibiotic producer [1]. At least, five species and 18 strains of *Rhodococcus* have been demonstrated to exhibit anti-microbial activity. *R. erythropolis*, in particular, includes three groups of strains that produce respective antibiotics [1]. One of the antibiotic-producing strains, *R. erythropolis* JCM 6824, and also the partially purified antibiotic isolated from this strain, exhibits strong antibiotic against a broad range of Gram-positive bacteria. Further investigations on the metabolites of this strain have resulted in the isolation of a new quinoline antibiotic compound designated aurachin RE [**1a**; IUPAC name: 1-hydroxy-2-methyl-3-(3,7,11-trimethyldodeca-9-hydroxy-2,6,10-trienyl)quinolin-4-one]. In this paper, we report on the isolation, structure elucidation, and biological properties of this compound.

R. erythropolis JCM 6824 was cultured in W-minimal medium [2] supplemented with succinate (0.2%, w/v), sucrose (0.2%, w/v), and casamino acids (0.2%, w/v). Stock seed cultures (10^6 cfu/ml) were maintained at −80°C in 10% glycerol. A 600-μl aliquot of stock seed was transferred into each of six 2-liters baffled Erlenmeyer flasks containing 600 ml of the culture medium. The fermentation was carried out on a rotary shaker at 28°C for 28 hours with agitation of 120 rpm.

The fermentation broth (3.6 liters) was centrifuged and any residual cells in the supernatant were removed by membrane filtration (0.2-μm pore size). The filtered supernatant was applied to a C18 cartridge column (SepPak® Vac 35 cc; Waters, Milford, MA), and the column was sequentially washed with 100 ml of 20% EtOH and 30 ml of 50% EtOH. The sample was eluted with 20 ml of EtOH and then evaporated to dryness. The residue was



Aurachin RE (**1a**) and aurachin C (**1b**: H instead of 9′-OH)

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dissolved in 1 ml of EtOH, and this solution was used for purification. The sample was subjected to reversed-phase HPLC [column: TSKgel ODS-80Ts, 4.6 mm I.D.×15 cm (TOSOH, Tokyo, Japan); mobile phase: 65～75% MeOH (0～8 minutes) and 90% MeOH (8～12 minutes); flow rate: 1.0 ml/minute; monitor wavelength: 220～600 nm] [1]. A single peak that retained antibiotic activity and eluted at a retention time of 10.6 minutes, was reproducibly collected. This process yielded 0.5 mg of pure aurachin RE (**1a**) from the original 3.6-liters culture.

The appearance of the purified compound **1a** is gray-brown, and it is soluble in EtOH, MeOH, CH_3CN, EtOAc, and DMSO, and moderately soluble in water. The molecular formula of **1a** was determined to be $C_{25}H_{33}NO_3$ (MW=395), on the basis of positive ESI/MS data (*m*/*z* found, 396.2527 $[M+H]^+$; calcd, 396.2533) obtained using a QSTAR XL mass spectrometer (Applied Biosystems, Foster City, CA). The structure of **1a** was elucidated by extensive NMR analysis; the chemical shifts in the ^{1}H- and ^{13}C-NMR of **1a** are shown in Table 1. The ^{1}H-NMR spectrum and HSQC analysis indicated the presence of 15 methyl protons, eight methylene protons, and eight methine protons, the latter of which included three olefinic protons, and four aromatic protons. The ^{13}C-NMR spectrum indicated the presence of 25 carbon atoms, which were assigned to five methyls, four methylenes, eight methines, and eight quaternary carbons using DEPT spectrum. DQF COSY and HMBC experiments confirmed the partial structures of **1a**. As a result, the whole structure of **1a** was determined as shown in Fig. 1.

The structure of compound **1a** is highly similar to that of the antibiotic aurachin C (**1b**), which was isolated from the Gram-negative myxobacterium, *Stigmatella aurantiaca* Sg a15 [3]. The sole difference between the two compounds is the presence of a hydroxyl group on the hydrocarbon chain

Table 1 ^{1}H- and ^{13}C-NMR spectral data of **1a** in DMSO-d_6

Position	δ_C	δ_H
2	147.8	
3	117.9	
4	173.1	
4a	123.6	
5	125.5	8.11 (1H, dd, *J*=1.4, 6.9 Hz)
6	122.9	7.29 (1H, t, *J*=7.3 Hz)
7	131.6	7.64 (1H, dd, *J*=1.4, 6.9 Hz)
8	114.7	7.77 (1H, d, *J*=8.2 Hz)
8a	139.7	
9	14.7	2.39 (3H, s)
1′	24.2	3.29 (2H, d, *J*=6.8 Hz)
2′	123.1	4.98 (1H, m)
3′	134.3	
4′	39.3	1.90 (2H, m)
5′	26.4	1.99 (2H, m)
6′	126.1	5.02 (1H, m)
7′	132.1	
8′	48.3	1.88 (1H, m)
		2.06 (1H, m)
9′	66.1	4.21 (1H, q, *J*=7.3 Hz)
10′	130.0	5.00 (1H, m)
11′	131.1	
12′	25.6	1.59 (3H, s)
13′	16.2	1.72 (3H, s)
14′	16.6	1.53 (3H, s)
15′	18.1	1.52 (3H, s)

^{1}H- and ^{13}C-NMR were measured at 400 MHz and 100 MHz, respectively. Chemical shifts are shown in δ values relative to DMSO-d_6 at δ=2.49 for ^{1}H-NMR and at δ=39.7 for ^{13}C-NMR values.

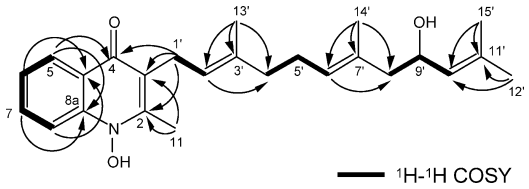

Fig. 1 COSY and HMBC correlations of **1a**.

Table 2 Antimicrobial spectrum of **1a**

Test organism	Inhibition zone (mm)[a]
Sphingomonas paucimobilis IAM 12576^T	trace
Sinorhizobium meliloti IAM 12611^T	24
Paracoccus aminovorans IAM 14244^T	—
Comamonas testosteroni IAM 12419^T	—
Ralstonia eutropha IAM 12368	—
Pseudomonas putida IAM 1236^T	—
Escherichia coli K-12[b]	—
Acinetobacter calcoaceticus IAM 12087^T	—
Deinococcus grandis IAM 13005^T	35
Bacillus subtilis IAM 12118^T	20
Paenibacillus polymyxa IAM 13419^T	38 (turbid)
Arthrobacter atrocyaneus IAM 12339^T	38
Corynebacterium glutamicum IAM 12435^T	29
Nocardia pseudosporangifera IAM 501^T	35
Streptomyces griseus IAM 12311^T	30
Rhodococcus erythropolis IAM 12122^T	28
Saccharomyces cerevisiae W303[b]	—

[a] 10 μg of antibiotic were applied on paper disks (6 mm diameter).
[b] Laboratory stock.
^{T} Type strain.

(position C-9′< substituted) in compound **1a**. The antimicrobial spectrum of compound **1a** was tested by an agar diffusion assay using 10 μg of compound (Table 2). Compound **1a** exhibit wide and strong antibiotic activity against Gram-positive bacteria. Compound **1b** has also been reported to exhibited antibiotic activity against Gram-positive bacteria; however, in comparison, compound **1a** appeared to possess considerably stronger activity. For example, 10 μg of **1a** produced large growth inhibition zones against *Nocardia* sp. and *Streptomyces* sp. (Table 2), whereas 40 μg of **1b** exhibited only trace antibiotic activity against these species according to the literature [3]. In addition, compound **1a** exhibited activity against the Gram-negative bacteria, *Sinorhizobium* sp. and *Deinococcus* sp. Though several aurachin analogs have previously been isolated and synthesized [3, 4], no hydroxylated analogs (on the hydrocarbon chain) have been reported and tested. The hydroxyl group might increase the solubility of the compound in the aqueous phase and may also increase its ability to permeate bacterial cell walls. Thus, introducing a hydroxyl group might be a key factor in improving the activities of these compounds. The MIC values of compound **1a** against various organisms were also examined and the determined values are listed in Table 3. This study again demonstrated that this antibiotic is highly active against Gram-positive bacteria, particularly against the *Actinobacteria*. Compound **1b** has been reported to exhibited MIC values of 0.2 μg/ml and 0.8 μg/ml against *Arthrobacter* sp., and *Corynebacterium* sp., respectively [3]. In contrast, compound **1a** exhibited a minimum MIC value of 0.01 μg/ml against *Arthrobacter* sp., *Corynebacterium* sp., and *Nocardia* sp.

Recently, biosynthetic genes for aurachins (*auaA*∼*E*) have been isolated and published [5]. Preliminary PCR screening for these genes in *R. erythropolis* JCM 6824 gave no amplified products (data not shown). These results indicated that this *Rhodococcus* strain does not contain *aua*-type biosynthetic genes or biosynthetic genes similar to those that have been isolated from *Stigmatella*. Although to date, two antibiotics have been isolated from the genus *Rhodococcus* [6∼8], the biosynthetic genes for these compounds have not been identified.

Table 3 Minimal inhibitory concentrations of **1a**

Test organism	MIC (μg/ml)
Sinorhizobium meliloti IAM 12611^T	0.4
Escherichia coli K-12	>50
Deinococcus grandis IAM 13005^T	0.2
Bacillus subtilis IAM 12118^T	3.1
Arthrobacter atrocyaneus IAM 12339^T	0.01
Corynebacterium glutamicum IAM 12435^T	0.01
Nocardia pseudosporangifera IAM 501^T	0.01
Streptomyces griseus IAM 12311^T	0.1
Rhodococcus erythropolis IAM 12122^T	0.1
Saccharomyces cerevisiae W303	>50

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