Structure of Sch 419560, a Novel α-Pyrone Antibiotic Produced by *Pseudomonas fluorescens*

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(Received for publication September 26, 2001)

Rapid increase of resistance to antibacterial agents in recent years has become a major global public health problem.[1,2] The development of antibiotics with novel mechanisms of action is one of the most important measures leading to the control of bacterial infectious diseases in the new millennium. The genomic antibacterial approach plays a critical role in the discovery of new targets by searching for genes essential to the growth of bacteria. In the course of screening with a broad set of genomic-derived targets, a new metabolite Sch 419560 (**1**), was discovered from the fermentation culture broth of a bacterial strain identified as *Pseudomonas fluorescens*. The level of expression of the *rpoE* gene affected the biological activity for **1** against an *E. coli* laboratory strain. In *E. coli*, *rpoE* encodes a sigma factor that is responsible for the regulation of expression of a subset of genes.[3] The genes known to be regulated by *rpoE* encompass stress response-related activities described as extra-cytoplasmic-functions.[4] *rpoE* activity is essential for the viability of *E. coli* and is considered an attractive target for the development of novel antibacterial agents.[5] This paper describes fermentation, isolation, structure elucidation and biological activity of **1**.

The frozen whole broth of the producing organism (1 ml) was transferred to a germination medium consisting of BBL Trypticase soy broth 30 g/liter. The culture was incubated at 30°C for 48 hours on a rotary shaker at 250 rpm. Approximately 1% inoculum of this culture was transferred to fermentation medium consisting of (g/liter): Traders Pharmamedia, 20; cerelose, 20; and $CaCO_3$, 4. The culture was fermented at 30°C for 48 hours on a rotary shaker at 250 rpm to produce the *rpoE* active components.

The fermentation broth (10 liters) was extracted with ethyl acetate at harvest pH (7.0). The crude extract was separated by modified Kupchan liquid partition as follows:[6] the EtOAc extract was first dissolved in MeOH - H_2O (9 : 1) and partitioned with an equal volume of hexane. After layer separation, the aqueous MeOH lower phase was adjusted to 20% of water and partitioned with an equal volume of CH_2Cl_2. The upper MeOH layer was separated and adjusted to 40% of water, and them partitioned with an equal volume of CH_2Cl_2 again. The combined CH_2Cl_2 layers were found to be active in the *rpoE* assay, and chromatographed on Amberchrom CG 161 resin (Toso Haas) eluting with aqueous MeOH in step gradient. The active 80% MeOH fraction was further separated by reversed-phase HPLC (YMC-ODS semi-preparative column 250×20 mm, S-5, 120Å, with a guard column 50×20 mm, 85% MeOH in H_2O isocratic elution, 15 ml/minute, UV=290 nm). The enriched active mixture was purified by Sephadex LH-20 column eluting with CH_2Cl_2 - MeOH (7 : 3) to obtain pure **1** as pale yellow solid.

The physico-chemical properties of **1** are listed in Table 1. The molecular weight of **1** was determined to be 294 by LC-MS method that indicated the protonated ion peak

Table 1. Physico-chemical properties of Sch 419560 (**1**).

	Sch 419560 (**1**)
Appearance	Pale-yellow solid
Molecular weight	294
MS (APCI+) *m/z*	295 $(M+H)^+$
MS (APCI-) *m/z*	293 $(M-H)^-$
HRFAB-MS *m/z*	
found:	295.2282 $(M+H)^+$
calcd.:	295.2273 ($C_{18}H_{31}O_3$)
Molecular formula	$C_{18}H_{30}O_3$
UV λ_{max} MeOH nm(ε)	292 (15200)
IR ν_{max} (KBr) cm^{-1}	3300,2920,2850,1660, 1630,1570,1410,1300

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Table 2. ^{1}H and ^{13}C NMR spectral data of **1** and **2**[a].

	^{13}C (δ)		^{1}H (δ)	
	1	**2**	**1**	**2**
1	168.00 s[b]	167.34 s	--	--
2	103.42 s	115.64 s	--	--
3	166.63 s	164.84 s	--[c]	--
4	100.62 d	101.21 d	6.15 s	5.90 s
5	163.61 s	158.26 s	--	--
1'	23.11 t	24.32 t	2.42 m	2.37 t, J=6 Hz
2'	28.04 t	27.63 t	1.50 m	1.48 m
3'	29.32 t	29.10 t	1.30 m	1.30 m
4'	31.65 t	31.53 t	1.30 m	1.30 m
5'	22.57 t	22.55 t	1.30 m	1.30 m
6'	14.07 q	14.04 q	0.86 t, J=6 Hz	0.88 t, J=6 Hz
1"	33.53 t	33.58 t	2.42 m	2.45 t, J=6 Hz
2"	26.81 t	26.68 t	1.63 m	1.64 m
3"	28.97 t	28.93 t	1.30 m	1.30 m
4"	28.92 t	28.88 t	1.30 m	1.30 m
5"	31.80 t	31.62 t	1.30 m	1.30 m
6"	22.65 t	22.58 t	1.30 m	1.30 m
7"	14.00 q	14.02 q	0.88 t, J=6 Hz	0.88 t, J=6 Hz
CO	--	163.24 s	--	--
CH_3	--	20.82 q	--	2.31 s

a. Recorded in $CDCl_3$ at 400 MHz for ^{1}H, and 100 MHz for ^{13}C NMR, respectively.
b. Multiplicity was determined by APT data.
c. The broad peak of hydroxyl proton at position-3 was observed at δ 11.15 by using DMSO-d_6.

at *m/z* 295 $(M+H)^+$ in positive atmospheric pressure chemical ionization mode (APCI+). This was also confirmed by negative APCI mode (APCI−) that showed the deprotonated molecular ion at *m/z* 293 $(M-H)^-$. The molecular formula was deduced as $C_{18}H_{30}O_3$ based on HRFAB-MS data (calcd. for $C_{18}H_{31}O_3$: 295.2773. Found: 295.2282). The UV spectrum of **1** showed a maximum absorption at 292 nm as typical α-pyrone class of compounds. IR absorptions at 3300 and 1660 cm^{-1} revealed the presence of hydroxyl and conjugated carbonyl (lactone) functionalities, respectively. Based on the physico-chemical data, literature search was conducted and found that **1** matched with an antibiotic, BN-213, reported in a patent.[7] However, the structure of BN-213 was not disclosed in the literature. The structure elucidation of **1**, therefore, was carried out by the analysis of spectroscopic data. As shown in Table 2, ^{1}H and ^{13}C NMR data were consistent with the observation of MS, UV and IR data. In the ^{1}H NMR spectrum, the presence of only one vinyl proton singlet at δ 6.15 indicated that the pyrone ring was highly substituted with a hydroxyl group and two aliphatic carbon chains. In order to complete the assignment of **1**, 2D-NMR studies were conducted including HMQC-TOCSY and HMBC experiments. The carbon numbers of two aliphatic chains were established as six and seven carbons by HMQC-TOCSY experiments, respectively. The positions of these two chains on the pyrone ring were further determined by the analysis of ^{1}H and ^{13}C long range correlation in HMBC experiment as shown in Fig. 2. There are two dominant possible tautomeric Structures I (α-

Fig. 1. Structures of Sch 419560 (**1**) and its acetate (**2**).

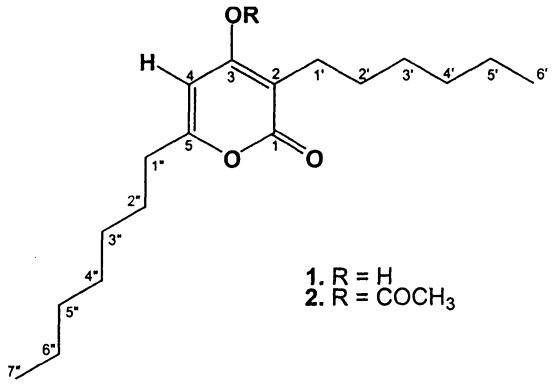


Fig. 2. Some important HMBC data of **1**.

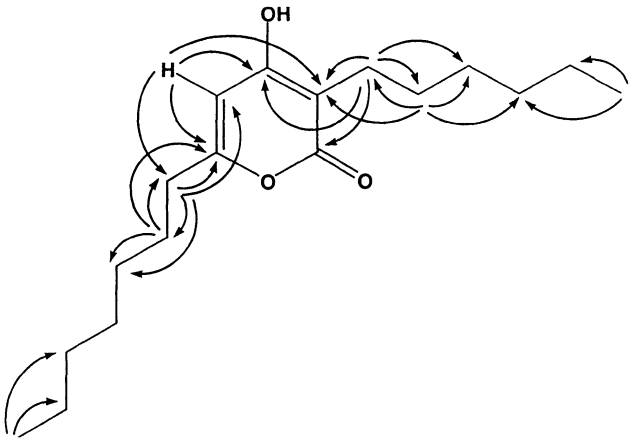
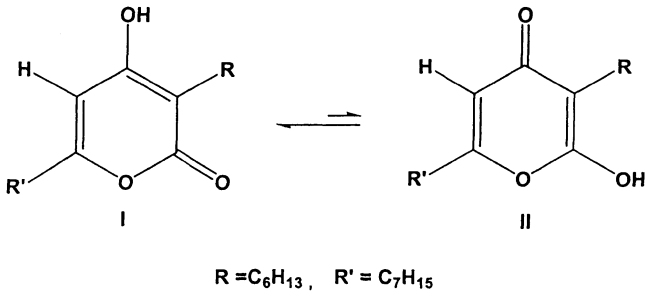
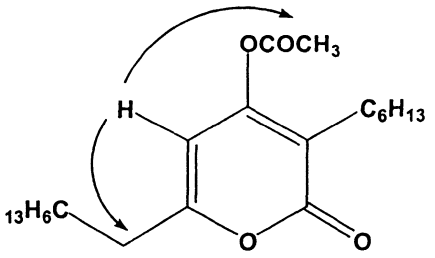


Fig. 3. Possible tautomerization of **I** and **II**



$R = C_6H_{13}$, $R' = C_7H_{15}$

Fig. 4. Difference NOE data of **2**.



pyrone) and II (γ-pyrone) for compound **1** (Fig. 3). In order to determine the dominant tautomer, **1** was acetylated by acetic anhydride/pyridine to form the derivative **2** (see ^1H and ^{13}C NMR data in Table 2). The NOE spectral data of **2** revealed the strong couplings of proton-4 to acetyl CH_3 group and proton-1″ (Fig. 4). This evidence suggested that structure **I** is the dominant tautomer of **1**. The UV absorption of **1** at 292 nm also supported the assignment, because the typical UV absorption of α-pyrones should be in the range of 280~314 nm. For γ-pyrones, the range of maximal UV absorption is typically at 240~276 nm.[8]

Compound **1** displayed an MIC_{50} of 2.5 μg/ml against a *Staphylococcus aureus* strain, and >64 μg/ml against *E. coli*. When the *rpoE* protein was constitutively overexpressed in *E. coli*, the MIC_{50} of **1** was determined to be 5 μg/ml.

Acknowledgement

The authors are grateful to Ms. C. CRAMER and Dr. M. BEYAZOVA for taxonomical information of the producing strain, and Mrs. D. SCOTT for the preparation of this manuscript.

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