J. Antibiot. 60(5): 321–324, 2007

NOTE

THE JOURNAL OF ANTIBIOTICS

New Aureothin Derivative, Alloaureothin, from *Streptomyces* sp. MM23

Jun-ya Ueda, Junko Hashimoto, Aya Nagai, Takuji Nakashima, Hisayuki Komaki, Kozue Anzai, Shigeaki Harayama, Takayuki Doi, Takashi Takahashi, Kazuo Nagasawa, Tohru Natsume, Motoki Takagi, Kazuo Shin-ya

Received: February 28, 2007 / Accepted: April 25, 2007

Abstract A new polypropionate alloaureothin (**1**) possessing a nitro group, together with a known polypropionate aureothin (**2**), was isolated from mycelium of *Streptomyces* sp. MM23. The structure was determined on the basis of spectroscopic data. **1** exhibited growth inhibitory effect against human fibrosarcoma HT1080 cells with an IC_{50} value of 30 μM.

Keywords polypropionate, alloaureothin, *Streptomyces* sp., cytotoxic

Polypropionates with a nitro group, a class of polyketides, were isolated from several actinomycetes and displayed interesting biological activities. Aureothin (**2**) isolated from the mycelium of *Streptomyces thioluteus* [1] was reported to exhibit antifungal, antitumor, and anti-*Helicobacter pylori* activities [2]. Spectinabilin (**3**) isolated from the culture broth of *Streptomyces spectinabilis* [3] showed inhibitory activity against reverse-transcriptase in Rausche leukemia virus and antimalarial activity [4]. In the course of our screening program for biological active compounds of microbial origin, we isolated a new aureothin derivative, designated as alloaureothin (**1**), from mycelium of *Streptomyces* sp. MM23 (Fig. 1).

The *Streptomyces* sp. MM23 isolated from soil sample collected in Hiroshima Prefecture, Japan, was cultured at 27°C for 5 days in 500-ml Erlenmeyer flasks containing a medium consisting of 2.5% starch, 1.5% soy bean meal, 0.2% dry yeast, 0.4% $CaCO_3$ (pH 6.2 before sterilization). The whole culture broth (2 liters) was centrifuged, and the mycelial cake was extracted with acetone (400 ml). The extract was evaporated *in vacuo*, and the residual aqueous concentrate was extracted with ethyl acetate. The organic layer (157 mg) was separated by silica gel flash column (Purif-Pack SI-60, Moritex) with a *n*-hexane - ethyl acetate linear gradient system (0～100% EtOAc). The 50～100% EtOAc eluate was further purified by reversed-phase HPLC (70% aqueous MeOH) with Senshu Pak PEGASIL ODS column (20 mm i.d.×250 mm) to give a new compound **1** (Rt 38.8 minutes, 2.5 mg) and **2** (Rt 41.8 minutes, 8.3 mg) [1]. In both isolation procedures, peak detection was carried out by UV absorption at 254 nm. A structurally

K. Shin-ya (Corresponding author)**, T. Natsume:** Biological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan,
E-mail: kshinya@jbirc.aist.go.jp
M. Takagi (Corresponding author)**, J. Ueda, J. Hashimoto, A. Nagai:** Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan,
E-mail: mtakagi@jbirc.aist.go.jp
T. Nakashima, H. Komaki, K. Anzai, S. Harayama: NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan
T. Doi, T. Takahashi: Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo 152-8552, Japan
K. Nagasawa: Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan

related compound, **3** was obtained from another strain in the course of our chemical screening program. The structure elucidation of **1** was carried out mainly by NMR spectral analyses and a comparison with these compounds as follows.

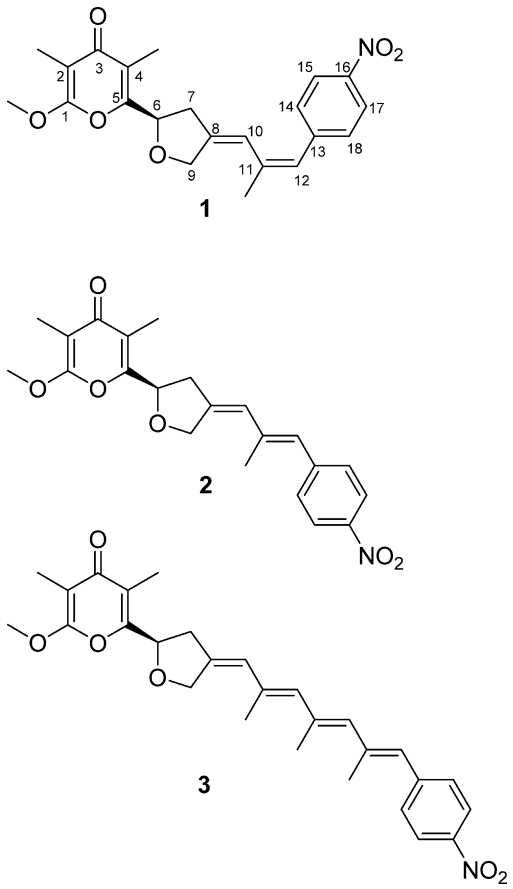

Fig. 1 Structures of alloaureothin (**1**), aureothin (**2**) and spectinabilin (**3**).

1 was obtained as a yellowish amorphous solid and showed similar UV spectrum (λ_{max}, 255, 334 nm) to that of **2** on HPLC (detector: Hitachi L-2455 diode array detector). Its physico-chemical properties are summarized in Table 1. The molecular formula was established as $C_{22}H_{23}NO_6$ from HR-ESI-MS data (*m*/*z* 398.1628). The IR spectra revealed the characteristic absorption of a nitro groups (ν_{max} 1592, 1342 cm^{-1}), together with carbonyl group (ν_{max} 1666 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **1** (Table 2) showed the signals of a 1,4-disubstituted phenyl group [C-13 (δ_C 144.6), C-14,18 (δ_H 7.39; δ_C 129.7), C-15,17 (δ_H 8.17; δ_C 123.7), C-16 (δ_C 146.3)], a conjugated ketone group C-3 (δ_C 180.7), eight olefins [C-1 (δ_C 162.3), C-2 (δ_C 100.4), C-4 (δ_C 120.4), C-5 (δ_C 154.6), C-8 (δ_C 142.1), C-10 (δ_H 6.35; δ_C 119.9), C-11 (δ_C 138.0), C-12 (δ_H 6.39; δ_C 127.6)], an oxymethine group C-6 (δ_H 5.10; δ_C 73.9), an oxygenated methylene C-9 (δ_H 4.65, 4.49; δ_C 70.5), a methoxyl group 1-*O*-Me (δ_H 3.92; δ_C 55.5), a methylene C-7 (δ_H 2.96, 2.89; δ_C 38.1), and three vinyl methyl groups [2-Me (δ_H 1.85; δ_C 7.1), 4-Me (δ_H 2.01; δ_C 9.7), and 11-Me (δ_H 2.07; δ_C 24.3)]. The HMBC together with ^{1}H-^{1}H COSY spectra established a tetrasubstituted γ-pyrone, a 2,4-disubstituted furan, a 1,3-butadiene, and a 1,4-disubstituted benzene units as shown in Fig. 2A. The connectivity between these substructures was established by the long-range ^{1}H-^{13}C couplings from 12-H to C-13 and C-14,18, from 10-H to C-7 and C-9, and from 7-H and 6-H to C-5. The methoxyl proton 1-*O*-Me was long-range coupled to C-1. Long-range couplings between the methyl proton 2-Me and C-1, C-2, and C-3, together with the long-range couplings between 4-Me and C-3, C-4, and C-5 revealed the substituted pattern in the γ-pyrone moiety. The remaining nitro functional group was determined to be substituted at C-16 by IR absorption and comparison of the chemical shifts with **2**.

Table 1 Physico-chemical properties of alloaureothin (**1**) and aureothin (**2**)[a]

	1	**2**[a]
Appearance	Yellowish amorphous solid	Yellow prism
MP	50.5∼55.0°C	158°C
Optical rotation	$[\alpha]_D^{25}$ −29.7° (*c* 0.12, $CHCl_3$)	$[\alpha]_D^{18}$ +51° ($CHCl_3$)
Molecular formula	$C_{22}H_{23}NO_6$	$C_{22}H_{23}NO_6$
HR-ESI-MS (*m*/*z*)		
found	398.1628 $(M+H)^+$	
calcd	398.1604	
UV λ_{max}^{MeOH} nm (log ε)	255 (4.3), 334 (4.0)	257 (4.39), 346 (4.27)
IR ν_{max} (KBr) cm^{-1}	1666, 1592, 1516, 1342	1505, 1321

[a] Reported data by Hirata *et al.* [1].

Table 2 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** in $CDCl_3$

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		162.3		162.0
2		100.4		100.0
3		180.7		180.5
4		120.4		120.2
5		154.6		154.6
6	5.10 (t, 7.1)	73.9	5.14 (t, 7.0)	73.3
7	2.96 (dd, 15.8, 6.5)	38.1	3.06 (dd, 16.1, 6.1)	38.2
	2.89 (dd, 16.4, 6.8)		2.96 (dd, 15.6, 6.1)	
8		142.1		138.5
9	4.65 (d, 14.3)	70.5	4.87 (d, 14.2)	70.1
	4.49 (d, 14.3)		4.75 (d, 14.2)	
10	6.35 (br s)	119.9	6.20 (br s)	125.9
11		138.0		140.6
12	6.39 (br s)	127.6	6.37 (br s)	128.3
13		144.6		144.2
14, 18	7.39 (d, 8.8)	129.7	7.39 (d, 8.6)	129.5
15, 17	8.17 (d, 8.6)	123.7	8.20 (d, 8.8)	123.6
16		146.3		146.1
1-*O*-Me	3.92 (s)	55.5	3.95 (s)	55.2
2-Me	1.85 (s)	7.1	1.86 (s)	6.9
4-Me	2.01 (s)	9.7	2.04 (s)	9.4
11-Me	2.07 (s)	24.3	2.05 (d, 1.5)	17.7

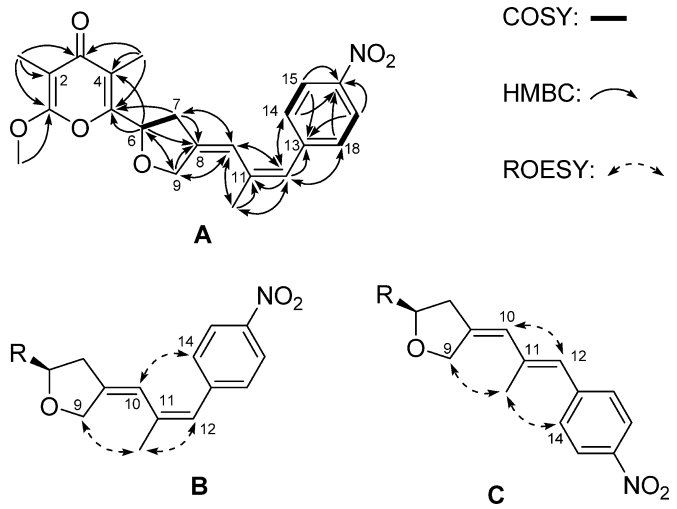

Fig. 2 Key correlations in ^{1}H-^{1}H COSY and HMBC experiments of **1** (A), and ROESY experiments of **1** (B) and **2** (C), respectively.

The stereochemistry of olefins at C-8 and C-11 proved to be *E* and *Z*, respectively, based on the ROE correlations between 9-H and 11-Me, between 11-Me and 12-H. ROE between 10-H and 14,18-H and low-field ^{13}C chemical shift at 11-Me (δ_C 24.3) compared with high-field ^{13}C chemical shift in **2** also supported the stereochemistry as shown in Fig. 2B (also shown ROE correlations of **2** in Fig. 2C). The same Cotton effects among **1**, **2** and **3** in CD spectra (**1**, $[\theta]_{240}$ 4510, $[\theta]_{281}$ −2118; **2**, $[\theta]_{223}$ 5152, $[\theta]_{281}$ −3034; **3**, $[\theta]_{215}$ 4845, $[\theta]_{249}$ −2172, $[\theta]_{281}$ −3626) revealed that the absolute configuration at C-6 to be *R*. In addition to the CD spectra, **1** was gradually transformed to **2** in methanol solution, indicating that they possess the same configuration. Thus, the structure of **1** was established to be 11-*cis* aureothin as shown in Fig. 1. As the derivative of **2**, an isomeric compound RP-18051 was reported in French Patent 1,516.739. Although this compound showed the different optical rotation value from **2**, the melting point was much higher than that of **1**. Taking into consideration these results, this compound could be 6*S* aureothin.

The three isolated compounds were examined for their growth inhibitory activity toward the highly metastatic human HT-1080 fibrosarcoma cell line. Compounds **1** and **2** inhibited the cell growth in a concentration-dependent manner with the IC_{50} values of 30 and 60 μM, respectively. To the contrary, **3** which consist of a highly resembled structure did not show any cytotoxic activity in HT1080 cells at concentration of 100 μM. Studies on detailed biological activities of **1**, **2**, and **3** are now underway.

Acknowledgement This work was supported by the grant from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

1. (a) Maeda K. Chemical studies on antibiotic substances, IV. A crystalline toxic substance of *Streptomyces thioluteus* producing aureothricin. J Antibiot, Ser. A 6: 137–138 (1953) (b) Hirata Y, Nakata H, Yamada K, Okuhara K, Naito T. The structure of aureothin, a nitro compound obtained from *Streptomyces thioluteus*. Tetrahedron 1961: 252–274 (1961)
2. (a) Schwartz JL, Tishler M, Arison BH, Shafer HM, Omura S. Identification of mycolutein and pulvomycin as aureothin and labilomycin respectively. J Antibiot 29: 236–241 (1976) (b) Taniguchi M, Watanabe M, Nagai K, Suzumura K, Suzuki K, Tanaka A. γ-Pyrone compounds with selective and potent anti-*Helicobacter pylori* activity. J Antibiot 53: 844–847 (2000)
3. Kakinuma K, Hanson CA, Rinehart Jr KL. Spectinabilin, a new nitro-containing metabolite isolated from *Streptomyces spectabilis*. Tetrahedron 32: 217–222 (1976)
4. Isaka M, Jaturapat A, Kramyu J, Tanticharoen M, Thebtaranonth Y. Potent *in vitro* antimalarial activity of metacycloprodigiosin isolated from *Streptomyces spectabilis* BCC 4785. Antimicrob Agents Chemother 46: 1112–1113 (2002)