

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

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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 μ M.

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% CaCO₃ (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

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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.

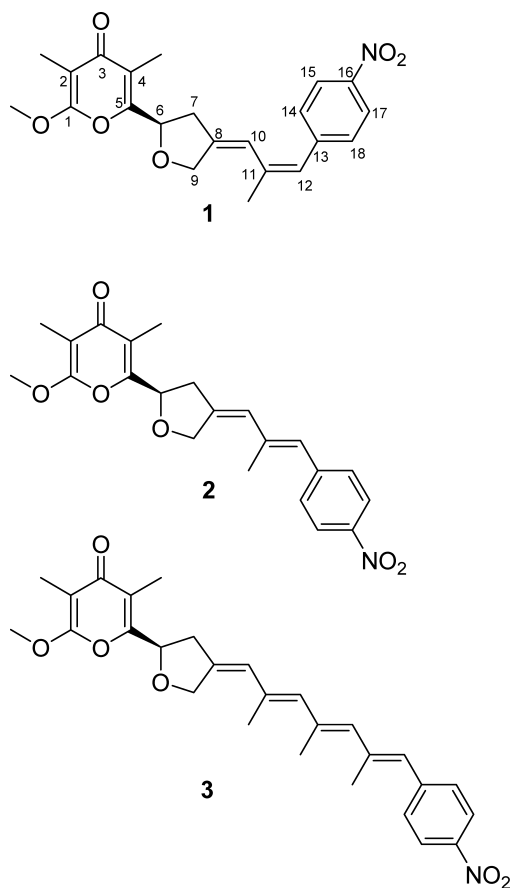


Fig. 1 Structures of alloaureothin (**1**), aureothin (**2**) and spectinabilin (**3**).

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 ^1H - 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 ^1H - ^1H 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 ^1H - ^{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**.

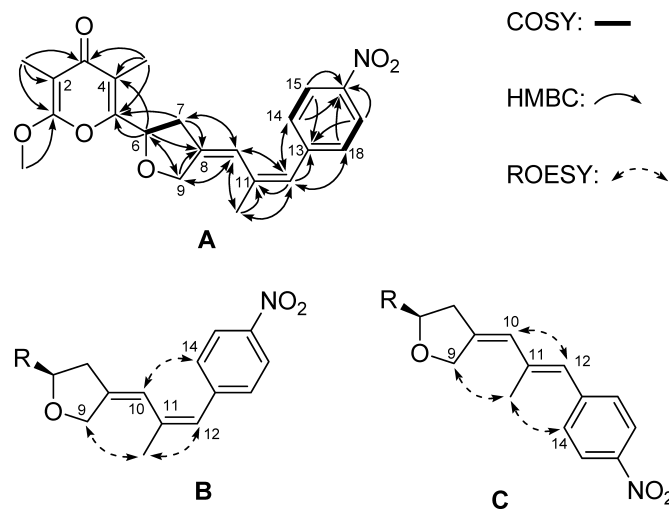
Table 1 Physico-chemical properties of alloaureothin (**1**) and aureothin (**2**)^a

	1	2 ^a
Appearance	Yellowish amorphous solid	Yellow prism
MP	50.5~55.0°C	158°C
Optical rotation	$[\alpha]_{\text{D}}^{25} -29.7^\circ$ (c 0.12, CHCl_3)	$[\alpha]_{\text{D}}^{18} +51^\circ$ (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 $\lambda_{\max}^{\text{MeOH}}$ 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 ^1H (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) 2.89 (dd, 16.4, 6.8)	38.1	3.06 (dd, 16.1, 6.1) 2.96 (dd, 15.6, 6.1)	38.2
8		142.1		138.5
9	4.65 (d, 14.3) 4.49 (d, 14.3)	70.5	4.87 (d, 14.2) 4.75 (d, 14.2)	70.1
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 ^1H - ^1H 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.

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