

## NOTE

# JBIR-69, a new metabolite from *Streptomyces* sp. OG05

Takeshi Fujiwara<sup>1</sup>, Aya Nagai<sup>1</sup>, Motoki Takagi<sup>1</sup> and Kazuo Shin-ya<sup>2</sup>

*The Journal of Antibiotics* (2010) 63, 95–96; doi:10.1038/ja.2009.132; published online 15 January 2010

**Keywords:** cytotoxicity; HL-60; *Streptomyces*; thioester

Actinobacteria have been extensively studied thus far, and they are known to produce pharmaceutically important compounds.<sup>1,2</sup> Soil serves as the primary source of actinobacteria and, therefore, we attempted to obtain novel metabolites from actinobacteria isolated from soil samples. During our screening program for novel metabolites, a new metabolite possessing a thioester named JBIR-69 (**1**, Figure 1a), together with a known compound, 3-isopropylmalate methyl ester, was isolated from the culture broth of *Streptomyces* sp. OG05. This paper describes the fermentation, isolation, structural elucidation, and, in brief, the biological activity of **1**.

*Streptomyces* sp. OG05 was isolated from a soil sample collected in Okinawa Prefecture, Japan, and was cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of 2% glycerol (Nacalai Tesque, Kyoto, Japan), 1% molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan), 0.5% casein (Kanto Chemical, Tokyo, Japan), 0.1% polypeptone (Nihon Pharmaceutical, Tokyo, Japan), and 0.4% CaCO<sub>3</sub> (Kozaki Pharmaceutical, Tokyo, Japan; pH 7.2 before sterilization).

The mycelium collected from the culture broth (1 l) by centrifugation was extracted with Me<sub>2</sub>CO (200 ml). After concentration in vacuo, the residue aqueous concentrate with pH adjusted to 2–3 using 2 N HCl was extracted twice with EtOAc. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The dried residue (180 mg) was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI-60, size: 20, Moritex, Tokyo, Japan) with a Hexane-EtOAc (0–30% EtOAc) linear gradient system, followed by elution with a CHCl<sub>3</sub>–MeOH (0–90% MeOH) linear gradient system; then, peak detection was carried out with UV absorption at 254 nm. The 10–20% MeOH elute fractions (30 mg) were further purified by reversed-phase high-pressure liquid chromatography (HPLC) using a PEGASIL ODS column (Senshu Pak, 20 i.d. × 150 mm, Senshu Scientific, Tokyo, Japan) with a H<sub>2</sub>O–MeOH (0–100% MeOH) linear gradient system containing 0.1% formic acid

to yield JBIR-69 (**1**, 15 mg) together with 3-isopropylmalate methyl ester (6 mg).<sup>3</sup>

Compound **1** was obtained as a colorless oil ([ $\alpha$ ]<sub>D</sub> –11.8, 29 °C, c 0.28 (CHCl<sub>3</sub>), UV (MeOH)  $\lambda_{\max}$  ( $\epsilon$ ) 233 (15 800)). The infrared (IR) spectrum (KBr) of **1** revealed the characteristic absorptions of esters ( $\nu_{\max}$  1730 cm<sup>–1</sup>), amide ( $\nu_{\max}$  1670 cm<sup>–1</sup>), hydroxyl, and/or amide NH ( $\nu_{\max}$  3400 cm<sup>–1</sup>) groups. Its molecular formula was established as C<sub>13</sub>H<sub>21</sub>NO<sub>7</sub>S ( $m/z$  [M–H]<sup>–</sup> 334.0960, +0.2 mmu) by high-resolution electrospray ionization-mass spectrum.

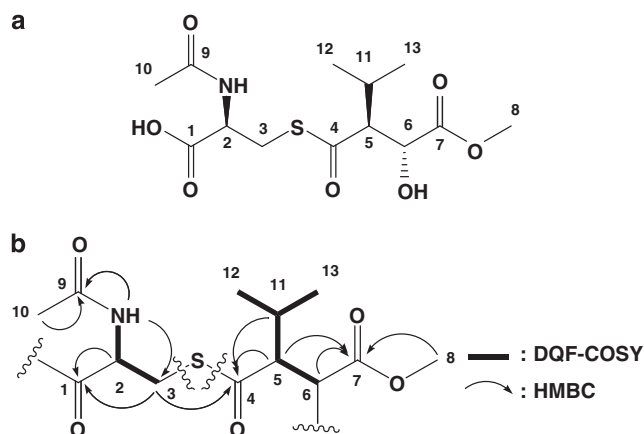
The structure of **1** was mainly elucidated by the analyses of NMR spectra, including heteronuclear single-quantum coherence, double-quantum-filtered correlation (DQF-COSY), and constant-time heteronuclear multiple-bond correlation (HMBC).<sup>4</sup> The <sup>1</sup>H and <sup>13</sup>C NMR spectral data revealed by the heteronuclear multiple quantum coherence spectrum for **1** are listed in Table 1. The DQF-COSY and HMBC analyses revealed the two partial structures (Figure 1b). The sequence from an amide proton 2-NH ( $\delta_{\text{H}}$  6.68) to methylene protons 3-H ( $\delta_{\text{H}}$  3.67 and 3.22) through an  $\alpha$ -methine proton 2-H ( $\delta_{\text{H}}$  4.91,  $\delta_{\text{C}}$  52.2) was observed in the DQF-COSY of **1**. In the HMBC spectrum, <sup>1</sup>H–<sup>13</sup>C long-range couplings from the  $\alpha$ -methine proton 2-H and the methylene protons 3-H to a carbonyl carbon C-1 ( $\delta_{\text{C}}$  171.6), and from the amide proton 2-NH and a singlet methyl proton 10-H ( $\delta_{\text{H}}$  2.12) to an amide carbonyl carbon C-9 ( $\delta_{\text{C}}$  172.4) were observed. Thus, the carbonyl and acetyl moieties were elucidated to be substituted at the C-2 and 2-N positions, respectively. In a similar manner, the sequence from an oxymethine proton 6-H ( $\delta_{\text{H}}$  4.46) to a methyl proton 12-H ( $\delta_{\text{H}}$  0.96) through methine protons 5-H ( $\delta_{\text{H}}$  2.64) and 11-H ( $\delta_{\text{H}}$  2.30), which in turn spin coupled to a methyl proton 13-H ( $\delta_{\text{H}}$  1.06), was established. <sup>1</sup>H–<sup>13</sup>C long-range couplings from 5-H and 11-H to a carbonyl carbon C-4 ( $\delta_{\text{C}}$  200.3) and from 5-H and 6-H to an ester carbonyl carbon C-7 ( $\delta_{\text{C}}$  174.7) were recognized in the HMBC spectrum of **1**. A methoxyl proton 8-H ( $\delta_{\text{H}}$  3.81) was long-range coupled to the ester carbonyl carbon C-7, which established the substitution position of the methoxyl

<sup>1</sup>Biomedical Information Research Center (BIRC), Japan Biological Informatics Consortium (JBIC), Koto-ku, Tokyo, Japan and <sup>2</sup>Biomedical Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), Koto-ku, Tokyo, Japan

Correspondence: Dr M Takagi, Biomedical Information Research Centre (BIRC), Japan Biological Informatics Consortium (JBIC), 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan or Dr K Shin-ya, Biomedical Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan.

E-mails: motoki-takagi@aist.go.jp or k-shinya@aist.go.jp

Received 7 November 2009; revised 9 December 2009; accepted 14 December 2009; published online 15 January 2010



**Figure 1** (a) Structure of 1. (b) Key correlations in the DQF-COSY (bold line) and HMBC (arrow) spectra of 1.

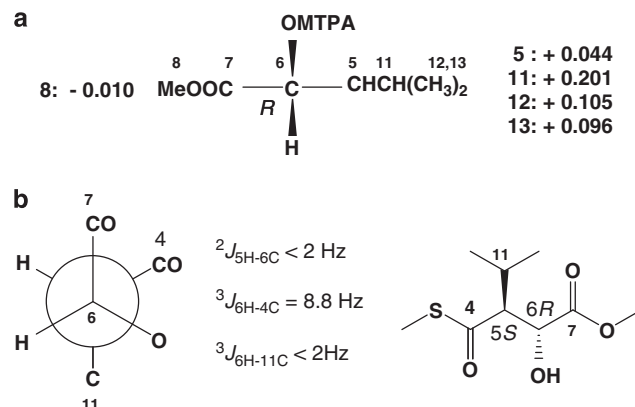
**Table 1**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data for 1 and 3-isopropylmalate methyl ester

No.	1		3-Isopropylmalate methyl ester	
	$^{13}\text{C}$	$^1\text{H}$ (J in Hz)	$^{13}\text{C}$	$^1\text{H}$ (J in Hz)
1	171.6			
2	52.2	4.91 (1H, m)		
3	29.7	3.22 (1H, dd, 14.5, 4.4) 3.67 (1H, dd, 14.5, 3.9)		
4	200.3		177.7	
5	63.9	2.64 (1H, dd, 10.1, 2.9)	55.8	2.67 (1H, dd, 9.9, 2.8)
6	70.1	4.46 (1H, d, 3.1)	69.7	4.50 (1H, d, 2.9)
7	174.7		174.0	
8	52.9	3.81 (3H, s)	52.2	3.82 (3H, s)
9	172.4			
10	22.5	2.12 (3H, s)		
11	27.5	2.30 (1H, m)	26.9	2.27 (1H, m)
12	20.9	0.96 (3H, d, 6.6)	20.9	1.06 (3H, d, 6.4)
13	19.9	1.06 (3H, d, 6.6)	19.8	1.03 (3H, d, 6.4)
NH		6.68 (1H, d, 6.6)		

$^{13}\text{C}$  (125 MHz) and  $^1\text{H}$  (500 MHz) NMR spectra were obtained using an NMR System 500 NB CL (Varian, Palo Alto, CA, USA) in  $\text{CDCl}_3$ , and the solvent peak was used as an internal standard ( $\delta_{\text{C}}$  77.0,  $\delta_{\text{H}}$  7.24).

functional group. By taking into consideration the molecular formula of 1 and the long-range coupling from 3-H to C-4, together with the  $^{13}\text{C}$  chemical shifts of C-3 ( $\delta_{\text{C}}$  29.7) and C-4, these partial structures should be connected through a sulfur atom. Finally, the structure of 1 was determined to be 2-acetyl-3-(3-hydroxy-2-isopropyl-4-methoxy-4-oxobutanoylthio)propanoic acid, as shown in Figure 1. This structure was also supported by alkaline hydrolysis (2 N NaOH, 40 °C, 1 h), which yielded an *N*-acetyl cysteine and 2-hydroxy-3-isopropylsuccinic acid residues.

The absolute stereochemistry of 1 was established as follows. The *N*-acetyl cysteine obtained from 1 by alkaline hydrolysis was determined as *R* by comparing the optical rotations ( $[\alpha]_{\text{D}}$  6.25,  $c$  0.24 (MeOH), 25 °C; authentic sample:  $[\alpha]_{\text{D}}$  6.08,  $c$  0.25 (MeOH), 25 °C). The absolute stereochemistry at C-5 and C-6 was determined by the modified-Mosher method<sup>5</sup> and the *J*-based method.<sup>6–9</sup> Compound 1 was treated with trimethylsilyldiazomethane to afford a methyl ester of 1. This methyl ester compound was then reacted with (*R*)- and (*S*)-MTPA chloride in pyridine. The differences in chemical shift



**Figure 2** (a) Absolute configuration at C-6 revealed by modified Mosher's method. (b) Absolute configuration at C-5 established by *J*-based analysis.

values obtained by subtracting the (*R*)-MTPA ester values from (*S*)-MTPA ester values ( $\delta\Delta = \delta(\text{S})\text{-MTPA} - \delta(\text{R})\text{-MTPA}$ ) are summarized in Figure 2a. From these values, the absolute configuration at C-6 was concluded to be 6*R*. A small coupling constant (<2 Hz) between 5-H and C-6 revealed that the oxygen atom and 5-H were in an *anti* relationship. A large coupling constant between 6-H and C-4 ( $^3J_{\text{H6-C4}} = 8.8\text{ Hz}$ ) and a small one between 6-H and C-11 ( $^3J_{\text{H6-C11}} < 2\text{ Hz}$ ) indicated that they were in *anti* and *gauche* locations, respectively, as shown in Figure 2b. These results revealed the absolute configuration at C-5 to be 5*S*. Thus, the absolute structure of 1 was established, as shown in Figure 1a.

The cytotoxic activity of 1 against human acute myelogenous leukemia HL-60 cells was tested by the WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium, monosodium salt] colorimetric assay (Cell Counting Kit, Dojindo, Kumamoto, Japan). It was found that 1 exhibited a weak cytotoxic effect against HL-60 cells for 48 h with an  $\text{IC}_{50}$  value of 210  $\mu\text{M}$ . We also attempted to investigate the antimicrobial activity of 1. However, 1 did not exhibit antimicrobial activity against *Micrococcus luteus*, *Escherichia coli*, and *Schizosaccharomyces pombe*.

## ACKNOWLEDGEMENTS

This work was supported in part by the New Energy and Industrial Technology Development Organization of Japan (NEDO) and a Grant-in-Aid for Scientific Research (20380070 to KS) from The Japan Society for the Promotion of Science (JSPS).

- Berdy, J. Bioactive microbial metabolites—a personal view. *J. Antibiot.* **58**, 1–26 (2005).
- Dernain, A. L. & Sanchez, S. Microbial drug discovery: 80 years of progress. *J. Antibiot.* **62**, 5–16 (2009).
- Dumlaio, D. S., Hertz, N. & Claecke, S. Secreted signals invasive growth during amino acid starvation in *Saccharomyces cerevisiae*. *Biochemistry* **47**, 698–709 (2008).
- Furihata, K. & Seto, H. Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett.* **39**, 7337–7340 (1998).
- Ohtani, I., Kusumi, T., Kashman, Y. & Kakisawa, H. High-field FT NMR application of Mosher method—the absolute-configurations of marine terpenoids. *J. Am. Chem. Soc.* **113**, 4092–4096 (1991).
- Kobayashi, H., Shin-ya, K., Furihata, K., Hayakawa, Y. & Seto, H. Absolute configuration of a novel glutamate receptor antagonist kaitocephalin. *Tetrahedron Lett.* **42**, 4021–4023 (2001).
- Murata, M., Matsuoka, S., Matsumori, N., Paul, G. K. & Tachibana, K. Absolute configuration of amphotidinol 3, the first complete structure determination from amphotidinol homologues: application of a new configuration analysis based on carbon-hydrogen spin-coupling constants. *J. Am. Chem. Soc.* **121**, 870–871 (1999).
- Park, H. R., Chijiwa, S., Furihata, K., Hayakawa, Y. & Shin-ya, K. Relative and absolute configuration of versiplostatin, a down-regulator of molecular chaperone GRP78 expression. *Org. Lett.* **9**, 1457–1460 (2007).
- Umeda, Y. *et al.* Absolute structure of prunostatin A, a novel GRP78 molecular chaperone down-regulator. *Org. Lett.* **9**, 4239–4242 (2007).