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NOTE

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

Takeshi Fujiwara[1], Aya Nagai[1], Motoki Takagi[1] and Kazuo Shin-ya[2]

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Actinobacteria have been extensively studied thus far, and they are known to produce pharmaceutically important compounds.[1,2] 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% $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan; pH 7.2 before sterilization).

The mycelium collected from the culture broth (1 l) by centrifugation was extracted with Me_2CO (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_2SO_4 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 $CHCl_3$–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_2O–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).[3]

Compound **1** was obtained as a colorless oil ($[\alpha]_D$ −11.8, 29 °C, c 0.28 ($CHCl_3$), UV (MeOH) λ_{max} (ε) 233 (15 800)). The infrared (IR) spectrum (KBr) of **1** revealed the characteristic absorptions of esters (ν_{max} 1730 cm^{-1}), amide (ν_{max} 1670 cm^{-1}), hydroxyl, and/or amide NH (ν_{max} 3400 cm^{-1}) groups. Its molecular formula was established as $C_{13}H_{21}NO_7S$ (*m/z* $[M\text{-}H]^-$ 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).[4] The ^1H and ^{13}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 (δ_H 6.68) to methylene protons 3-H (δ_H 3.67 and 3.22) through an α-methine proton 2-H (δ_H 4.91, δ_C 52.2) was observed in the DQF-COSY of **1**. In the HMBC spectrum, ^1H–^{13}C long-range couplings from the α-methine proton 2-H and the methylene protons 3-H to a carbonyl carbon C-1 (δ_C 171.6), and from the amide proton 2-NH and a singlet methyl proton 10-H (δ_H 2.12) to an amide carbonyl carbon C-9 (δ_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 (δ_H 4.46) to a methyl proton 12-H (δ_H 0.96) through methine protons 5-H (δ_H 2.64) and 11-H (δ_H 2.30), which in turn spin coupled to a methyl proton 13-H (δ_H 1.06), was established. ^1H–^{13}C long-range couplings from 5-H and 11-H to a carbonyl carbon C-4 (δ_C 200.3) and from 5-H and 6-H to an ester carbonyl carbon C-7 (δ_C 174.7) were recognized in the HMBC spectrum of **1**. A methoxyl proton 8-H (δ_H 3.81) was long-range coupled to the ester carbonyl carbon C-7, which established the substitution position of the methoxyl

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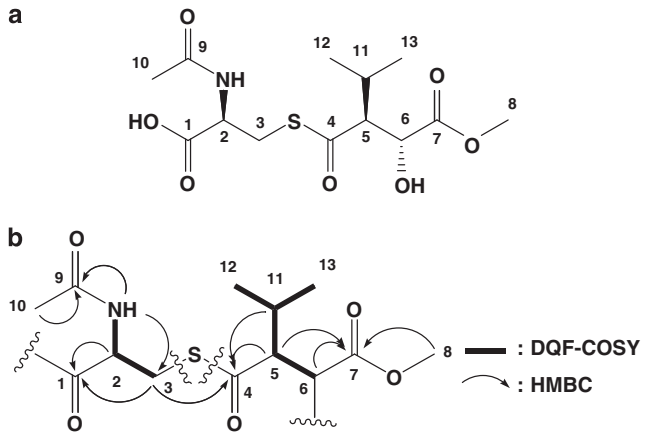

Figure 1 (**a**) Structure of **1**. (**b**) Key correlations in the DQF-COSY (bold line) and HMBC (arrow) spectra of **1**.

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

	1		*3-Isopropylmalate methyl ester*	
No.	^{13}C	^{1}H *(J in Hz)*	^{13}C	^{1}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}C (125 MHz) and ^{1}H (500 MHz) NMR spectra were obtained using an NMR System 500 NB CL (Varian, Palo Alto, CA, USA) in $CDCl_3$, and the solvent peak was used as an internal standard (δ_C 77.0, δ_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}C chemical shifts of C-3 (δ_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-acetamido-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 cystein and 2-hydroxy-3-isopropylsuccinic acid residues.

The absolute stereochemistry of **1** was established as follows. The *N*-acetyl cystein obtained from **1** by alkaline hydrolysis was determined as *R* by comparing the optical rotations ($[\alpha]_D$ 6.25, c 0.24 (MeOH), 25 °C; authentic sample: $[\alpha]_D$ 6.08, *c* 0.25 (MeOH), 25 °C). The absolute stereochemistry at C-5 and C-6 was determined by the modified-Mosher method[5] and the *J*-based method.[6–9] 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

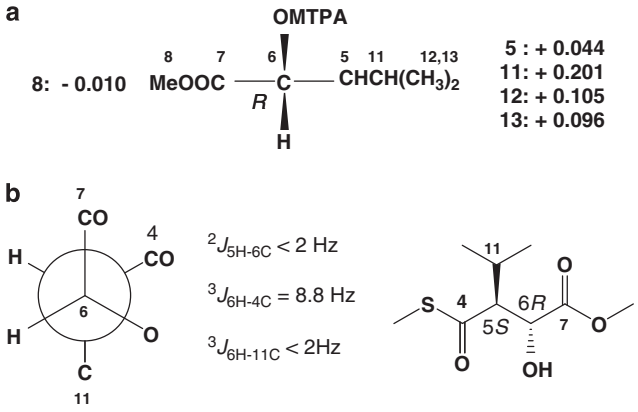

Figure 2 (**a**) Absolute configuration at C-6 revealed by modified Mosher's method. (**b**) Absolute configuration at C-5 established by *J*-based analysis.

values obtained by subtracting the (*R*)-MTPA ester values from (*S*)-MTPA ester values ($\delta\Delta = \delta(S)$-MTPA$-\delta(R)$-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 ($^{3}J_{H6-C4} = 8.8$ Hz) and a small one between 6-H and C-11 ($^{3}J_{H6-C11} < 2$ 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-disulfophenyl)-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 IC_{50} value of 210 μM. We also attempted to investigate the antimicrobial activitiy of **1**. However, **1** did not exhibit antimicrobial activity against *Micrococcus luteus*, *Escherichia coli*, and *Schizosaccharomyces pombe*.

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