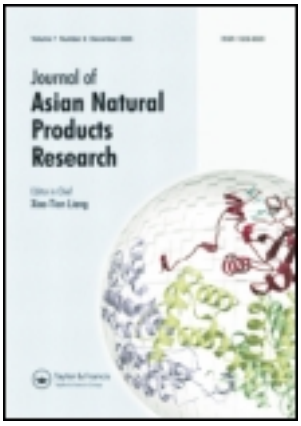

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Two new compounds from the metabolites of a marine-derived actinomycete *Streptomyces cavourensis* YY01-17

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Our current marine natural product program investigated the second metabolites of an actinomycete *Streptomyces cavourensis* YY01-17 originating from the Antarctic ecological niche to discover potential antitumor chemical entities. Two new compounds, along with a known compound, were isolated from the ethyl acetate extract of the fermentation broth of the marine-derived actinomycete, and their structures were elucidated, respectively, as 2(*S*)-3′-hydroxybutan-2′-yl 2-hydroxypropanoate (**1**), (*E*)-3-hydroxy-2,4-dimethylhept-4-enamide (**2**), and 2-hydroxy-3-methylbutanoic acid (**3**) on the basis of spectroscopic data interpretation.

Keywords: marine actinomycete; *Streptomyces cavourensis*; amide

1. Introduction

Marine micro-organisms continue to be both a major focus of many research efforts and a rich source of novel metabolites all over the world [1]. Recent years, actinomycetes isolated from the marine environment have attracted considerable attention. Extensive exploration of the second metabolites of marine actinomycetes has led to the isolation of many important drug leads, and some are developed into antimicrobial, anticancer, and immunosuppressive drugs [2].

As part of our ongoing screening for new antitumor compounds from marine micro-organisms [3], we have isolated a marine actinomycete *Streptomyces cavourensis* YY01-17 from the lichens, grown in the Antarctic area. Studies on the ethyl acetate extract of the fermentation broth of the actinomycete afforded three compounds, among which compounds **1** and **2** (Figure 1) were new linear compounds.

2. Results and discussion

Compound **1** was obtained as a colorless oil with $[\alpha_D^{20}] + 6.0$ (*c* 0.05, MeOH), for which HR-ESI-MS established its molecular formula as $C_7H_{14}O_4$ at *m/z* 163.0972 $[M + H]^+$. Its UV spectrum showed absorption maximum at 219 nm; and the IR spectrum revealed absorption bands at 3393, 2982, 2939, 1733, 1452, and 1377 cm^{-1}. Analysis of the NMR spectra (Table 1) revealed chemical shifts indicative of a carboxyl moiety (δ_C 174.2), two hydroxyl groups (δ_H 4.73 and δ_H 5.27), three methyl groups [(δ_H 1.02, δ_C 19.1), (δ_H 1.12, δ_C 15.5), and (δ_H 1.23, δ_C 20.5)], and three oxygenated methine moieties [(δ_H 3.57, δ_C 68.0), (δ_H 4.08, δ_C 66.2), and (δ_H 4.60, δ_C 74.2)]. The presence of these groups was supported by HSQC experiments. On the basis of the HMBC correlations (Figure 2) of H-3 (δ_H 1.23) with C-2 (δ_C 66.2); H-1′ (δ_H 1.12) with C-2′ (δ_C 74.2), C-3′ (δ_C 68.0); H-4 (δ_H 1.02) with C-3′ (δ_C 68.0), C-2′ (δ_C 74.2), and the

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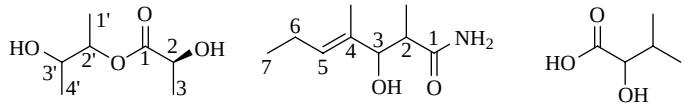

1 **2** **3**

Figure 1. The structures of compounds **1**–**3**.

correlations of OH-2 (δ_H 5.27) with C-2 (δ_C 66.2), C-3 (δ_C 20.5); OH-3′ (δ_H 4.73) with C-2′ (δ_C 74.2), C-4′ (δ_C 19.1), C-3′ (δ_C 68.0), the complete carbon connectivity was established (Figure 1). On alkaline hydrolysis of **1**, the optical rotation of lactic acid was $[\alpha_D^{20}]$ −13.8 (*c* 0.3, H_2O) and L-lactic acid was confirmed by comparing with the data in the literature [4], so the absolute configuration of C-2 was confirmed as *S*. Thus, compound **1** was finally elucidated as 2(*S*)-3′-hydroxybutan-2′-yl 2-hydroxypropanoate.

Compound **2** was also obtained as a colorless oil with $[\alpha_D^{20}]$ + 12.0 (*c* 0.05, MeOH), and its molecular formula was established to be $C_9H_{17}NO_2$ by HR-ESI-MS at *m/z* 172.1334 $[M + H]^+$, thereby indicating two degrees of unsaturation. Its UV spectrum showed absorption maximum at 210 and 238 nm; and the IR spectrum revealed absorption bands at 3339, 2966, 2934, 1662, 1609, 1459, and 862 cm^{-1}. The NMR spectra (Table 1) of **2** illustrated a trisubstituted double bond [δ_H 5.24 (1H, t, $J = 6.9$ Hz)/δ_C 128.5, δ_C 135.5], an amide carbonyl moiety [δ_H 7.17 (1H, s, —NH), 6.65 (1H, s, —NH), and δ_C 177.2], an ethyl group [δ_H 0.91 (3H, t, $J = 7.5$ Hz)/δ_C 14.2, δ_H 1.96 (2H, m)/δ_C 20.3], an oxygenated alcohol methine group [δ_H 3.81 (1H, d, $J = 9.3$ Hz)/δ_C 78.9, δ_H 4.68 (1H, br s, —OH)], a methyl moiety adjacent to an unsaturated quaternary carbon [δ_H 1.49 (3H, s)/δ_C 10.6], and a tertiary aliphatic methyl group [δ_H 0.74 (3H, d, $J = 7.2$ Hz)/δ_C 15.0, δ_H 2.27 (1H, m)/δ_C 43.1]. The complete connectivity of these groups was confirmed by HMBC correlations (Figure 2) of H-5 at δ_H 5.24 with 4-CH_3 at δ_C 10.6, C-7 at δ_C 14.2, C-6 at δ_C 20.3, C-3 at δ_C 78.9; 4-CH_3 at δ_H 1.49 with C-3 at δ_C 78.9, C-5 at δ_C 128.5, C-4 at δ_C 135.5; and H-3 at δ_H 3.81 with 4-CH_3 at δ_C 10.6, 2-CH_3 at δ_C 15.0, C-2 at δ_C 43.1, C-5 at δ_C 128.5, C-4 at δ_C 135.5, C-1 at δ_C 177.2. The absence of any correlation between CH_3-4 and H-5 in the NOESY spectrum (Figure 3), meanwhile the obvious cross-peak between CH_3-4 and

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of compounds **1** and **2** in DMSO-d_6.

	1			**2**	
Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
1	174.2	–	1	177.2	–
2	66.2	4.08 (1H, m)	2	43.1	2.27 (1H, m)
3	20.5	1.23 (3H, d, 6.6)	3	78.9	3.81 (1H, d, 9.3)
1′	15.5	1.12 (3H, d, 6.0)	4	135.5	–
2′	74.2	4.60 (1H, m)	5	128.5	5.24 (1H, t, 6.9)
3′	68.0	3.57 (1H, m)	6	20.3	1.96 (2H, m)
4′	19.1	1.02 (3H, d, 6.3)	7	14.2	0.91 (3H, t, 7.5)
3′-OH	–	4.73 (1OH, d, 5.1)	2-CH_3	15.0	0.74 (3H, d, 7.2)
2-OH	–	5.27 (1OH, d, 5.7)	4-CH_3	10.6	1.49 (3H, s)
			3-OH	–	4.68 (1H, br s)
			—NH_2	–	7.17 (1H, s), 6.65 (1H, s)

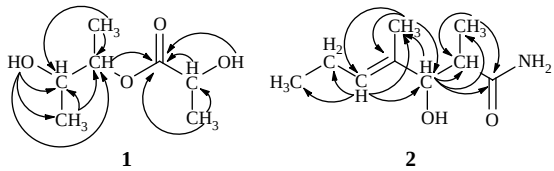

Figure 2. The key HMBC correlations of new compounds **1** and **2**.

H-6, supported the (*E*)-double-bond geometry. Hence, the structure of compound **2** was finally determined as (*E*)-3-hydroxy-2,4-dimethylhept-4-enamide. Meanwhile, the relative configuration of this compound was confirmed by the NOESY spectrum in Newman projection (Figure 3). The appearance correlations of H-3 with H-2 and CH_3-2 declared H-3 was in place between H-2 and CH_3-2 in Newman projection. The obvious cross-peak between CH_3-2 and CH_3-4 supported that CH_3-4 was surrounding CH_3-2. So the elative configuration of compound **2** was confirmed as shown in Figure 3.

The known compound 2-hydroxy-3-methylbutanoic acid (**3**) was identified by the comparison of the spectral data (^{1}H and ^{13}C NMR) with those reported in the literature [5].

3. Experimental

3.1 General experimental procedures

UV spectra were measured on a Shimadzu UV-1601 (Shimadzu, Tokyo, Japan). IR spectra were measured on a Bruker IFS-55 infrared spectrophotometer (Bruker Co., Zurich, Switzerland). Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Perkin-Elmer Co., Jena, Germany). The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) with TMS (tetramethylsilane) as the internal standard (Bruker Co.). The HR-ESI-MS data were obtained on the Microssmass AutoSpec-UltimaE TOF mass spectrophotometer (Bruker Co.). Chromatography was carried out on silica gel (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA), and reversed-phase HPLC (Shimadzu LC-8A vp, Kyoto, Japan).

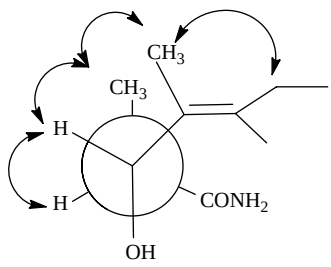

Figure 3. The key NOESY correlations of new compound **2**.

3.2 Actinomycete material

The actinomycete was isolated from the lichens, collected from Antarctica in December 1999. A voucher specimen (No. HTTA-F99123), the genomic DNA of strain, was prepared, and 16S rDNA genes were amplified with a method described by Kataoka *et al*. [6].

The 16S rDNA sequences were aligned with published sequences from the GeneBank database (http://www.ncbi.nlm.nih.gov) using the NCBI BLASTN comparison software. Phylogenetic trees were constructed by the neighbor-joining method using the DNAMAN software (version 5.1, Lynnon Biosoft, Quebec, Canada) (Figure 4). The closest relatives of the remaining sequences were obtained from the GeneBank database using BLAST program and sequence similarity was 99% between strain YY01-17 and *S. cavourensis* NR04385.1. From phylogenetic analysis, physiological and biochemical tests, and

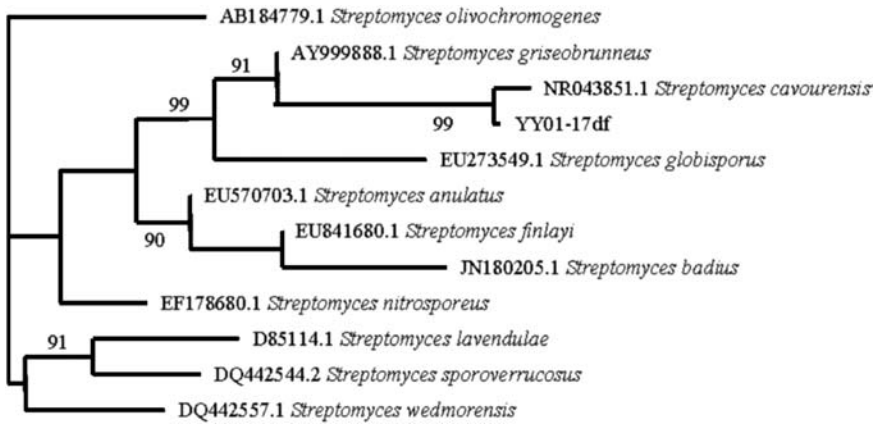

Figure 4. Phylogenetic tree of species YY01-17 based on 16S rDNA gene sequences.

morphological characteristics, the strain was identified as *S. cavourensis* (GeneBank No. GU575124), and has been deposited in the Marine Microbial Medicinal Resource Library of the First Institute of Oceanography SOA funded by the Ministry of Science and Technology.

3.3 Fermentation, extraction, and isolation

The inoculum prepared by putting Petri dish cultures of YY 01-17 into 500-ml baffled flasks containing 150 ml of the broth (soluble starch 1.8%, peptone 0.2%, seawater 25% in deionized water, pH 7.0) was fermented by shaking at 150 rpm and 20°C for 20 days in incubator shaker.

The supernatant of the fermentation broth of the strain YY 01-17 (50 liters) was concentrated to 5 liters *in vacuo* and extracted with ethyl acetate and *n*-butanol, successively. The EtOAc crude extract (10.7 g) was subjected to silica gel column and eluted with $CHCl_3$—CH_3OH (100:1–0:1), yielding eight fractions. Fraction 3 (1.2 g) was purified by Sephadex LH-20 column chromatography (CH_3OH) and preparative HPLC (CH_3OH–H_2O 40:100, flow rate 1 ml/min, wavelength 210 nm) to obtain compounds **1** (15.8 mg, retention time 25 min) and **3** (15 mg, retention time 32 min). Fraction 4 (2.3 g) was purified by Sephadex LH-20 column chromatography (CH_3OH) and preparative HPLC (CH_3OH—H_2O 58.5:100, flow rate 1.0 ml/min, wavelength 210 nm) to obtain **2** (7.7 mg, retention time 34 min).

*3.3.1 2(S)-3′-Hydroxybutan-2′-yl 2-hydroxypropanoate (**1**)*

Colorless oil, $[\alpha_D^{20}] + 6.0$ (*c* 0.05, MeOH); UV (MeOH) λ_{max}: 219 nm; IR (KBr) ν_{max} (cm^{-1}): 3393, 2982, 2939, 1733, 1452, 1377; ^1H NMR and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS *m/z*: 163.0972 $[M + H]^+$ (calcd for $C_7H_{15}O_4$, 163.0970).

*3.3.2 (E)-3-Hydroxy-2,4-dimethylhept-4-enamide (**2**)*

Colorless oil, $[\alpha_D^{20}] + 12.0$ (*c* 0.05, MeOH); UV (MeOH) λ_{max}: 210, 238 nm; IR (KBr) ν_{max} (cm^{-1}): 3339, 2966, 2934, 1662, 1609, 1459, 862; ^1H NMR and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS *m/z*: 172.1334 $[M + H]^+$ (calcd for $C_9H_{18}NO_2$, 172.1338). This compound could get CAS Registry Number as 1246085-99-3 but without any reference.

*3.3.3 Alkaline hydrolysis of compound **1***

Compound **1** was hydrolyzed with 5% KOH in dioxane (1 ml) for 5 h. The

mixture was extracted with *n*-butanol, and then aqueous fraction was neutralized with 5% HCl. The aqueous fraction was detected by optical rotation analysis and L-lactic acid was confirmed by comparing with the data in the literature.

Acknowledgements

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