

ORIGINAL ARTICLE

# 03219A, a new $\Delta^{8,9}$ -pregnene isolated from *Streptomyces* sp. SCSIO 03219 obtained from a South China Sea sediment

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**03219A (1), a new pregnene steroid possessing a rare  $\Delta^{8,9}$ -double bond in the skeleton, together with the known naphthoquinone antibiotic (+)-cryptosporin (2) have been isolated from the fermentation broth of *Streptomyces* sp. SCSIO 03219, which was isolated from a marine sediment collected in the South China Sea. The structure of 03219A was elucidated using a combination of NMR, MS and X-ray crystallographic methods.**

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**Keywords:** 03219A; pregnene steroid; South China Sea sediment; *Streptomyces*

## INTRODUCTION

Actinomycetes obtained from marine environments have proven to be promising sources of new natural products for use in drug discovery. Indeed, in the last decade a large number of novel metabolites with various biological activities have been identified from such actinomycetes.<sup>1–8</sup> Some of these compounds, such as salinosporamide A, have been of such significant bioactive potency and uniqueness that they have been fast-tracked into clinical trials as potential anticancer agents.<sup>9</sup> As part of our program to discover novel structures and bioactive metabolites originating from actinomycetes obtained from the South China Sea, we have reported antimalarial  $\beta$ -carboline and indolactam alkaloids from *Marinactinospora thermotolerans* SCSIO 00652,<sup>10</sup> and cytotoxic angucyclines from *Streptomyces lusitanus* SCSIO LR32.<sup>11</sup> Recently, an actinomycete strain was isolated from a marine sediment sample collected in the South China Sea and subsequently identified as *Streptomyces* sp. SCSIO 03219 on the basis of 16S ribosomal DNA sequence analysis. This strain was fermented on a 6 L-scale, resulting in the purification of a new pregnene-type sterol 03219A (1) and a known naphthoquinone antibiotic (+)-cryptosporin (2) (Figure 1). We report herein, the fermentation, isolation and structural characterization of these natural products.

## RESULTS

### Taxonomy of the producing strain

16S rRNA gene of strain SCSIO 03219 was PCR amplified in order to achieve precise taxonomic identification by using bacterial universal primers, sequenced and deposited in GenBank as accession number JQ970427. BLAST analysis of 16S rRNA gene sequences revealed that

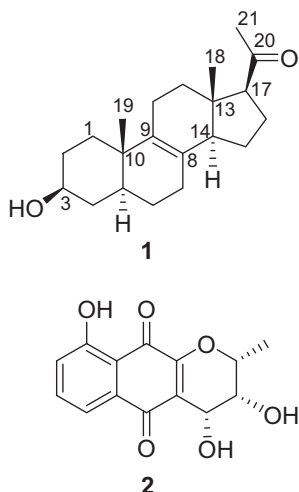
this strain has the highest similarity as 98.67% with the validly described species of *Streptomyces olivaceus* NBRC 12805. Phylogenetic tree (Figure 2) showed that strain SCSIO 03219 clustered with the most close species of *S. olivaceus* NBRC 12805 and *Streptomyces pactum* NBRC 13433<sup>T</sup>. Besides the phylogenetic analysis, the morphological characteristics of strain SCSIO 03219, such as the branching spore chains and spore in sizes  $0.5\ \mu\text{m} \times 2\text{--}6\ \mu\text{m}$ , also showed the classical properties of genus *Streptomyces* (Figure 3).<sup>12</sup> On the basis of these data, strain SCSIO 03219 was identified as one member of genus *Streptomyces* (see Supplementary Figure S1 in Supplementary Material for more strain pictures and scanning electron micrographs).

### Structural elucidation

Compound 1 was obtained as white needles. Its molecular formula,  $\text{C}_{21}\text{H}_{32}\text{O}_2$ , was determined by HR-TOF-ESIMS (electron spray ionization-time of flight-mass), which revealed a quasi-molecular peak at  $m/z$  317.2464  $[\text{M} + \text{H}]^+$  (calcd 317.2475), requiring 6° of unsaturation. The CD spectrum of 1 (Figure 4) indicated it is a single enantiomer. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data (Table 1, for NMR spectra, see Supplementary Figures S2–S6 in Supplementary Material) for 1 revealed the presence of signals diagnostic for three methyls at  $\delta_{\text{H}}$  0.58,  $\delta_{\text{C}}$  13.1 (CH<sub>3</sub>-18),  $\delta_{\text{H}}$  0.98,  $\delta_{\text{C}}$  18.2 (CH<sub>3</sub>-19),  $\delta_{\text{H}}$  2.15,  $\delta_{\text{C}}$  32.1 (CH<sub>3</sub>-21), nine methylenes, four methines, including an oxygen-bearing methine at  $\delta_{\text{H}}$  3.55,  $\delta_{\text{C}}$  71.6 (CH-3), two aliphatic quaternary carbons at  $\delta_{\text{C}}$  36.9 (C-10) and  $\delta_{\text{C}}$  44.9 (C-13), two olefinic quaternary carbons at  $\delta_{\text{C}}$  128.9 (C-8) and  $\delta_{\text{C}}$  136.6 (C-9), and one carbonyl moiety at  $\delta_{\text{C}}$  212.4 (C-20). The <sup>1</sup>H-<sup>1</sup>H COSY experiment suggested three <sup>1</sup>H-<sup>1</sup>H spin systems of H-1–H-7, H-11–H-12 and H-14–H-17,

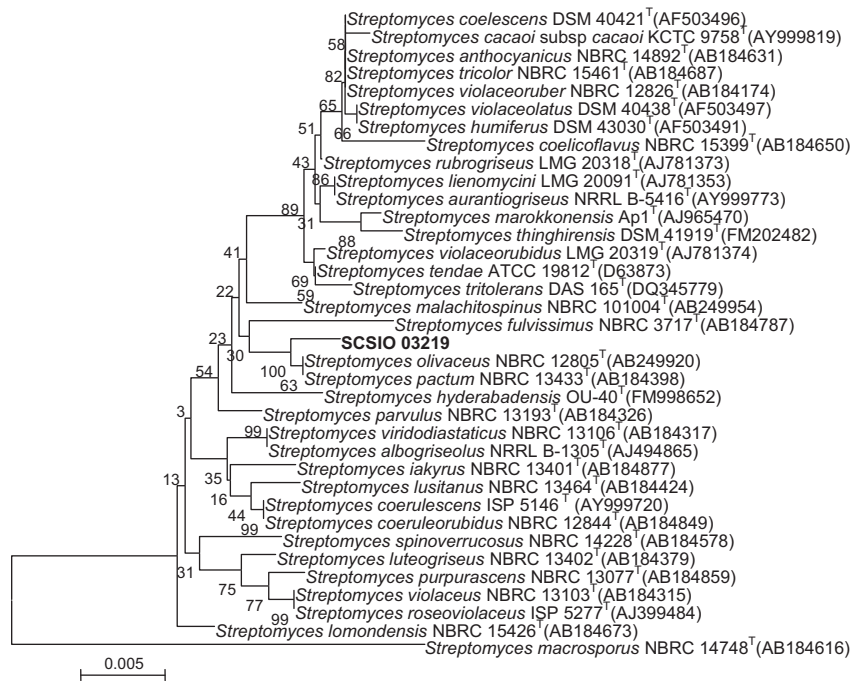
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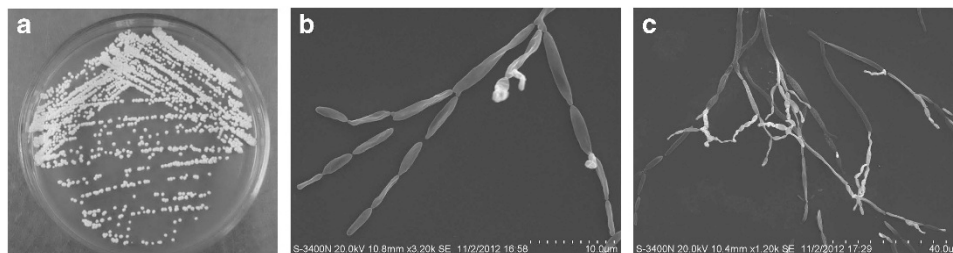


**Figure 1** Chemical structures of compounds **1** and **2**.

consistent with the indicated structural fragments of C-1–C-7, C-11–C-12 and C-14–C-17 (Figure 5). In the heteronuclear multiple bond correlation (HMBC) spectrum, the correlations that originated from H<sub>3</sub>-19 to C-1, 5, 9, 10, and from H<sub>2</sub>-7 to C-8, 9 helped to establish the presence of six-membered rings A and B. The HMBC correlations of H<sub>2</sub>-11/C-8, 9, H<sub>2</sub>-12/C-13, 14, and of H-14/C-8, 13 supported the assigned structure of ring C. Additional HMBC correlations of H<sub>3</sub>-18/C-12, 13, 14, 17, and of H-17/C-13 supported the assignment of five-membered ring D. An acetyl moiety was assigned at C-17 on the basis of HMBC correlations involving H-17/C-20 and H<sub>3</sub>-21/C-20. Consequently, **1** was elucidated to be  $\Delta^{8,9}$ -3-hydroxy-17-acetyl steroid and its structure was confirmed by X-ray crystallography (Figure 6). This new compound was named 03219A. 03219A (**1**) was tested for antibacterial activities against four bacteria *Staphylococcus aureus* ATCC 29213, *Bacillus thuringiensis* ATCC 39765, *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 25922 using a disk diffusion method, but none of them showed an inhibition zone at a concentration of 10  $\mu$ g/6 mm paper disk.



**Figure 2** Phylogenetic dendrogram of the strain SCSIO 03219<sup>T</sup> and its closest relatives as constructed using the neighbor-joining method based on 16S ribosomal DNA gene sequences.



**Figure 3** Pure culture in dish (**a**) and scanning electron micrographs of spore chains (**b**) and aerial mycelia (**c**) of *Streptomyces* sp. SCSIO 03219 after incubation for 7 days on modified International *Streptomyces* Projects (ISP), medium No. 2 at 28 °C. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

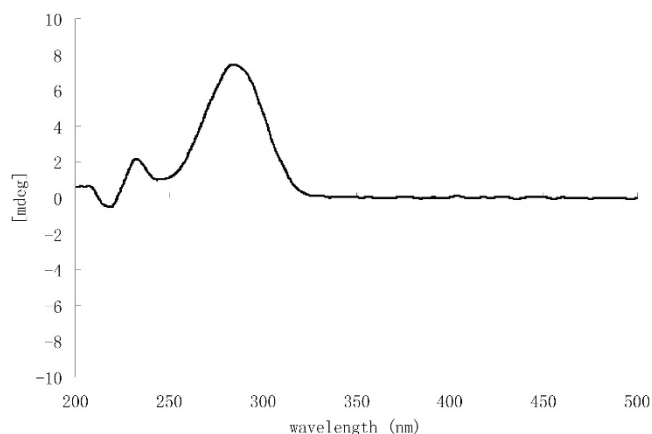
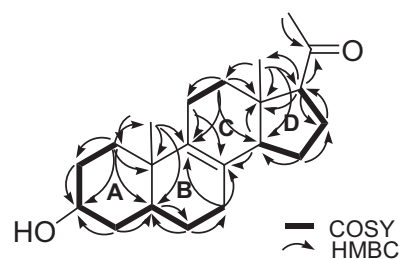
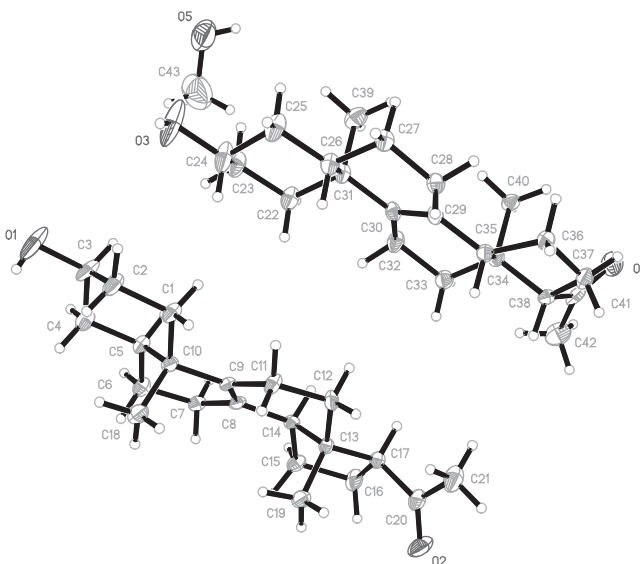


Figure 4 CD spectrum of compound 1.

Table 1 Summary of  $^1\text{H-NMR}$  (500 MHz) and  $^{13}\text{C-NMR}$  (125 MHz) data for 1 in  $\text{CD}_3\text{OD}/\text{CDCl}_3$ 

Position	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$	HMBC	$^1\text{H-}^1\text{H COSY}$
1	1.80 (1H, m) 1.24 (1H, td, $J=14.0, 4.5$ )	36.3 $\text{CH}_2$	C-2, 3, 5, 10, 19	H-2
2	1.83 (1H, m) 1.46 (1H, m)	32.1 $\text{CH}_2$	C-3	H-1, 3
3	3.55 (1H, m)	71.6 CH		H-2, 4
4	1.64 (1H, ddd, $J=10.0, 4.7, 2.2$ ) 1.33 (1H, br d, $J=10.0$ )	38.9 $\text{CH}_2$	C-3, 5	H-3, 5
5	1.42 (1H, m)	42.0 CH	C-6	H-4, 6
6	1.41 (1H, m) 1.53 (1H, dd, $J=10.8, 7.3$ )	26.5 $\text{CH}_2$	C-5, 7	H-5, 7
7	2.08 (1H, m) 2.02 (1H, m)	28.3 $\text{CH}_2$	C-8, 9	H-6
8		128.9 C		
9		136.6 C		
10		36.9 C		
11	2.25 (1H, m) 2.18 (1H, m)	24.1 $\text{CH}_2$	C-8, 9	H-12
12	2.06 (1H, m) 1.70 (1H, m)	37.1 $\text{CH}_2$	C-9, 11, 13, 14, 18	H-11
13		44.9 C		
14	2.30 (1H, m)	53.1 CH	C-8, 13	H-15
15	1.73 (1H, m) 1.45 (1H, m)	25.0 $\text{CH}_2$	C-14, 16	H-14, 16
16	2.23 (1H, m) 1.72 (1H, m)	23.8 $\text{CH}_2$	C-17	H-15, 17
17	2.69 (1H, t, $J=8.7$ )	63.4 CH	C-13, 16, 20	H-16
18	0.58 (3H, s)	13.1 $\text{CH}_3$	C-12, 13, 14, 17	
19	0.98 (3H, s)	18.2 $\text{CH}_3$	C-1, 5, 9, 10	
20		212.4 C		
21	2.15 (3H, s)	32.1 $\text{CH}_3$	C-20	

The known compound, (+)-cryptosporin (2), was identified on the basis of comparisons of the optical rotation, MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data with those previously reported.<sup>13,14</sup> Cryptosporin was initially isolated and identified by Closse<sup>13</sup> from

Figure 5 Selected key  $^1\text{H-}^1\text{H}$  COSY and heteronuclear multiple bond correlation (HMBC) correlations for compound 1.Figure 6 Oak ridge thermal ellipsoid plot (ORTEP) structure of compound 1. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

the fungus *Cryptosporium pinicola* in 1973 and was subsequently found to possess antibacterial activities against methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis* with MIC value of  $8 \mu\text{g ml}^{-1}$ .<sup>14</sup>

#### X-ray crystallographic analysis

A colorless crystal of 1 was obtained in MeOH. The crystal data of 1 were recorded on an Oxford Gemini A Ultra single-crystal diffractometer (Oxford Diffraction, Ltd., Abingdon, UK) using Cu K $\alpha$  radiation ( $\lambda = 1.54184 \text{ \AA}$ ). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least squares difference fourier techniques.<sup>15</sup> Crystallographic data for 1 have been deposited in the Cambridge Crystallographic Data Center with the deposition number (CCDC 877992). A copy of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44(0)-1233-336033 or E-mail: deposit@ccdc.cam.ac.uk).

*Crystal data of 1.* Orthorhombic,  $2(\text{C}_{21}\text{H}_{32}\text{O}_2) \cdot \text{CH}_4\text{O}$ , space group  $P2(1)2(1)2$ ,  $a = 7.5505(2) \text{ \AA}$ ,  $b = 50.0504(10) \text{ \AA}$ ,  $c = 9.7193(2) \text{ \AA}$ ,  $\alpha = 90^\circ$ ,  $\beta = 90^\circ$ ,  $\gamma = 90^\circ$ ,  $V = 3672.23(14) \text{ \AA}^3$ ,  $Z = 4$ ,  $D_{\text{calcd}} = 1.203 \text{ mg m}^{-3}$ ,  $\mu = 0.591 \text{ mm}^{-1}$ , and  $F(000) = 1464$ . Crystal size:  $0.43 \times 0.35 \times 0.32 \text{ mm}^3$ . Independent reflections: 6537 [ $R_{\text{int}} = 0.0277$ ]. The final indices were  $R_1 = 0.0634$ ,  $wR_2 = 0.1604$  [ $I > 2\sigma(I)$ ].

## DISCUSSION

Strain SCSIO 03219 was isolated from a deep-sea sediment collected from the South China Sea at a depth of 1350 m and subsequently identified as a member of *Streptomyces* on the basis of 16S rRNA gene clade and morphology analysis. From the fermentation broth of this deep-ocean sediment-derived actinomycete, we have isolated a polyketide compound (+)-cryptosporin and a new steroid congener 03219A that possesses a rare  $\Delta^{8,9}$ -double bond in the steroid skeleton. We report the isolation of (+)-cryptosporin from an actinomycete strain for the first time, indicating strain SCSIO 03219 has acquired enzymes to biosynthesize this polyketide compound. Numerous aromatic polyketides have been characterized to be biosynthesized by type II polyketide synthases from actinomycete strains.<sup>16</sup> Actinomycetes have not been reported to produce steroids, however, the actinobacteria have proven to be efficient biocatalysts for steroid transformation due, in large part, to their compatibility with a broad spectrum of steroid substrates. Transformations executed by actinobacteria strains include, but not limited to, hydroxylation, dehydrogenation, alcohol oxidations and side chain degradation.<sup>17</sup> We envision, the pregnene-type steroid congener 03219A was a biotransformed product from the cleavage of C-20/C-22 side chain of steroids that originated from the fermentation media components by strain SCSIO 03219. Strain SCSIO 03219, like the above reported actinobacteria,<sup>17</sup> may harbor biosynthetic machinery to degrade steroid with a side chain to 03219A.

## MATERIALS AND METHODS

### General experimental procedures

Column chromatography was performed using silica gel (100–200 mesh; Jiangyou Silica Gel Development, Yantai, China), Sephadex LH-20 (40–70  $\mu$ m; GE Healthcare, Uppsala, Sweden). TLC was conducted with precoated glass plates (0.1–0.2 mm; silica gel GF<sub>254</sub>, 10–40 nm, Jiangyou, China). Medium pressure liquid chromatography was performed with a CHEETAH 100 automatic flash chromatography (Bonna-Agela Technology, Tianjin, China) with a YMC ODS-A (Kyoto, Japan; S-50  $\mu$ m, 12 nm) flash column (100  $\times$  20 mm). HPLC analyses were performed with a ProStar 210 solvent delivery module (Varian, Palo Alto, CA, USA) equipped with a ProStar 335 photodiode array detector (Varian), using a Phenomenex Prodigy ODS (2) column (Torrance, CA, USA; 150  $\times$  4.6 mm, 5  $\mu$ m), and a YMC-Pack ODS-A column (250  $\times$  10 mm, 5  $\mu$ m). Melting points were determined with a SGW X-4 apparatus (Yidian, Shanghai, China) and are uncorrected. UV spectra were recorded on a U-2910 spectrometer (Hitachi, Tokyo, Japan); IR spectra were obtained on an IRAffinity-1 spectrophotometer (Shimadzu, Tokyo, Japan). Low-resolution and high-resolution mass spectral data were obtained on amaZon SL ion trap mass spectrometer (Bruker, Raleigh, NC, USA) and MaXis quadrupole-time-of-flight mass spectrometer (Bruker), respectively. Optical rotations were recorded with a MCP 300 polarimeter (Anton Paar, Graz, Austria). NMR spectra were recorded on an Avance 500 spectrometer (Bruker) at 500 MHz for <sup>1</sup>H nuclei and 125 MHz for <sup>13</sup>C nuclei. Chemical shifts ( $\delta$ ) are given with reference to TMS. Coupling constants (*J*) are given in Hz. CD spectra were measured with a J-810 spectrophotometer (JASCO, Tokyo, Japan).

### Isolation, characterization and fermentation of strain SCSIO 03219

Sediment sample was collected from a depth of 1350 m in South China Sea (E 111°54.693', N 08°56.003') in July 2007, and was processed for cultivation experiments by using a standard dilution plating method on ship within 2 h. Strain SCSIO 03219 was isolated on gauze no. 1 medium prepared with seawater instead of distilled water, incubated at 28 °C for 3 weeks. After purification, the strain SCSIO 03219 was subsequently deposited into the type culture collection of the Center for Marine Microbiology, Research Network of Applied Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China, at –80 °C (deposition number SCSIO 03219). Genomic DNA isolation, PCR amplification of 16S ribosomal DNA, sequence comparison and phylogenetic tree construction of the strain

SCSIO 03219 were performed as described previously.<sup>18</sup> Cellular morphology was examined by scanning electron microscope (Hitachi, S-3400N) using cells incubated for 7 days on International *Streptomyces* Projects (ISP), medium No. 2 modified with natural seawater instead of distilled water.

Single colonies of SCSIO 03219 grown on ISP medium No. 2 agar plates were inoculated (with the mycelia) into 50 ml seed medium in 250 ml Erlenmeyer flasks, and were incubated at 28 °C on a rotary shaker at 200 r.p.m. for 2 days as seed culture. Thirty 1-liter Erlenmeyer flasks each containing 200 ml of production medium were then individually inoculated with 50 ml of seed culture, and incubated at 28 °C on a rotary shaker at 200 r.p.m. for 7 days. Both seed medium and production medium consist of 1.0% glucose (Guangzhou Chemical Reagent Factory, Guangzhou, China), 2.0% soluble starch (Guangdong Huankai, Guangzhou, China), 1.0% malt meal (Guangdong Huankai), 1.0% maltose (Beijing Dingguo, Beijing, China), 0.5% corn powder (Wal-Mart, Guangzhou, China), 3.0% sea salt (Guangdong Province Salt Industry Group, Guangzhou, China), 0.01% (v/v) trace element solution (40 mg ZnCl<sub>2</sub>, 200 mg FeCl<sub>3</sub>·6H<sub>2</sub>O, 10 mg CuCl<sub>2</sub>·2H<sub>2</sub>O, 10 mg MnCl<sub>2</sub>·4H<sub>2</sub>O, 10 mg Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, 10 mg (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O in 1-liter H<sub>2</sub>O), pH 7.2–7.4 before sterilization.

### Isolation

The whole culture broth (6 l) was extracted with equal volume of butanone three times and the combined organic layers then evaporated in vacuo to yield 15.8 g of crude extract. The extract was subjected to silica gel column chromatography using gradient elution with CHCl<sub>3</sub>–MeOH mixtures (100:0, 98:2, 96:4, 94:6, 92:8, 9:1, 8:2, 5:5, v/v, 300 ml for each gradient), to afford eight fractions (Fr.1–Fr.8). Fr.2 was purified by gradient column chromatography over silica gel using increasingly polar mixtures of petroleum ether–EtOAc (10:0, 8:2, 6:4, 4:6, 2:8, 0:10, v/v, 50 ml for each gradient) to afford 60 fractions (Fr.2-1–Fr.2-60). The resulting fractions were then analyzed by HPLC-UV and Fr.2-8–Fr.2-10 were combined and separated by HPLC with a semi-preparative ODS column eluted with CH<sub>3</sub>CN–H<sub>2</sub>O (30:70 to 100:0 over the course of 30 min at a flow rate of 2.5 ml min<sup>-1</sup>) to render 03219A (1) (7 mg, *t*<sub>R</sub> 8 min). Fr.2-21–Fr.2-60 were combined and further purified by medium pressure liquid chromatography with an ODS flash column, eluted with MeOH–H<sub>2</sub>O (20:80 to 100:0 over the course of 1 h at a flow rate of 15 ml min<sup>-1</sup>) to afford (+)-cryptosporin (2) (35 mg, *t*<sub>R</sub> 20 min).

### Physio-chemical properties of 1 and 2

03219A (1). White crystalline needles; m.p. 168–169 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +108 (*c* 0.22, MeOH); UV (MeOH)  $\lambda$ <sub>max</sub> (log *ε*) 206 (4.01); IR (ATR)  $\nu$ <sub>max</sub> 3400, 2930, 1703, 1466, 1360, and 1031 cm<sup>-1</sup>; CD (0.332 mm, MeOH),  $\lambda$ <sub>max</sub> ( $\Delta\epsilon$ ), 282 (6.76), 230 (1.92), 220 (–0.55); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>) data, see Table 1; (+)HR-ESI-MS *m/z* 317.2464 ([M + H]<sup>+</sup>, calcd for C<sub>21</sub>H<sub>32</sub>O<sub>2</sub>, 317.2475).

(+)-Cryptosporin (2). Deep yellow crystals; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +190 (*c* 0.10, MeOH) [lit. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +237.9 (*c* = 0.1, acetone)];<sup>13</sup> lit. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +271.2 (*c* = 0.00125, CHCl<sub>3</sub>);<sup>14</sup> (+)ESI-MS *m/z* 277.3 [M + H]<sup>+</sup>.

### Antibacterial activity assay

03219A (1) was tested for antibacterial activity against *S. aureus* ATCC 29213, *B. thuringiensis* ATCC 39765, *Bacillus subtilis* ATCC 6633 and *E. coli* ATCC 25922 using a disk diffusion assay.<sup>19</sup>

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- 1 Fenical, W. *et al.* Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* **2**, 666–673 (2006).
- 2 Bull, A. T. & Stach, J. E. M. Marine actinobacteria: new opportunities for natural product search and discovery. *Trends Microbiol.* **15**, 491–499 (2007).
- 3 Lam, K. S. Discovery of novel metabolites from marine actinomycetes. *Curr. Opin. Microbiol.* **9**, 245–251 (2006).
- 4 Williams, P. G. Panning for chemical gold: marine bacteria as a source of new therapeutics. *Trends Biotechnol.* **27**, 45–52 (2009).
- 5 Liu, X. *et al.* Bioprospecting microbial natural product libraries from the marine environment for drug discovery. *J. Antibiot.* **63**, 415–422 (2010).
- 6 Lane, A. L. & Moore, B. S. A sea of biosynthesis: marine natural products meet the molecular age. *Nat. Prod. Rep.* **28**, 411–428 (2011).
- 7 Mayer, A. M. S. *et al.* Marine pharmacology in 2007–8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. *Comp. Biochem. Physiol. C* **153**, 191–222 (2011).
- 8 Zotchev, S. B. Marine actinomycetes as an emerging resource for the drug development pipelines. *J. Biotechnol.* **158**, 168–175 (2012).
- 9 Chauhan, D. *et al.* A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell* **5**, 407–419 (2005).
- 10 Huang, H. *et al.* Antimalarial  $\beta$ -carboline and indolactam alkaloids from *marinactinospira thermotolerans*, a deep sea isolate. *J. Nat. Prod.* **74**, 2122–2127 (2011).
- 11 Huang, H. *et al.* Cytotoxic angucycline class glycosides from the deep sea actinomycete *Streptomyces lusitanus* SCSIO LR32. *J. Nat. Prod.* **75**, 202–208 (2012).
- 12 Kämpfer, P. in *Order XIV. Streptomycetales Ord. Nov. Bergey's Manual of Systematic Bacteriology*. (Goodfellow, M. *et al.* eds) (Springer, New York 1446–1804 (2012)).
- 13 Closse, A. *et al.* Isolierung und strukturaufklärung von (+)-cryptosporin. *Helv. Chim. Acta* **56**, 619–625 (1973).
- 14 Zhou, J. *et al.* Fermentation, physico-chemical properties and anti-bacteria activity of SP-1, a naphtho-[2, 3-b]pyrandonones group antibiotics produced by strain Z-2002. *J. Biol.* **24**, 29–31 (2007).
- 15 Sheldrick, G. M. *SHELXTL-97, Program for Crystal Structure Solution and Refinement* (University of Göttingen: Göttingen, Germany, 1997).
- 16 Kharel, M. K. *et al.* Angucyclines: biosynthesis, mode-of-action, new natural products, and synthesis. *Nat. Prod. Rep.* **29**, 264–325 (2012).
- 17 Donova, M. V. Transformation of steroids by Actinobacteria: a review. *Appl. Biochem. Microbiol.* **43**, 1–14 (2007).
- 18 White, T. J. *et al.* *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York 315–322, 1990).
- 19 Huang, H. *et al.* Halogenated anthranquinones from the marine-derived fungus *Aspergillus* sp. SCSIO F063. *J. Nat. Prod.* **75**, 1346–1352 (2012).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (<http://www.nature.com/ja>)