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Diketopiperazines from two strains of South China Sea sponge-associated microorganisms

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ABSTRACT

The paper reports the isolation and structural elucidation of seven diketopiperazines from the title microorganisms. Although all isolates are known, three of which were isolated from the actinomycetes for the first time. And this is also the first report to isolate four DKPs from the *D. avara*-associated microorganism.

1. Subject and source

Sponge *Craniella australiensis* and *Dysidea avara* were collected by SCUBA diving at depth of about 20 m off Sanya Island in the South China Sea in Nov. 2002 and identified by Professor Jinhe Li at Institute of Oceanology, Chinese Academy of Sciences. *Streptomyces* sp. DA18 (GenBank No. DQ180133) was isolated from *C. australiensis* (Li and Liu, 2006). *Bacillus vallismortis* C89 was isolated from *D. avara* and identified as *B. vallismortis* by 16S rDNA sequencing (GenBank No. DQ091007) (Li et al., 2007a).

2. Previous work

It is well-known that marine microbes are an excellent resource for the discovery of potential new drugs (Blunt et al., 2009). Marine sponges harbor various microbial symbiosis (Taylor et al., 2007; Lee et al., 2001), which are perhaps the true producers of some natural products isolated from sponges (Piel, 2009). To our knowledge, the reported metabolites from sponge-associated actinomycetes and bacteria are relatively rare, compared to those from sponge-associated fungi (Liu et al., 2005; Lee et al., 1998; Saleem et al., 2007). In particular, there is no report about metabolites of South China Sea sponge-associated actinomycetes.

3. Present study

Streptomyces sp. DA18 was cultured on solid-plates using M1 medium (Mincer et al., 2002). *B. vallismortis* C89 was incubated on solid-plates using a medium containing 5 g of beef extract, 10 g of peptone, 20 g of agar in every 1000 ml of

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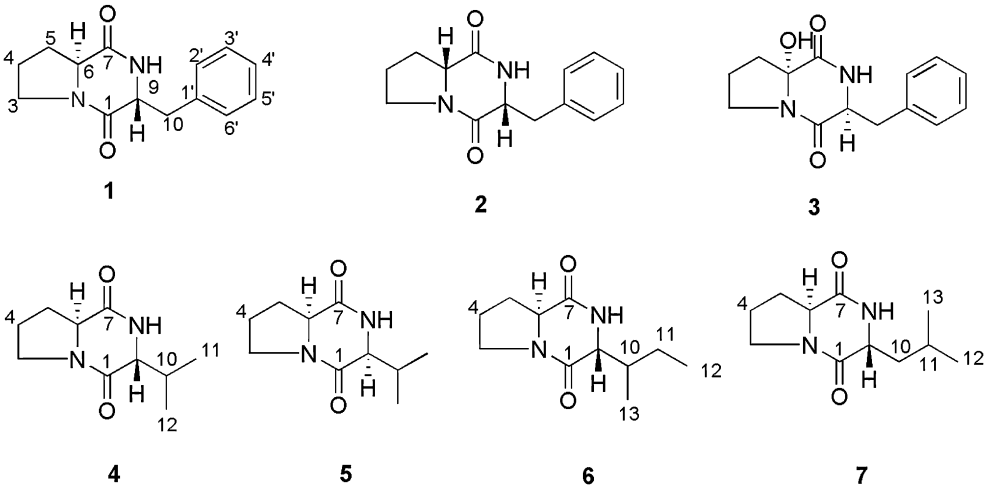

Fig. 1. Structures of compounds 1–7.

artificial seawater with pH 7.0–7.2. After fermentation for 5 days at 28 °C, the whole culture medium was extracted four times with EtOAc, respectively. After being evaporated *in vacuo*, 6.3 g of *Streptomyces* sp. DA18 and 6.4 g of *B. vallismortis* C89 extracts were obtained. The extracts were then subjected to silica gel column chromatography, and eluted with stepwise gradient of $CHCl_3$:MeOH (95:5, 90:10, 80:20, 0:100) to yield fifteen fractions (*A*—*O*) of *Streptomyces* sp. DA18 and five fractions (*Fr.1-5*) of *B. vallismortis* C89. The fraction *I* (998.4 mg) from *Streptomyces* sp. DA18 was rechromatographed over Sephadex LH-20 using MeOH as eluent to give four subfractions. The subfraction *I-3* (154.4 mg) was then further purified on a silica gel column, $CHCl_3$-MeOH (9:1 v/v) as eluent, and followed by reversed-phase preparative HPLC with MeOH-H_2O (35:65, v/v) as the mobile phase at 2.0 ml/min. *Fr. 2* (595.0 mg) from *B. vallismortis* C89 was further chromatographed over Sephadex LH-20 columns with $CHCl_3$-MeOH (1:1). Then the subfraction (*Fr. 2-1*) was further purified on semi-prep. HPLC was at a flow rate of 2.0 ml/min with MeOH-H_2O (40:60). As a result, seven pure compounds, dikeropiperazines (DKPs) **1–7** (Fig. 1), were obtained. Compounds **2–4** were from *Streptomyces* sp. DA18, whereas compounds **5–7** were from *B. vallismortis* C89. Compound **1** was isolated from both of the microbes. Compounds **1–7** were identified by interpretation of their spectral data, and comparison with those reported in the literature.

Compound **1** (7.0 mg), a colorless amorphous solid, $[\alpha]_D^{20}$-10 (*c* 0.02, EtOH), showed molecular formula $C_{14}H_{16}N_2O_2$ requiring eight double bond equivalent according to pseudo-molecular ion peak $[M+H]^+$ *m/z* 245.0 in ESI-MS combined with ^1H, ^{13}C (DEPT) NMR data. The identical ^1H NMR data reported in literature (Lin et al., 2008) suggested its planar structure was *cyclo*-(Pro–Phe). Its optical rotation was in agreement with the reported value (Xie et al., 2008), The structure of compound **1** was identified as *cyclo*-(L-Pro-D-Phe).

Compound **2** (2.5 mg) was a colorless amorphous solid with optical rotation $[\alpha]_D^{20}$ + 143 (*c* 0.023, EtOH). It was identified as *cyclo*-(D-Pro-D-Phe) from its opposite optical rotation and ^{13}C NMR spectral data with reported values (Adamczeski et al., 1995).

Compound **3** (2.1 mg), colorless solid with optical rotation $[\alpha]_D^{20}$-13 (c 0.04, MeOH), showed similar ^1H NMR and ^{13}C NMR spectra as compound **1** but lacking the H-6 proton resonance. The ^{13}C NMR chemical shift value for C-6 in **3** (δ_C 88.7) suggested the presence of a hydroxyl group attached to this position. EI-MS supported the molecular formula $C_{14}H_{16}N_2O_3$ (*m/z* 260). Therefore we confirm its planar structure as *cyclo*-(6-Hyp-Phe). The absolute configurations of C-6 and C-9 were determined by comparing its optical rotation with values from the literature (Park et al., 2006). Finally the structure of **3** was identified as *cyclo*-(D-6-Hyp-L-Phe). To the best of our knowledge this is the second report of isolation of *cyclo*-(D-6-Hyp-L-Phe).

Compound **4** (2.0 mg), colorless solid with optical rotation $[\alpha]_D^{20}$-93 (*c* 0.04, MeOH), contained the same DKP ring system as compound **1** according to the ^1H NMR data. EI-MS supported the molecular formula $C_{10}H_{16}N_2O_2$ (*m/z* 196). Its structure was identified to be *cyclo*-(L-Pro-D-Val) as its physical and spectral data were in accordance with the reported values (Adamczeski et al., 1995).

The spectral data of compound **5** (4.4 mg) are consistent with **4** except for higher optical rotation $[\alpha]_D^{20}$-131 (*c* 0.02, MeOH) due to the substitution of D-Val for L-Val (Siemion, 1971). Thus, compound **5** was identified to be *cyclo*-(L-Pro-L-Val).

The structures of compounds **6** [5.8 mg, $[\alpha]_D^{20}$-111 (*c* 0.04, MeOH)] and **7** [1.5 mg, $[\alpha]_D^{20}$-38 (*c* 0.02, MeOH)] were identified as *cyclo*-(L-Pro-D-Ile) (**6**) and *cyclo*-(L-Pro-D-Leu) (**7**), respectively, by comparison of their spectroscopic data with those reported in the literature (Siemion, 1971; Xie et al., 2008).

4. Chemotaxonomic and ecological significance

Diketopiperazines (DKPs), the smallest cyclic peptides, represent an important class of biologically active natural products (Fischer, 2003; Li et al., 2007b). Compounds **1-3** from *Streptomyces* sp. DA18 were isolated from actinomycetes for the first

time. Compound **3** has been previously obtained only from a marine-derived fungus *Chromoleista* sp. (Park et al., 2006). Furthermore, it was the first time that compounds **1**, **5**, **6** and **7** were isolated from the *D. avara*-associated microorganism as well as *B. vallismortis*.

The synthetic methods used for the preparation of DKPs are now being exploited in combinatorial chemistry strategies (Fischer, 2003). Besides the synthesis of DKPs, many DKPs have been isolated from natural sources (Martins and Carvalho, 2007), for instance, proline-containing DKPs were isolated from sponges (Adamczeski et al., 1995; Fu et al., 1997, 1998), marine microorganisms (Adamczeski et al., 1995; De Rosa et al., 2003; Fdhila et al., 2003; Ovenden et al., 2004; Li et al., 2006; Li et al., 2008b; Xie et al., 2008) and marine actinomycetes (Li et al., 2007b). *Cyclo*-(L-Pro-L-Phe), the isomer of compound **2**, and the derivative of compound **4**, were isolated from bacterium *Pseudomonas aeruginosa* associated with sponge *Ipoinoea setifera* (Jayatilake et al., 1996). Particularly, compounds **2**, **4** and **7** were also found in the sponge *Calyx* cf. *podatypa* (Adamczeski et al., 1995), and one similar compound, compound **3**, was isolated from the sponge *Jarpis digonoxea* (Rudi et al., 1994), which suggested the microbial origin of DKPs found in sponges. Compound **5**, *cyclo*-(L-Pro-L-Val), was also isolated from a marine sponge-associated bacterium *Psychrobacter* sp. (Li et al., 2008a).

DKPs play an important ecological role in antifouling (Wang et al., 1999; Li et al., 2006), antifungi (Musetti et al., 2007) and antibacterial (Fdhila et al., 2003). Compound **2** from *Streptomyces* sp. DA18 was previously found in marine bacteria associated with *Pecten maximus* and proved to exhibit bioactivity against *Vibrio anguillarum* (Fdhila et al., 2003). It was also obtained in a South China Sea sponge *Acanthella cavernosa*-associated fungus and proved to have antifouling activity (Yang et al., 2007). *Cyclo*-(L-Pro-D-Val) (**5**) and *cyclo*-(L-Pro-D-Leu) (**7**) inhibit the production of aflatoxin by *Aspergillus parasiticus* (Yan et al., 2004). The isomer of compound **1** was found to have antifungal activity (Wang et al., 1999). *Streptomyces* sp. DA18, from which compounds **1** and **2** were isolated, showed moderate antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Candida albican* (Li and Liu, 2006). The bacterium *B. vallismortis* C89 producing compound **1** showed significant activity against *Aspergillus niger* and *Paecilomyces* variotii. The above results suggest that *Streptomyces* sp. DA18 and *B. vallismortis* C89 might provide antimicrobial defense for their, respective, host sponges. Similarly, *cyclo*-(L-Pro-L-Phe) and *cyclo*-(L-Pro-L-Leu) isolated from a South China Sea sponge *Stelletta tenuis* and the associated bacterium *Alcaligenes faecalis* A72, showed moderate inhibitory activity against *Staphylococcus aureus* (Li et al., 2008b). Considering the structural similarity between some cyclodipeptides and endogenous signaling peptides, such as thyrotropine-releasing hormone, oxytocine and melanocyte-stimulating hormone release inhibiting factor, an interaction of DKPs with receptors of sponge cells was also suggested (De Rosa et al., 2003). Li et al. (2006) have proved the antifouling activity of DKPs. Further studies need to be done for understanding the role of DKPs play in the relationships between sponge and their associated microorganisms.

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References

Adamczeski, M., Reed, A.R., Crews, P., 1995. J. Nat. Prod. 58, 201.
Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T., Princep, M.R., 2009. Nat. Prod. Rep. 26, 170.
De Rosa, S., Mitova, M., Tommonaro, G., 2003. Biomol. Eng. 20, 311.
Fischer, P.M., 2003. J. Pept. Sci. 9, 9.
Fu, X., Ferreira, M.L.G., Schmitz, F.J., Kelly-Borges, M.K., 1998. J. Nat. Prod. 61, 1226.
Fu, X., Zeng, L., Su, J., Pais, M., 1997. J. Nat. Prod. 60, 695.
Fdhila, F., Vazquez, V., Sanchez, J.L., Riguera, R., 2003. J. Nat. Prod. 66, 1299.
Jayatilake, G.S., Thornton, M.P., Leonard, A.C., Grimwade, J.E., Baker, B.J., 1996. J. Nat. Prod. 59, 293.
Lee, H.K., Lee, D., Lim, J., Kim, J.S., Sik Im, K., Jung, J.H., 1998. Arch. Pharm. Res. 21, 729.
Lee, Y.K., Lee, J., Lee, H.K., 2001. J. Microbiol. 39, 254.
Li, H.Y., Lee, B.C., Kim, T.S., Bae, K.S., Hong, J.K., Choi, S.H., Bao, B.Q., Jung, J.H., 2008a. Biomolecules Ther. 16, 356.
Li, Z.Y., Hu, Y., Huang, Y.Q., Huang, Y., 2007a. Mikrobiologiia 76, 560.
Li, Z.Y., Liu, Y., 2006. Lett. Appl. Microbiol. 43, 410.
Li, Z.Y., Peng, C.S., Shen, Y., 2008b. Biochem. Systematics Ecol. 36, 230.
Li, X., Sergey, D., Ying, X., Xiao, X., Oi, H., Qian, P., 2006. Biofouling 22, 201.
Li, D., Zhu, W., Gu, Q., Cui, C., Zhu, T., Liu, H., Fang, Y., 2007b. Marine Sci. 31 (5), 45.
Lin, Z.J., Lu, X.M., Zhu, T.J., Fang, Y.C., Gu, Q.Q., Zhu, W.M., 2008. Arch. Pharm. Res. 31 (9), 1108.
Liu, R., Cui, C., Duan, L., Gu, Q., Zhu, W., 2005. Arch. Pharm. Res. 28, 1341.
Martins, M.B., Carvalho, I., 2007. Tetrahedron 63, 9923.
Mincer, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W., 2002. Appl. Environ. Microbiol. 68, 5005.
Musetti, R., Polizzotto, R., Vecchione, A., Borselli, S., Zulini, L., Ambrosio, M.D., Sanita di Toppi, L., Pertot, I., 2007. Micron 38, 643.
Ovenden, S.P.B., Sberna, G., Tait, R.M., Wildman, H.G., Patel, R., Li, B., Steffy, K., Nguyen, N., Meurer-Grimes, B.M., 2004. J. Nat. Prod. 67, 2093.
Park, Y.C., Gunasekera, S.P., Lopez, J.V., McCarthy, P., Wright, A.E., 2006. J. Nat. Prod. 69, 580.
Piel, 2009. J. Nat. Prod. Rep. 26, 338.
Rudi, A., Kashman, Y., Enayahu, Y., Chleyer, M., 1994. J. Nat. Prod. 57, 829.
Saleem, M., Ali, S.M., Hussain, S., Jabbar, A., Ashraf, M., Lee, Y.S., 2007. Nat. Prod. Rep. 24, 1142.
Siemion, I.Z., 1971. Org. Magn. Res. 3, 545.

Taylor, M.W., Radax, R., Steger, D., Wagner, M., 2007. Microbiol. Mol. Biol. Rev. 71, 295.
Wang, Y., Mueller, U.G., Clardy, J., 1999. J. Chem. Ecol. 25, 935.
Xie, H.H., Dan, Y., Wei, X.Y., 2008. Chin. J. Nat. Med. 6, 395.
Yang, L.H., Miao, Y.L., Lee, O.O., Li, X., 2007. Appl. Microbiol. Biotechnol. 74, 1221.
Yan, P.S., Song, Y., Sakuno, E., Nakajima, H., Nakagawa, H., Yabe, K., 2004. Appl. Environ. Microbiol. 70, 7477.