

Dysamide U, a new trichlorinated diketopiperazine from the sponge *Dysidea* sp.

FU, Xiong(符雄) SU, Jing-Yu(苏镜娱) ZENG, Long-Mei*(曾陇梅)

Department of Chemistry, Zhongshan University, Guangzhou, Guangdong 510275, China

Dysamide U, a new trichlorinated diketopiperazine (1) along with seven known ones (2–8) were isolated from the sponge of *Dysidea* sp. collected from the Pacific ocean. The structure of dysamide U (1) was elucidated by high resolution FABMS and ^1H NMR data including COSY and RCT-COSY.

Keywords Sponges, *Dysidea*, dipeptide, diketopiperazine

Introduction

Sponges of the genus *Dysidea* (Order *Dendroceratida*, Family *Dysideidae*) have been sources of a variety of organic compounds,¹ such as terpenoids,² steroids,³ polybrominated diphenyl ethers,⁴ and polychlorinated peptides.^{5–10} It has been confirmed that the terpenoids and steroids isolated from *Dysidea* sponges are their true metabolites, but the polyhalogenated metabolites are produced by cyanobacterial symbionts.^{11–13}

In our earlier papers, we reported sesquiterpene,¹⁴ sterol,¹⁵ and chlorinated diketopiperazine^{8,9} from the sponge *D. fragilis* collected from the South China Sea. In continuation of our studies on *Dysidea* species, we have obtained specimens which possibly contained cyanobacterial symbionts because of the greenish interior.⁴ The study led to the isolation of dysamide U (1), a new trichlorodiketopiperazine, and seven known ones 2–8. In this paper, we describe the isolation of these compounds and the structure elucidation of dysamide U.

Results and discussion

The specimens were extracted twice with MeOH and

then twice with MeOH-CH₂Cl₂ (1:1), and the combined extracts, after removal of solvents, were subjected to solvent partitioning¹⁶ to give hexane, CH₂Cl₂ and *n*-BuOH soluble fractions. The CH₂Cl₂ solubles were chromatographed over a silica gel open column. Selected fractions from this chromatography were rechromatographed on reversed phase HPLC to yield dysamides 1–8.

All the compounds we report here are polychlorinated, a feature readily recognized by diagnostic clusters of isotope peaks in their mass spectra.¹⁷ All the compounds have one double bond, whose stereochemistry could be distinguished by the chemical shift values of 3'-H and 4'-H. In the *Z* isomers 7 and 8, 3'-H (δ ca. 6.1) was observed farther downfield than in the *E* isomers 1–6 (δ ca. 5.6), due to deshielding by the carbonyl group. Conversely, in the *Z* isomers 4'-H resonated farther upfield ($\sim \delta$ 3.65) than in the *E* isomer ($\sim \delta$ 5.20). Compound 5 has a hydrogen at 7-N in places of methyl groups in all other compounds, thus the methyl singlet for the methyl at 7-N disappeared, and instead of observing an exchangeable broad singlet at δ 6.6 in the ^1H NMR spectrum of 5. In addition, a doublet of triplets at δ 4.1 for 2-H in 5 was observed instead of a doublet of doublets for 2-H in 2. Unlike other compounds, 6 has a hydroxyl group attached to 2-C, therefore no signal around δ 4 was observed in the ^1H NMR spectrum of 6. However, there was a singlet at δ 4.5 corresponding to the proton of a hydroxyl group. The ultimate identification of all known compounds was based on comparison of their MS and ^1H NMR data with those reported in the

* E-mail: ceszlm@zsu.edu.cn

Received March 7, 2000; accepted June 12, 2000.

Project (No. 29932030 and 29772058) supported by the National Natural Science Foundation of China.

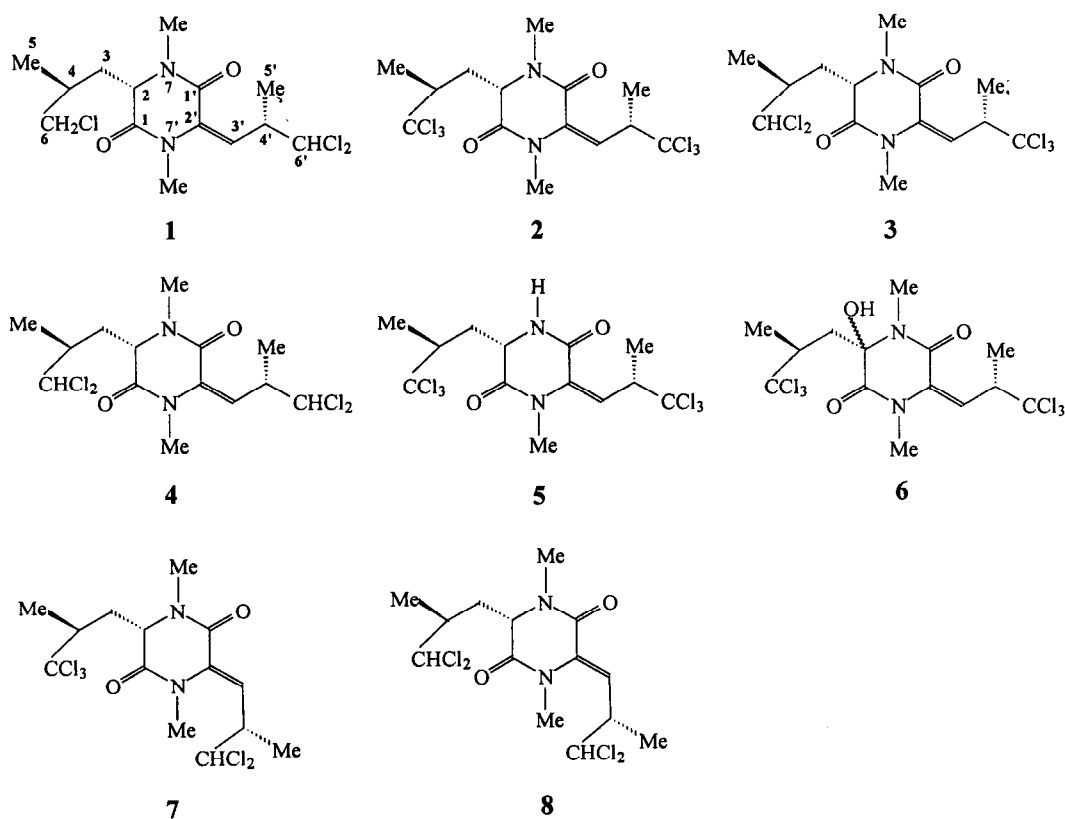
literatures.^{7,10}

Dysamides **1**, **2**, **3** and **6** showed antibiotic activities toward *Staphylococcus*, *Bacillus* and *Escherichia* at a concentration 1.0 $\mu\text{g}/\text{dish}$.

Dysamide U (**1**) was obtained as a trace component. Its molecular formula $\text{C}_{14}\text{H}_{21}\text{Cl}_3\text{N}_2\text{O}_2$ determined by HRFABMS (m/z 355.0745; calcd for $\text{C}_{14}\text{H}_{22}\text{Cl}_3\text{N}_2\text{O}_2$ 355.0747) contained one less chlorine than that of 6, 6'-didechloro-2, 3-dihydrodysamide C (**4**).⁷ The ^1H NMR data of **1** were similar to those of compound **4** except that the doublet at δ 6.03 ($J = 3$ Hz) in the ^1H NMR spectrum of **4** was missing in the data for **1**.

Instead, two geminal proton signals were observed at δ 3.82(dd, $J = 10.8, 4.9$ Hz, 6-H) and 3.49(dd, $J = 10.8, 3.9$ Hz, 6'-H). Two spin systems (3'-H to 6'-H and 2-H to 6-H) could be delineated from the COSY and RCT (relay coherence transfer)-COSY¹⁸ spectra of **1**. Therefore, structure **1** was assigned to dysamide U. The *E*-configuration of the carbon-carbon double bond was assigned on the basis of the chemical shifts of 3'-H (δ 5.40) and 4'-H (δ 4.12).

Dysamide U (**1**) is the first example of trichlorinated compounds in the diketopiperazine family.



Experimental

General experimental procedures

All solvents were redistilled. Merck Si gel 60 (230–240 mesh) was used for vacuum flash chromatography. HPLC was conducted using a UV detector and a Spherex 5 C_{18} column. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3 mm $^1\text{H}/^{13}\text{C}$ switchable gradient microprobe (MDG-500-3) and a pulsed field gradient driver; sig-

nals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB-E mass spectrometer, and optical rotations on a Rudolph Autopol III Automatic Polarimeter.

Animal material

The sponge *Dysidea sp.* was collected in August, 1995, at Yap, Federated States of Micronesia, and frozen shortly after collection. The sponge was identified by Dr Michelle Kelly, National Institute of Water and Atmospheric Research, Private Bag 109-695, Newmar-

ket, Auckland, New Zealand.

Extraction and isolation

Freshly thawed specimens of the sponge (2.8 kg. wet wt.; 350 g dry wt. after extraction) were cut in small pieces and soaked in MeOH (2 × 3 L) followed by MeOH-CH₂Cl₂ (1:1) (2 × 3 L). The extracts were concentrated under vacuum and combined to give a residue which was dissolved in 700 mL of 10% aqueous MeOH. The slurry solution was partitioned against hexane (2 × 700 mL) to afford hexane-solubles (6.83 g). The resulting aqueous solution was diluted to 30% water in MeOH and partitioned against CH₂Cl₂ (2 × 910 mL). This gave CH₂Cl₂ (4.17 g) soluble fraction and aqueous MeOH which was concentrated *in vacuo* and extracted with *n*-BuOH (2 × 300 mL) to give 1.98 g of *n*-BuOH-solubles. This partitioning process effectively extracted the nonpolar fatty acids and their esters in the hexane fraction, and the polar nucleosides in the *n*-BuOH fraction. The CH₂Cl₂ solubles were fractionated by flash chromatography over silica gel using increasing amounts of EtOAc in hexane as eluent (10% EtOAc-hexane to EtOAc). Nine fractions were collected. The third, fourth and sixth fractions were each rechromatographed over reversed phase C₁₈ HPLC using 30% H₂O-CH₃CN as eluent. Fraction three yielded dysamides 2–4, fraction four afforded compounds 2, and 4–6, and fraction six yielded compounds 7 and 8.

Dysamide U (1) 0.6 mg. $[\alpha]_D - 23^\circ$ (*c* 0.05, EtOH). δ_H (CDCl₃): 6.18(d, *J* = 3.0 Hz, 6'-H), 5.40(d, *J* = 8.8 Hz, 3'-H), 4.08–4.16(m, 4'-H), 3.92(dd, *J* = 3.9, 9.8 Hz, 2-H), 3.82(dd, *J* = 10.8, 4.9 Hz, 6-H), 3.49(dd, *J* = 10.8, 3.9 Hz, 6-H), 3.18(s, NMe), 3.04(s, NMe), 1.96–2.08(m, 3-H and 4-H), 1.45–1.55(m, 3-H), 1.29(d, *J* = 6.5 Hz, 5'-H), 1.05(d, *J* = 6.5 Hz, 5-H). HRFABMS *m/z* 355.0745 (calcd for C₁₄H₂₂Cl₃N₂O₂ 355.0747).

2,3-Dihydrodysamide C (2) 10.8 mg. $[\alpha]_D - 150^\circ$ (*c* 1.00, EtOH), ¹H NMR, and MS data identical with literature data.^{8,10}

Dysamide K (3) 1.2 mg. $[\alpha]_D - 70.3^\circ$ (*c* 0.10, EtOH). ¹H NMR, and MS data identical with literature data.¹⁰

6,6'-Didechloro-2,3-dihydrodysamide C (4)

0.9 mg. ¹H NMR, and MS data identical with literature data.⁷

7-*N*-Demethyl-2,3-dihydrodysamide C (5)

0.7 mg. ¹H NMR, and MS data identical with literature data.⁷

Dysamide Q (6) 1.3 mg. ¹H NMR, and MS data identical with literature data.¹⁰

Dysamide I (7) 0.8 mg. ¹H NMR, and MS data identical with literature data.¹⁰

Dysamide M (8) 0.6 mg. ¹H NMR, and MS data identical with literature data.¹⁰

Acknowledgement

We gratefully acknowledge Dr Michelle Kelly for sponge identification. We thank Professor Francis J. Schmitz for providing us with the specimens for this work.

References and notes

- Faulkner, D. J., *Nat. Prod. Reports*, **15**, 113 (1998) and previous reviews in this series.
- Recent examples see:
 - Gunasekera, S. P.; McCarthy, P. J.; Kelly-Borges, M.; Lobkovsky, E.; Clardy, J., *J. Am. Chem. Soc.*, **118**, 8759 (1996).
 - Patil, A. D.; Freyer, A. J.; Killmer, L.; Offen, P.; Carte, B.; Jurewicz, A. J.; Johnson, R. K., *Tetrahedron*, **53**, 5047 (1997).
- Recent examples see:
 - Casapullo, A.; Minale, L.; Zollo, F.; Roussakis, C.; Verbist, J., *Tetrahedron Lett.*, **36**, 2669 (1995).
- Recent examples see:
 - Fu, X.; Schmitz, F. J.; Govindan, M.; Abbas, S. A.; Hanson, K. M.; Horton, P. A.; Crews, P.; Laney, M.; Schatzman, R. C., *J. Nat. Prod.*, **58**, 1384 (1995).
 - Fu, X.; Schmitz, F. J., *J. Nat. Prod.*, **59**, 1102 (1996).
- Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J., *J. Org. Chem.*, **58**, 6336 (1993).
- Kazlauskas, R.; Murphy, P. T.; Walls, R. J., *Tetrahedron Lett.*, 4945 (1978).
- Dumdei, E. J.; Simpson, J. S.; Garson, M. J.; Byriel, K. A.; Kennard, C. H. L., *Aust. J. Chem.*, **50**, 139 (1997).
- Su, J. Y.; Zhong, Y. L.; Zeng, L. M.; Wei, X.; Wang, Q. W.; Mak, T. C. W.; Zhou, Z. Y., *J. Nat.*

- Prod.*, **56**, 637(1993).
9. Fu, X.; Zeng, L. M.; Su, J. Y.; Pais, M., *J. Nat. Prod.*, **60**, 695(1997).
 10. Fu, X.; Ferreira, M. L. G.; Schmitz, F. J.; Kelly, M., *J. Nat. Prod.*, **61**, 1226(1998).
 11. Faulkner, D. J.; He, H.; Unson, M. D.; Bewley, C. A.; Garson M. J., *Gazz. Chim. Ital.*, **123**, 301(1993).
 12. Unson, M. D.; Faulkner, D. J., *Experientia*, **49**, 349 (1993).
 13. Unson, M. D.; Holland, N. D.; Faulkner, D., *J. Mar. Biol.*, **119**, 1(1994).
 14. Su., J. Y.; Zeng, L. M.; Zhong, Y. L.; Fu, X., *J. Chin. Chem. Soc.*, **42**, 735(1995).
 15. Zhong, Y. L.; Su, J. Y.; Zeng, L. M., *Chin. J. Chem.*, **11**, 560(1993).
 16. Fu, X.; Schmitz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L., *J. Nat. Prod.*, **57** 1591(1994).
 17. Hesse, M.; Meier, H.; Zeeh, B., *Spektroskopische Methoden in der Organischen Chemie*, Georg Thieme Verlag: Stuttgart, New York, 1984, p.382.
 18. RCT-COSY shows geminal, vicinal and longer range couplings simultaneously depending on mixing time used. Crews, P.; Rodriguez, J.; Jaspars, M., *Organic Structure Analysis*, Oxford University Press, New York, 1998, p.185.

(E200003048 SONG, J. P.; DONG, L. J.)