

A New Diketopiperazine Derivative from the South China Sea Sponge *Dysidea fragilis*

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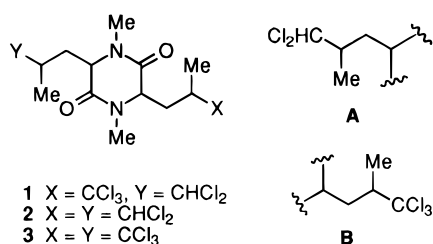
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A new diketopiperazine, dysamide D (**1**), has been isolated from the marine sponge *Dysidea fragilis*, collected in the South China Sea. The structure of dysamide D was determined by spectral data.

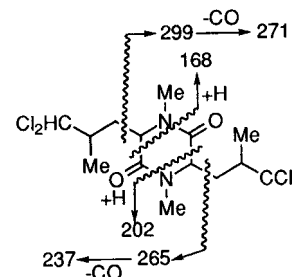
Sponges of the genus *Dysidea* (order Dictyoceratida, family Dysideidae) have been the subject of intense chemical study,^{1,2} and its members have been reported previously as a source of terpenoids,^{2,3} steroids,^{2,4,5} polybrominated diphenyl ethers,^{6,7} and peptide⁸ metabolites, including polychlorinated compounds.⁹ It is generally accepted that the true metabolites of *Dysidea* are terpenoids and steroids; however, the polybrominated diphenyl ethers and polychlorinated peptides have been frequently suggested to be produced by epiphytes, such as blue-green algae and bacteria, because their structure is similar or identical to metabolites of blue-green algae^{8,10,11} and bacteria.¹² Recent investigations^{11,13,14} have provided further evidence for this suggestion.

The diversity of metabolites of sponges of the genus *Dysidea* has drawn our interest. Our earlier investigation on *Dysidea fragilis* Montagu led to the isolation of a furano sesquiterpenoid, (–)-furodysin,^{15,16} an antibacterial dihydroxysterol,⁵ and dysamides A–C and 2,3-dihydrodysamide C.⁹ Further work on the sponge has resulted in an additional novel diketopiperazine for which we proposed the name dysamide D (**1**). The structure elucidation of this compound is the subject of this paper.



Dysamide D (**1**) was obtained as colorless needles from the MeOH extract of the sponge *D. fragilis*, mp 141–142 °C, [α]_D + 4° (*c* 1.5, CHCl₃). The molecular formula C₁₄H₂₁Cl₅N₂O₂ was established by HREIMS (*m/z* [M]⁺ 425.9991, C₁₄H₂₁³⁵Cl₄³⁷ClN₂O₂ requires 426.0016). The CIMS showed a cluster of peaks around *m/z* 427 (100, [M]⁺) with a pattern characteristic of a pentachlorinated compounds.¹⁷ The presence of a trichloromethyl group and a dichloromethyl group was indicated by the ¹³C-NMR spectrum, which showed a

Scheme 1. MS Fragmentation Pathway for **1**



singlet at δ 105.6 typical of trichloromethyl and a doublet at δ 77.5 typical of dichloromethyl.⁹ In support of the presence of the dichloromethyl group, the ¹H-NMR spectrum revealed a downfield signal at δ 6.20 (d, *J* = 3.2 Hz), which is virtually identical to that of the corresponding proton in related compounds.⁹

All ¹H- and ¹³C-NMR data (see Experimental Section) of dysamide D (**1**) were unambiguously assigned by COSY, TOCSY, HETCOR, and NOESY experiments. The presence of substructures **A** and **B** was suggested by the correlations observed in the COSY and TOCSY spectra. The NMR data of substructures **A** and **B** are in good agreement with those reported⁹ for dysamide B (**2**) and dysamide A (**3**), respectively. Two –CONMe residues were evident from ¹³C resonances at δ 165.9 (s, 2 × C), 33.7 (q), and 33.1 (q), together with proton signals at δ 3.01 (3H, s) and 3.05 (3H, s). The IR band at 1669 cm⁻¹ was attributed to the amide groups in dysamide D. With the structures of dysamides A–B in hand, and in consideration of all data mentioned-above, structure **1** was assigned to dysamide D, and this was confirmed by EIMS data (Scheme 1).

Polychlorinated compounds containing trichloromethyl or dichloromethyl groups occur rarely in nature. On the basis of asymmetric synthesis,¹⁸ X-ray diffraction analysis,⁹ and chemical correlations,^{19,20} it seems very likely that the absolute configuration of the carbon that bears the trichloromethyl (or dichloromethyl) in the polychlorinated metabolites isolated from *Dysidea* sponges is *S*. We assumed that dysamide D (**1**) has the same conformation as dysamide A.⁹ Thus, four asymmetric centers of dysamide D were presumed to have the same *S* absolute configuration as those of dysamide A, the structure of which was determined by X-ray.⁹

Experimental Section

General Experimental Procedures. All solvents used were of analytical grade. Si gel (60–100 mesh)

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and TLC grade Si gel-H were used for chromatography. The melting point was determined with an X4 micro-melting point apparatus. NMR spectra were recorded on Bruker AC 250 and AC 400 NMR spectrometers, the IR spectrum on a Nicolet 205 FT-IR spectrophotometer, and mass spectra on Kratos MS 80 and Finigan MAT 90 instruments.

Animal Material. The sponge *D. fragilis* was collected in the waters off Lingshui County, Hainan Province, People's Republic of China. A voucher specimen (# 91-08) was deposited in the Research Center for Organic Natural Products Chemistry, Zhongshan University, Guangzhou, China.

Isolation of Dysamide D. The specimens (700 g dry wt after extraction) were extracted repeatedly with MeOH at room temperature. The MeOH extracts were concentrated under reduced pressure to afford a residue that was partitioned between EtOAc and H₂O. The organic fraction was separated, and the solvent was removed *in vacuo* to obtain an oil that was subjected to flash chromatography on Si gel, eluted with increasing amounts of MeOH in CH₂Cl₂. The third fraction (1–2% MeOH in CH₂Cl₂), which contained **1**, was purified by flash chromatography on Si gel using 5% Me₂CO in CH₂Cl₂ as eluent to yield dysamide D.

Dysamide D(1): fine needles (19 mg) from ethyl ether, mp 141–142 °C; [α]_D + 4° (*c* 1.5, CHCl₃); IR(CHCl₃) ν_{\max} 1669 cm⁻¹; ¹H NMR (CDCl₃) δ 6.20 (1H, d, *J* = 3.2 Hz, H-6'), 3.97 (1H, dd, *J* = 8, 7 Hz, H-2), 3.90 (1H, dd, *J* = 10, 6 Hz, H-2'), 3.05 (3H, s, NMe), 3.01 (3H, s, NMe), 3.00 (1H, m, H-4), 2.58 (1H, m, H-4'), 2.52 (1H, m, H-3), 2.20 (1H, m, H-3'), 1.83 (1H, m, H-3), 1.70 (1H, m, H-3'), 1.42 (3H, d, *J* = 6.5 Hz, H-5), 1.20 (3H, d, *J* = 6.5 Hz, H-5'); ¹³C NMR (CDCl₃) δ 15.8 (q, C-5'), 18.1 (q, C-5), 33.1 (q, NMe), 33.7 (q, NMe), 37.7 (t, C-3'), 39.2 (t, C-3), 40.4 (d, C-4'), 52.3 (d, C-4), 60.4 (d, C-2'), 61.8 (d, C-2), 77.5 (d, C-6'), 105.6 (s, C-6), 165.9 (2C, s, C-1 and C-1'); HREIMS *m/z* [M]⁺ 425.9991, calcd for C₁₄H₂₁³⁵Cl₄³⁷ClN₂O₂, 426.0016; CIMS *m/z* (rel int) 425 (52), 427 (100), 429 (65), 431 (20), 433 (3); EIMS *m/z* (rel int) 424 (7), 426 (10), 428 (7), 430 (2), 389 (50), 391 (66), 393 (34), 395 (8), 353 (40), 355 (43), 357 (20), 299 (63), 301 (63), 303 (23), 271 (40), 273 (40), 275 (10),

265 (100), 267 (66), 269 (13), 237 (83), 239 (57), 241 (10), 202 (21), 204 (20), 206 (7), 168 (32), 170 (21), 172 (4).

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References and Notes

- (1) Blunt, J. W.; Munro, M. H. B. A. *MarinLit Database on Marine Natural Products*, 1996; Department of Chemistry, University of Canterbury, Christchurch, New Zealand; 192 records found containing the genus *Dysidea*.
- (2) Faulkner, D. J. *Nat. Prod. Rep.* **1996**, *13*, 75–125 and previous reviews in this series.
- (3) For a recent example, see: Searle, P. A.; Jamal, N. M.; Lee, G. M.; Molinski, T. F. *Tetrahedron* **1994**, *50*, 3879–3888.
- (4) For a recent example, see: Casapullo, A.; Minale, L.; Zollo, F.; Roussakis, C.; Verbist, J. *Tetrahedron Lett.* **1995**, *36*, 2669–2672.
- (5) Zhong, Y. L.; Su, J. Y.; Zeng, L. M. *Chin. J. Chem.* **1993**, *11*, 560–564.
- (6) 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.* **1995**, *58*, 1384–1391, and the references cited therein.
- (7) Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1996**, *59*, 1102–1103.
- (8) Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 7969–7972.
- (9) Su, J. Y.; Zhong, Y. L.; Zeng, L. M.; Wei, S.; Wang, Q. W.; Mak, T. C. W.; Zhou, Z. Y. *J. Nat. Prod.* **1993**, *56*, 637–642, and the references cited therein.
- (10) Schwartz, R. E.; Hiesch, C. F.; Sesin, D. F.; Flor, J. E.; Chartrain, M.; Fromtling, R. E.; Harris, G. H.; Salvatore, M. J.; Liesch, J. M.; Yudin, K. *J. Indust. Microbiol.* **1990**, *5*, 113.
- (11) Faulkner, D. J.; He, H.; Unson, M. D.; Bewley, C. A.; Garson, M. *J. Gazz. Chim. Ital.* **1993**, *123*, 301–307.
- (12) Elyakov, G. B.; Kuznetsova, T.; Mikahilov, V. V.; Maltsev, I. I.; Voinov, V. G.; Fedoreyev, S. A. *Experientia* **1991**, *47*, 632–633.
- (13) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349–353.
- (14) Unson, M. D.; Holland, N. D.; Faulkner, D. J. *Mar. Biol.* **1994**, *119*, 1–11.
- (15) Guella, G.; Mancini, L.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta.* **1985**, *68*, 1276–1282.
- (16) Horton, P.; Inman, W. D.; Crews, P. *J. Nat. Prod.* **1990**, *53*, 143–151.
- (17) Hesse, M.; Meier, H.; Zeeh, B. *Spektroskopische Methoden in der Organischen Chemie*, Georg Thieme: Stuttgart, 1984; p 382.
- (18) de Laszlo, S. E.; Williard, P. E. *J. Am. Chem. Soc.* **1985**, *107*, 199–203.
- (19) Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302–2304.
- (20) Biskupiak, J. E.; Ireland, C. M. *Tetrahedron Lett.* **1984**, 2935–2936.

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