Three New Diketopiperazines from a Marine Sponge Dysidea fragilis

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THREE NEW DIKETOPIPERAZINES FROM A MARINE SPONGE *DYSIDEA FRAGILIS*

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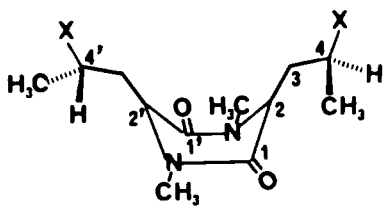
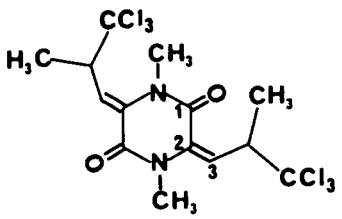
ABSTRACT.—Three new polychlorinated diketopiperazines, dysamides A [**1**], B [**2**], and C [**3**], and the known compound **4** have been isolated from a marine sponge *Dysidea fragilis* collected off Hainan Island. Their structures were established by spectroscopic methods, and complete nmr assignments were made. The absolute configuration and conformation of **1** were determined by X-ray crystallographic diffraction analysis.

Marine sponges of the genus *Dysidea* have proved to contain many interesting polychlorinated metabolites (1–7). Among them, a diketopiperazine derived from a polychlorinated amino acid has been reported (5). From the marine sponge *Dysidea fragilis* Montagu (family Dysideidae) collected off Hainan Island, the South China Sea, three new polychlorinated diketopiperazines, named dysamides A [**1**], B [**2**], and C [**3**], along with the known compound 2,3-dihydrodysamide C, were isolated. All are derivatives of *N*-methyl trichloroleucine. In this paper we report the isolation, structure elucidation, and absolute configuration determination of these compounds.



1 X=CCl_3
2 X=$CHCl_2$



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RESULTS AND DISCUSSION

Dysamide A [**1**], which was obtained as colorless prisms, mp 118–119° [from Me_2CO-petroleum ether (1:1)], $[\alpha]_D$ −36.6° (c=0.265, MeOH), had a molecular formula of $C_{14}H_{20}Cl_6N_2O_2$, which was established by hreims. The ^{13}C-nmr spectra showed only seven resonances instead of fourteen, indicating that the molecule is highly symmetric. The multiplicities of each carbon atom were determined by DEPT experiments, which revealed the presence of two methyl, one methylene, two methine, and two quaternary carbons. The ^{13}C-nmr resonance at δ 105.35(s) together with a strong ir absorption at 790 cm^{-1} revealed the presence of trichloromethyl groups (3,8). The presence of two CONMe groups was suggested by a very strong ir band at 1663 cm^{-1} and a ^{13}C signal at δ 160.00(s), together with a methyl signal at δ 33.59 (q) and δ_H 3.07 (2 × 3H, s). Interpretation of the ^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments led to a

partial structure: CH-CH-CHMe-CCl_3. This molecule is highly symmetric but shows optical activity; thus it should have C_2 symmetry. Thus, structure **1**, containing a diketopiperazine ring with the two substituents occupying the 1,4 positions, was proposed for dysamide A. It was further confirmed by the ms data, including a base peak at *m/z* 301 due to the β cleavage of the amide function.

The absolute configuration of **1** was determined by X-ray crystallographic analysis. As expected, the central molecular skeleton consisted of two planar fragments folded about a line passing through C-1 and C-7, making a dihedral angle of 28.9°. Consequently, the six-membered ring took a boat conformation. Interestingly, both 3,3,3-trichloro-2-methylpropyl substituents occupied flagpole positions; however, their dispositions with respect to the central ring differed considerably, as shown by the torsion angles N-1–C-1–C-3–C-4 = 102.6(5)° and N-2–C-7–C-9–C-10 = −179.8(5)°. The absolute configuration of **1** was established as 2*S*, 4*S*, 2′*S*, 4′*S*.

Dysamide B [**2**], needles, mp 147–149° [from Me_2CO-petroleum ether (1:1)], $[\alpha]_D$ +13.7° (*c* = 0.117, MeOH), has a molecular formula of $C_{14}H_{22}C_{14}N_2O_2$ as determined by hreims. The ^{13}C-nmr spectrum also revealed only seven signals. In the ^{13}C-nmr spectrum, the singlet at δ 105.35 (s) in **1** was replaced by a doublet at δ 77.29 (d), accounting for the presence of $CHCl_2$ groups instead of CCl_3 groups (3). The structure **2** for dysamide B was confirmed by eims fragmentation ions at *m/z* 265 $[M - CH_2 - CHMe - CHCl_2]^+$, 237 $[M - CH_2 - CHMe - CHCl_2 - CO]^+$, and 168 $[M\text{-}NMe - CH - CH_2 - CHMe - CHCl_2]^+$.

Dysamide C [**3**], needles, mp 196–197° [from Me_2CO-petroleum ether (1:2)], $[\alpha]_D$ −7.3° (*c* = 0.041, MeOH), had a molecular formula of $C_{14}H_{16}Cl_6N_2O_2$ established by hreims. The ir spectrum showed intense absorptions at 1692, 1637 (amides), and 774 (CCl_a) cm^{-1}. The ^{13}C-nmr spectrum contained fourteen carbon resonances, most of them revealed in pairs. The ^1H and ^{13}C nmr indicated the presence of 2 × CCl_a and 2 × CONMe. In addition, vinyl proton signals at δ 5.622 (1H, d, *J* = 9.7 Hz) and 6.312 (1H, d, *J* = 10.6 Hz), together with ^{13}C resonances at δ 132.3 (s), 133.3 (s), 123.2 (d), and 122.3 (d), indicated the presence of two trisubstituted double bonds in the molecule. The ^1H-^1H COSY spectrum showed cross peaks between H-3 at δ 5.622 (H-3), and H-4 at 4.863, which was further correlated to a doublet at δ 1.484 (5-Me). Similarly, the connectivities δ 6.312 (H-3′)–3.842 (H-4′)–1.471 (H-5′) in the other part of

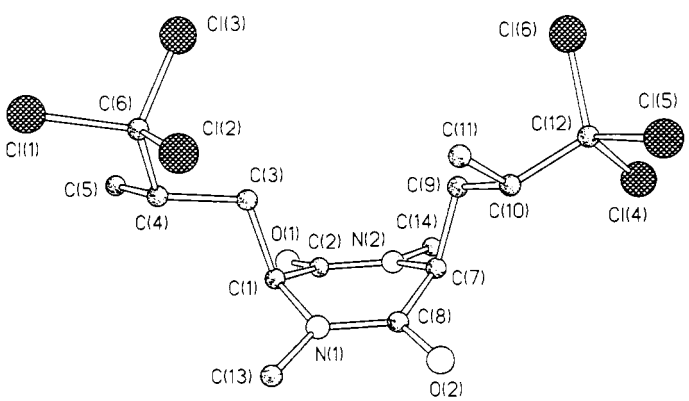

FIGURE 1. A perspective view of the structure of the molecule of dysamide A [**1**] showing its absolute configuration and the atom numbering scheme.

the molecule were found. Thus, the location of the two olefinic bonds was established at C-2 and C-2′. Based on the data shown above and the eims fragment ions at *m/z* 421 $[M-Cl]^+$, 385 $[M-H-2Cl]^+$ (100%), 339 $[M-CCl_3]^+$, and 82 $[CCl_2]^+$ (96%), the structure of dysamide C was determined as **3**. The characteristic fragment ion peak at *m/z* 339 was considered to arise from the β cleavage of the olefinic bonds.

The stereochemistry of the two double bonds could be determined in comparison with the chemical shifts between H-3 and H-3′; H-4 and H-4′. The resonance of H-3′ was lower field than that of H-3; this was due to deshielding by the amide carbonyl group. To the contrary, the H-4 signal resonated in a lower field than that of H-4′ due to the same effect. Apparently, these two olefinic bonds are in the opposite configuration as shown.

The hreims established a molecular formula of $C_{14}H_{18}Cl_6N_2O_2$ for the fourth compound. The ^1H- and ^{13}C-nmr and ir spectral data were consistent with the known 2,3-dihydrodysamide C from the sponge *Dysidea herbacea* (7), except for the melting point (which for our compound was 30° higher than that reported). No data are available concerning the relative and absolute configurations about three chiral centers.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr experiments were performed on a Bruker AMX-600 (600 MHz) spectrometer, using TMS as internal standard. Ms was measured with either a VG ZAB-HF-3F or a MAT-731 mass spectrometer. Ir spectra were recorded on a Nicolet 5DX FT-IR spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Preparative hplc was carried out by using μ-Porasil SiO_2 column with uv detection.

BIOLOGICAL MATERIAL.—The sponge *D. fragilis* was collected off Hainan Island, China. A voucher specimen (No. 91-21) was deposited in the Research Center of Organic National Products, Zhongshan University, Guangzhou, China.

EXTRACTION AND ISOLATION.—Chopped sun-dried specimens (1.5 kg) were extracted three times with EtOH at room temperature. The concentrated extract was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (57 g) was subjected to vacuum liquid chromatography on Si gel H with solvent mixtures of increasing polarity from petroleum ether to EtOAc.

The fraction eluted with 15% EtOAc/petroleum ether contained crude 2,3-dihydrodysamide C, from which 3 g of pure compound (5.26%) was obtained upon crystallization from Me_2CO-petroleum ether (1:1). The mother liquor, after separation of 2,3-dihydrodysamide C, was subjected to a repeat of vlc procedure using Et_2O-petroleum ether-Me_2CO (25:75:1) as eluent to give a white solid, which was further purified by pre-

TABLE 1. ^{13}C-nmr Data for Compounds **1–3** and 2,3-Dihydrodysamide C.

Carbon	Compound			
	1	**2**	**3**	2,3-Dihydrodysamide C
C-1	160.00 (s)	185.78 (s)	159.5 (s)	159.67 (s)
C-1′	160.00 (s)	165.78 (s)	160.0 (s)	165.37 (s)
C-2	61.85 (d)	60.03 (d)	132.3 (s)	131.65 (s)
C-2′	61.65 (d)	60.03 (d)	133.3 (s)	61.87 (d)
C-3	38.95 (t)	37.54 (t)	123.2 (d)	123.68 (d)
C-3′	38.95 (t)	37.54 (t)	122.3 (d)	37.55 (t)
C-4	52.03 (d)	40.13 (d)	52.3 (d)	51.34 (d)
C-4′	52.03 (d)	40.13 (d)	53.4 (d)	51.42 (d)
C-5	17.87 (q)	15.47 (q)	18.2 (q)	18.36 (q)
C-5′	17.87 (q)	15.47 (q)	18.0 (q)	17.51 (q)
C-6	105.35 (s)	77.29 (d)	104.0 (s)	104.05 (s)
C-6′	105.35 (s)	77.29 (d)	102.8 (s)	105.05 (s)
NMe	33.59 (q)	32.89 (q)	31.3 (q)	31.16 (q)
NMe	33.59 (q)	32.89 (q)	34.7 (q)	33.78 (q)

TABLE 2. ^{1}H-nmr Data for Compounds **1–3** and 2,3-Dihydrodysamide C.

Proton	Compound			
	1	**2**	**3**	2,3-Dihydrodysamide C
H-1	—	—	—	—
H-1′	—	—	—	—
H-2	4.00 (dd, 7.5, 6.5)	3.89 (dd, 10.1, 5.3)	—	4.07 (t, 7.0)
H-2′	4.00 (dd, 7.5, 6.5)	3.89 (dd, 10.1, 5.3)	—	—
H_a-3	2.53 (dddd,	2.20 (ddt,	5.62 (d, 9.7)	5.69 (d, 9.5)
H_b-3	1.83 (dt, 14.5, 7.5)	1.69 (ddt, 10.1, 6.3, 5.1)	—	—
H_a-3′	2.53 (dddd, 14.5, 6.5, 2.4, 2.3)	2.20 (ddt, 8.1, 6.3, 5.3)	6.31 (d, 10.6)	2.51 (dd, 14.2, 7.0)
H_b-3′	1.83 (dt, 14.5, 7.5)	1.69 (ddt, 10.1, 6.5, 5.1)	—	1.77 (dt, 124.2, 7.0)
H-4	3.02 (m)	2.58 (m)	4.86 (m)	5.20 (m)
H-4′	3.02 (m)	2.58 (m)	3.84 (m)	2.74 (m)
H-5	1.43 (d, 6.5)	1.22 (d, 6.6)	1.48 (d, 6.6)	1.38 (d, 6.5)
H-5′	1.43 (d, 6.5)	1.22 (d, 6.6)	1.47 (d, 6.6)	1.38 (d, 6.5)
H-6	—	6.20 (d, 2.9)	—	—
H-6′	—	6.20 (d, 2.9)	—	—
NMe	3.07 (s)	3.01 (s)	3.43 (s)	3.11 (s)
NMe	3.07 (s)	3.01 (s)	3.28 (s)	3.25 (s)

TABLE 3. Data Collection and Processing Parameters for Compound **1**.

Molecular formula	$C_{14}H_{20}N_2O_2Cl_6$
Mol wt	461.06
Color and habit	colorless transparent block
Crystal size	$0.22 \times 0.28 \times 0.34$ mm^3
Crystal system	monoclinic
Space group	$P2_1$ (No. 4)
Unit cell parameters	$a = 8.533(2)$ Å, $\beta = 99.91(2)°$; $b = 9.414(2)$, $V = 1028.1(4)$ Å^3; $c = 12.992(2)$, $Z = 2$, $F(000) = 472$
Density (calcd)	1.489 g·cm^{-3}
Radiation	graphite-monochromatized MoKα, λ = 0.71073 Å
Standard reflections	(1,-3,-3); (1,4,3)
Intensity variation	±1.0%
R_{int} (from merging of equiv. reflections)	0.025
Absorption coefficient	8.5 cm^{-1}
Scan type and rate	ω-scan; 2.02–19.53 deg·min^{-1}
Scan range	0.80° below Kα$_1$ to 0.80° above Kα$_2$
Background counting	stationary counts for one-fifth of scan time at each end of scan range
Collection range	$0 \le h \le 10$, $0 \le k \le 11$, $-15 \le l \le 15$; $2\theta_{max} = 50°$
Unique data points measured	1936
Observed data with $\lvert F_o\rvert \ge 3\sigma(\lvert F_o\rvert)$, n	1686
No. of variables, p	216
Weighting scheme	$w = [\sigma^2\lvert F_o\rvert + 0.0001\lvert F_o\rvert^2]^{-1}$
$R_F = \Sigma\lvert\lvert F_o\rvert - \lvert F_c\rvert\rvert/\Sigma\lvert F_o\rvert$	0.039
$R_w = [\Sigma w^2(\lvert F_o\rvert - \lvert F_c\rvert)^2/\Sigma w^2\lvert F_o\rvert^2]^{1/2}$	0.038
$S = [\Sigma w(\lvert F_o\rvert - \lvert F_c\rvert)^2/(n-p)]^{1/2}$	1.85
Residual extrema in final difference map	+0.33 to −0.33 eÅ^{-3}

TABLE 4. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Temperature Factors ($Å^2 \times 10^4$) for Compound **1**.

Atom	x	y	z	U_{eq}[a]
Cl-1	1583(2)	2057(2)	−1444(1)	812(4)
Cl-2	2472(2)	1565(2)	728(1)	833(4)
Cl-3	−198(1)	3374(3)	−52(1)	801(4)
Cl-4	2316(2)	6280(3)	5885(1)	897(4)
Cl-5	2027(2)	3279(3)	6185(1)	803(4)
Cl-6	−348(1)	4754(3)	4736(1)	738(4)
O-1	3810(3)	8196(4)	1014(2)	577(5)
O-2	6080(3)	4515(4)	3914(3)	664(5)
N-1	5435(4)	5000	2195(3)	474(5)
N-2	3887(4)	7444(4)	2676(3)	496(5)
C-1	4400(4)	5763(4)	1350(3)	428(5)
C-2	4022(4)	7252(4)	1670(3)	417(5)
C-3	2858(4)	4919(5)	932(3)	491(5)
C-4	2875(4)	4163(4)	−101(3)	430(5)
C-5	2489(5)	5202(5)	−1000(4)	751(5)
C-6	1745(4)	2894(5)	−203(3)	493(5)
C-7	4126(4)	6304(4)	3431(3)	457(5)
C-8	5305(4)	5199(4)	3202(3)	476(5)
C-9	2527(4)	5638(5)	3592(3)	509(5)
C-10	2690(4)	4403(4)	4387(3)	492(5)
C-11	2348(6)	2981(5)	3852(4)	768(5)
C-12	1708(5)	4662(5)	5230(3)	512(5)
C-13	6445(5)	3866(5)	1918(4)	645(5)
C-14	3412(5)	8833(5)	3010(4)	701(5)

[a]U_{eq} defined as one third of the trace of the orthogonalized U tensor.

parative tlc over SiO_2-G with CH_2Cl_2-EtOAc (40:1) as eluent to yield dysamide C [**3**] (30 mg) (0.0526%).

The more polar fractions eluted with 50% EtOAc/petroleum ether were subjected to vlc over Si gel H with $CHCl_3$-Me_2CO (15:1). Pure dysamide A [**1**] was obtained as colorless prisms (100 mg). The mother liquor, after separation of **1**, was purified by hplc [EtOAc-petroleum ether (1:1)] to give dysamide B [**2**] (80 mg).

Dysamide A [**1**].—Prisms: mp 118–119°; $[\alpha]_D$ −36.6° ($c = 0.265$, MeOH); ir (KBr) ν max 1663 (v. strong), 790, 780 (C-Cl) cm^{-1}; ^{13}C nmr ($CDCl_2$) see Table 1; ^1H nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 460 (5), 425 (44), 389 (35), 355 (25), 301 (100), 271 (72), 265 (78), 237 (60), 201 (54), 175 (45), 141 (70); hrms *m/z* 459.9590 (calcd for $C_{14}H_{20}{}^{35}Cl_5{}^{37}ClN_2O_2$, 459.9621).

Dysamide B [**2**].—Needles: mp 147–149°; $[\alpha]_D$ 13.7° ($c = 0.117$, MeOH); ir (KBr) ν max 1662 (vs), 760 (C-Cl) cm^{-1}; ^{13}C nmr ($CDCl_3$) see Table 1; ^1H nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 392 (8), 355 (17), 265 (100), 237 (97), 168 (70), 141 (33); hrms *m/z* 392.1367 (calcd for $C_{14}H_{22}{}^{35}Cl_3{}^{37}ClN_2O_2$, 392.1400).

Dysamide C [**3**].—Needles: mp 196–197°; $[\alpha]_D$ −7.3° ($c = 0.041$, MeOH); ir (KBr) ν max 1692, 1637, 1354, 774 (C-Cl) cm^{-1}; ^{13}C nmr ($CDCl_3$) see Table 1; ^1H nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 456 (5), 421 (18), 385 (100), 349 (14), 339 (33), 220 (24), 82 (96); hrms *m/z* 455.9313 (calcd for $C_{14}H_{16}{}^{35}Cl_5{}^{37}ClN_2O_2$, 455.9302).

2,3-Dihydrodysamide C.—Needles: mp 135–136°; $[\alpha]_D$ −156.5° ($c = 0.232$, $CHCl_3$), ^{13}C nmr ($CDCl_3$ see Table 1; ^1H nmr see Table 2; eims *m/z* (rel. int. %) 423 $[M-Cl]^+$ (13), 387 (100), 351 (9), 339 (16), 152 (17), 82 (42); hrms $[M-Cl]^+$ *m/z* 422.9756 (calcd for $C_{14}H_{18}{}^{35}CL_4{}^{37}ClN_2O_2$, 422.9774). *Anal.* found C 37.06, H 3.94, N 6.06 (calcd for $C_{14}H_{18}Cl_6N_2O_2$, C 36.63, H 3.95, N 6.10.

X-RAY STRUCTURE ANALYSIS OF DYSAMIDE A[1] [**1**].—Diffraction measurements were made on a Siemens P4 diffractometer (9), and inten-

[1]Atomic coordinates for this compound have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

sities were recorded at 20° and processed with the learnt-profile procedure (10) (Table 3).

Direct phase determination guided by negative quartets yielded the positions of all non-hydrogen atoms in the molecule, which were refined anisotropically. The hydrogen atoms were introduced at their idealized positions (C-H fixed at 0.96 Å) using the penultimate difference map as a guide and included in structure-factor calculations with assigned isotropic thermal parameters; the methyl groups were treated as rigid and the hydrogen atoms were allowed to ride on their respective parent carbon atoms. The absolute configuration of the molecule (Figure 1) was established by refining a parameter η that multiplies all $\Delta f''$ values (the imaginary components of the atomic scattering factors) (11). The refined value $\eta = 1.56$ (9) strongly indicates that the absolute configuration is correctly described by the set of atomic coordinates listed in Table 4.

All computations were performed with the SHELXTL-PC package (12). The R indices and other parameters at convergence of the least-squares refinement are listed in Table 3. The final atomic parameters of the nonhydrogen atoms are given in Table 4.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. S. Carmely, T. Gebreyesus, Y. Kashman, B.W. Skelton, A.H. White, and T. Yosief, *Aust. J. Chem.*, **43**, 1881 (1990).
2. T. Gebreyesus, T. Yosief, S. Carmely, and Y. Kashman, *Tetrahedron Lett.*, **29**, 3863 (1988).
3. K.L. Erickson and R.J. Wells, *Aust. J. Chem.*, **35**, 31 (1982).
4. C. Charles, J.C. Braekman, D. Daloze, and B. Tursh, *Tetrahedron*, **36**, 2133 (1980).
5. R. Kazlauskas, P.T. Murphy, and R.J. Wells, *Tetrahedron Lett.*, 4945 (1978).
6. C. Charles, J.C. Braekman, D. Daloze, B. Tursch, and R. Karlsson, *Tetrahedron Lett.*, 1519 (1978).
7. R. Kazlauskas, R.O. Lidgard, R.J. Wells, and W. Vetter, *Tetrahedron Lett.*, 3183 (1977).
8. N.B. Colthup, L.H. Daly, and S.E. Wiberley, "Introduction to Infrared and Raman Spectroscopy," Academic Press, New York and London, 1964, p. 316.
9. R.A. Sparks, in: "Crystallographic Computing Techniques." Ed. by F.R. Ahmed, Munksgaard, Copenhagen, 1976, p. 452.
10. R. Diamond, *Acta Crystallogr., Sect. A*, **25**, 43 (1969).
11. D. Rogers, *Acta Crystallogr., Sect. A*, **37**, 734 (1981).
12. G.M. Sheldrick, in: "Crystallographic Computing 3: Data Collection, Structure Determination, Proteins, and Databases." Ed. by G.M. Sheldrick, C. Krüger, and R. Goddard, Oxford University Press, New York, 1985, p. 175.

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