A New Lysine Derivative and New 3-Bromopyrrole Carboxylic Acid Derivative from Two Marine Sponges

Cong-Jun Li,[†] Francis J. Schmitz,*[,†] and Michelle Kelly-Borges[‡]

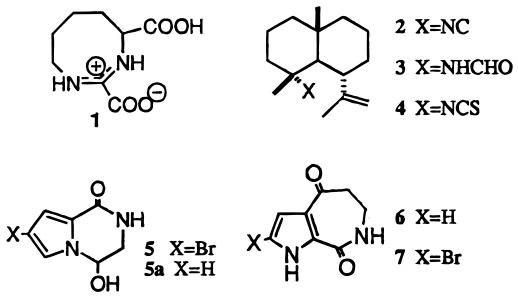
Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, Oklahoma 73019-0370, and Department of Civil and Environmental Engineering, Faculty of Health, Science and Technology, UNITEC Institute of Technology, Private Bag 92025, Auckland, New Zealand

Received October 24, 1997

A novel lysine derivative, **1**, has been isolated from the marine sponge *Axinyssa terpnis*, in addition to 4α-isocyanogorgon-11-ene and related compounds (**2**−**4**). From the marine sponge *Axinella carteri*, the new 3-bromopyrrole carboxylic acid derivative, **5**, was obtained along with the known compounds aldisin (**6**) and 2-bromoaldisin (**7**). Both sponges were collected from Chuuk Atoll, Federated States of Micronesia. The compounds were characterized by spectroscopic and chemical methods.

In an ongoing search for biologically active substances from marine invertebrates,[1] we found that the extracts of two marine sponges, *Axinyssa terpnis* de Laubenfels, 1954 (order Halichondrida, family Halichondriidae), and *Axinella carteri* (Dendy) (order Axinellida, family Axinellidae), inhibited the growth of brine shrimp.[2,3] The extracts of both sponges were partitioned (see Experimental Section) to give hexane-, CH_2Cl_2-, and MeOH-soluble fractions. The brine shrimp toxicity of the *A. terpnis* extract was traced to the hexane- and CH_2Cl_2-soluble fractions, and from these the known compounds 4α-isocyanogorgon-11-ene (**2**), 4α-formamidogorgon-11-ene (**3**), and 4α-isothiocyanatogorgon-11-ene (**4**)[4] were isolated, all of which were toxic to brine shrimp. From the MeOH-soluble fraction of this extract, a major, nontoxic component was isolated and identified as a new lysine derivative, **1**.

The MeOH-soluble part of the extracts of *Axinella carteri* showed limited toxicity to brine shrimp, and analysis of this fraction led to the isolation and identification of aldisin (**6**), 2-bromoaldisin (**7**)[5] and a new pyrrole-based metabolite (**5**). The structures of **1** and **5** are described herein.



Compound **1** crystallized as white needles from MeOH, mp 210−212 °C, and showed ions at *m*/*z* 201 $[M + 1]^+$ and 223 $[M + Na]^+$ in the laser desorption MS. A molecular formula of $C_8H_{12}N_2O_4$ was deduced from the HRFABMS and NMR data, requiring four degrees of unsaturation. Broad absorption in the region 3090−3500 and bands at 1660 and 1613 cm^{-1} in the IR spectrum taken together with ^{13}C NMR signals at δ 175.72, 159.26, and 156.66 were compatible with the presence of carboxyl groups or derivatives thereof. The ^1H NMR spectrum contained a series of coupled resonances that spanned the chemical shift region from δ 1.55 to 4.67. A DEPT spectrum confirmed that these protons were associated with one methine and four methylene groups. Analysis of DEPT, COSY, and HMQC identified the partial structure $C(X)HCH_2CH_2$-CH_2CH_2−Y, with X and Y likely being N on the basis of the associated ^{13}C NMR shifts, 56.60 and 41.57, respectively. The protons of each methylene are not equivalent and showed complicated coupling in the ^1H NMR spectrum, which indicated that this partial structure was integrated into a ring. An HMBC experiment (Figure 1) not only confirmed this partial structure but also revealed correlations from the methine proton (δ 4.67) and the adjacent methylene group protons (δ 1.81 and 2.38) to a carbonyl carbon signal at δ 175.72. The methine proton and the terminal methylene protons (δ 3.46 and 4.0) showed HMBC correlations to the carbon signal at δ 155.66. No long-range proton correlations were observed to the δ 159.26 ^{13}C signal. From these data structure **1** was proposed. Hydrolysis of **1** in aqueous 2 N HCl at 78° for 10 h afforded a product identified as lysine by comparison of its TLC behavior and ^1H NMR spectrum with those of an authentic sample. Esterification of compound **1** with BF_3·MeOH[6] gave a diester (**1a**) whose methoxy proton signals at δ 4.01 and 3.85 showed HMBC correlations to the carbonyl signals at δ 172.06 and 157.57, respectively. The remaining carbonyl signal at δ 151.83 was correlated with the H's-6 (δ 3.52 and 4.05). Thus, structure **1** was confirmed.

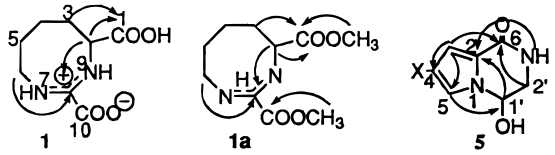


Figure 1. Selected HMBC correlations in **1**, **1a** and **5**.

Compound **5** was isolated as a yellow powder that

* To whom correspondence should be addressed. Tel: (405) 325-5581. Fax: (405) 325-6111. E-mail: fjschmitz@chemdept.chem.ou.edu.

S0163-3864(97)00479-5 CCC: $15.00
Published on Web 02/25/1998

gave a 1:1 pair of peaks in the laser desorption mass spectrum at *m/z* 231 and 233 $[M + 1]^+$, indicating the presence of one bromine. HRFABMS, together with ^1H NMR and ^{13}C NMR data, established the molecular formula as $C_7H_7BrN_2O_2$, which requires five sites of unsaturation. A comparison of the ^1H NMR and ^{13}C NMR data with those for hymenidin[7] suggested a 4-bromo-2-pyrrolecarboxyl partial structure in **5**. This was confirmed by an HMBC (J = 9 Hz) experiment (Figure 1). $^1H-^1H$ COSY, HMQC, and HMBC experiments revealed the remainder of the structure to consist of $-CH(OH)CH_2NH-$, of which the oxygenated methine carbon appears at δ 76.1 ppm, while the methylene carbon resonates at δ 47.6 ppm. The position of this fragment in the structure was established as shown in **5** by a second HMBC experiment (J = 6 Hz), which showed correlations from the oxygenated methine proton (δ 5.55) to C-2 (δ 124.7), H-5 (δ 7.15) to the oxygenated methine carbon, and the methylene protons to the amide carbonyl group (δ 161.5). The spectral data for compound **5** compare very favorably with those of longamide (**5a**) isolated from *Agelas longissima*.[8]

Brine shrimp lethality tests were carried out on the pure isolates.[2,3] Compounds **1**, **5**, **6**, and **7** were inactive. The known compounds **2**, **3**, and **4** showed modest toxicity, with LC_{50} values of approximately 50 μg/mL. Compounds **2** and **3** were toxic to murine leukemia P-388 cells, IC_{50} 8.3 and 1.2 μg/mL, respectively.

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Bio-Rad IR 3240-spc FT instrument. NMR spectra were measured at 300 and 500 MHz (^1H NMR) and 125 MHz (^{13}C), on Varian VXR-300 and 500 MHz spectrometers. ^1H and ^{13}C chemical shifts were assigned on the basis of COSY, HMQC, and HMBC experiments. FABMS were obtained on a VG ZAB-E instrument, and the laser desorption MS was recorded on a PerSeptive Biosystems Voyages Elite instrument. Preparative HPLC was performed using a Phenomenex C-18 column (250 × 10 mm) with UV detection.

Animal Material. The sponge 30-T-93 was collected from a depth of 60 m in Anaw Pass, Chuuk Atoll, Micronesia, in August 1993. It was identified as *Axinyssa terpnis* de Laubenfels 1954 (order Halichondrida, family Halichondriidae). The sponge forms a large amorphous mound and is dirty white to beige in life. Semisinuous oxea with strongylote ends, about 1000 μm long, are arranged in a confused fashion in the interior of the sponge, but are perpendicular to the surface of the sponge at the surface. The sponge oxidizes to a dark brown in EtOH. *Axinella carteri* (Dendy) was also collected in Chuuk Atoll in August 1993. It is a tall, stalked, bright orange sponge with flexible, softly conulose blades and has styles that range in length from 450 to 550 μm. Voucher specimens have been deposited at the Natural History Museum, London, U.K., and are also kept at the University of Oklahoma (*Axinella carteri*-BMNH 1997.9.20.3, University of Oklahoma 6-T-93; *Axinyssa terpnis*-BMNH 1997.9.20.4, University of Oklahoma 30-T-93).

Isolation. Freshly thawed specimens of *Axinyssa terpnis* (790 g wet wt) were extracted sequentially at room temperature with MeOH (700 mL × 2) and CH_2Cl_2–MeOH (1:1, 700 mL × 3). All extracts were combined and the solvents evaporated under vacuum to give a viscous brown concentrate. This total extract was dissolved in 350 mL of MeOH–H_2O (9:1), and the solution was extracted with hexane (100 mL × 2). The aqueous MeOH solution was then diluted to MeOH–H_2O (7:3) and extracted with CH_2Cl_2 (100 mL × 2). The aqueous MeOH was evaporated under vacuum to dryness, and the residue was triturated with MeOH (100 mL × 2). This MeOH-soluble material was subjected to a flash column chromatography over Si gel ($CHCl_3$–MeOH–H_2O, step gradient), followed by chromatography over a microcrystalline cellulose column using iPrOH–water (4:1) as eluent. Further purification chromatography over a Sephadex LH-20 column (MeOH as eluent) furnished 132 mg of **1**. Repeated chromatography of the hexane and CH_2Cl_2 extracts either on normal-pressure Si gel columns or HPLC eluted with hexane–EtOAc systems afforded the known compounds 4α-isocyanogorgon-11-ene (**2**, 13.1 mg), 4α-formamidogogon-11-ene (**3**, 6.3 mg), and 4α-isothiocyanatogorgon-11-ene (**4**, 11 mg).

Freshly thawed specimens of *Axinella carteri* (102 g dry wt) were extracted at room temperature with MeOH (1000 mL × 3) and MeOH–$CHCl_3$ (1:1) (1000 mL × 3). All extracts were combined and the solvents evaporated to give a total extract concentrate that was partitioned as described above. The residue obtained from evaporation of the MeOH–H_2O (7:3) solution was triturated with MeOH (100 mL × 2), and the MeOH-soluble portions were subjected to Si gel flash chromatography using a $CHCl_3$–MeOH step gradient (8:1; 6:1; 4:1, and 2:1) to yield fractions containing **5**–**7**. These fractions were then individually subjected to repeated reversed-phase HPLC, using MeOH–H_2O (1:10) to give compounds **5** (1.3 mg), **6** (2.7 mg), and **7** (1.2 mg).

Compound 1: $[\alpha]_D$ −66.3° (*c* 0.87, MeOH); IR (film) υ_{max} 3090–3500 (OH, NH), 1660 and 1613 (C=O, C–C=N–) cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 4.66 (1H, dd, 10.5, 5.5 Hz, H-2), 4.00 (1H, overlapping dt, 14.5, 9 Hz, H-6), 3.46 (1H, dt, 15.5, 4 Hz, H-6), 2.37 (1H, m, H-3), 1.97 (2H, m, H-5), 1.87–1.79 (2H, m, H-3 and H-4), 1.56 (1H, m, H-4); ^{13}C NMR (CD_3OD) δ 175.72 (C-1), 159.26 (C-10), 156.66 (C-8), 56.60 (C-2), 41.57 (C-6), 35.96 (C-3), 31.02 (C-5), 22.30 (C-4); ^1H NMR (DMSO-d_6, 300 MHz) δ 9.08 (1H, t, J = 6.1 Hz, H-7, exchangeable), 8.98 (1H, d, J = 4.8 Hz, H-9, exchangeable), 4.55 (1H, dd, J = 11.0, 5.1 Hz, H-2), 3.85 (1H, m, H-6), 3.34 (1H, m, H-6′), 2.20 (1H, m), 1.90–1.50 (4H, m), 1.35 (1H, m); MALDI-TOF MS *m/z* 223 ($[M + Na]^+$), 201 $[M + 1]^+$, 179 ($[M + Na - CO_2]^+$), 157 ($[M + 1 - CO_2]^+$); FABMS *m/z* 201 $[M + 1]^+$; HRFABMS *m/z* 201.0885 $[M + 1]^+$, calcd for $C_8H_{13}N_2O_4$, 201.0875.

Esterification of 1. Compound **1** (27 mg) was dissolved in 5 mL MeOH to which had been added 0.5 mL BF_3–MeOH complex (12% by wt). This solution was refluxed at 55 °C for 24 h and then was evaporated to dryness, leaving **1a** as a viscous residue: ^1H NMR (CD_3OD, 500 MHz) δ 5.21 (1H, dd, J = 10.8, 5.0 Hz, H-2), 4.05 (1H, m, H-6′), 4.01 (3H, s, MeO-10), 3.85 (3H, s, MeO-1), 3.52 (dt, J = 15.0 Hz, J = 3.7 Hz, H-6), 2.35 (1H, m, H-3), 2.05–1.75 (4H, m), 1.65 (1H, m, H-4); ^{13}C NMR (CD_3OD) δ 172.06 (C-1), 157.57 (C-10), 151.83 (C-

8), 56.25 (C-2), 55.61 (OMe), 54.17 (OMe), 42.43 (C-6), 34.21 (C-3), 30.32 (C-5), 21.20 (C-4); FABMS *m/z* 229 $[M + 1]^+$. The ^{13}C NMR assignments are based on a comparison with data for compound **1** and results of an HMBC experiment.

Hydrolysis of 1. Compound **1** (5 mg) was dissolved in 0.5 mL aqueous 2N HCl in a screw-capped vial, the vial was sealed, and the solution was heated at 78 °C for 10 h. The solution was dried under a nitrogen stream, and the residue was compared with an authentic sample of lysine by NMR and TLC (cellulose, 4:1 iPrOH−1 N NH_4OH and 4:1:1 BuOH−EtOH−1 N NH_4-OH. Both NMR and TLC comparisons were positive.

Compound 5: $[\alpha]_D$ −6° (*c* 0.083, MeOH); IR (film) υ_{max} 3100−3600 (br), 1650 (C═O) cm^{-1}; ^1H NMR (CD_3-OD, 500 MHz) δ 7.10 (1H, d, J = 1.5 Hz, H-5), 6.80 (1H, d, J = 1.5 Hz, H-3), 5.64 (1H, t, J = 3.5 Hz, H-1′), 3.73 (1H, dd, J = 13.5, 3.5 Hz, H-2′), 3.51 (1H, dd, J = 13.5, 3.5 Hz, H-2′); ^1H NMR (DMSO-d_6, 500 MHz) δ 7.75 (1H, br s, exchangeable), 7.15 (1H, br s, H-5), 6.60 (1H, br s, H-3), 7.0 (1H, br s, exchangeable), 5.55 (1H, br s, H-1′), 3.55 (1H, d, J = 13 Hz, H-2′), 3.3 (1H, overlapped with H_2O peak, H-2′); ^{13}C NMR (CD_3OD) δ 161.52 (C-6), 124.71 (C-2), 123.74 (C-5), 116.22 (C-3), 98.84 (C-4), 76.11 (C-1′), 47.65 (C-2′); MALDI-TOF MS *m/z* 231 and 233 $[M + 1]^+$; HRFABMS *m/z* 230.9767 $[M + 1]^+$ calcd for $C_7H_8N_2O_2Br$, 230.9769.

Acknowledgment. This work was supported by Department of Commerce, NOAA, Sea Grant Project NA66RG0172. We thank the Coral Reef Research Foundation for use of its facilities, the Government of Chuuk, Federated States of Micronesia for permission to collect specimens, and the NSF EPSCoR Laser Mass facility at the University of Oklahoma Health Sciences Center for MALDI-TOF mass spectra.

References and Notes

(1) Fu, X.; Ng, P.-L.; Schmitz, F. J.; Hossain, M. B.; van der Helm, D.; Kelly-Borges, M. *J. Nat. Prod.* **1996**, *59*, 1104−1106.
(2) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.
(3) McLaughlin, J. L.; Chang, C. J.; Smith, D. L. In *Studies in Natural Products Chemistry*; Rahman, A., Ed. Elsevier: Amsterdam, 1991; pp 383−405.
(4) Kassuhlke, K. E.; Potts, B. C. M.; Faulkner D. J. *J. Org. Chem.* **1991**, *56*, 3747−3750.
(5) Schmitz, F. J.; Gunasekera, S. P.; Lakshmi, V.; Tillekeratne, L. M. E. *J. Nat. Prod.* **1985**, *48*, 47−53.
(6) Marshall, J. L.; Erickson, K. C.; Folsom T. K. *Tetrahedron Lett.* **1970**, *46*, 4011−4012.
(7) Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. *Experientia* **1986**, *42*, 1176−1177.
(8) Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1995**, *36*, 7893−7896.

NP970479H