Isolation and Synthesis of 4-Bromopyrrole-2-carboxyarginine and 4-Bromopyrrole-2-carboxy-*N*(ϵ)-lysine from the Marine Sponge *Stylissa caribica*[§]

Achim Grube, Ellen Lichte, and Matthias Köck*

Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gemeinschaft, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Received July 28, 2005

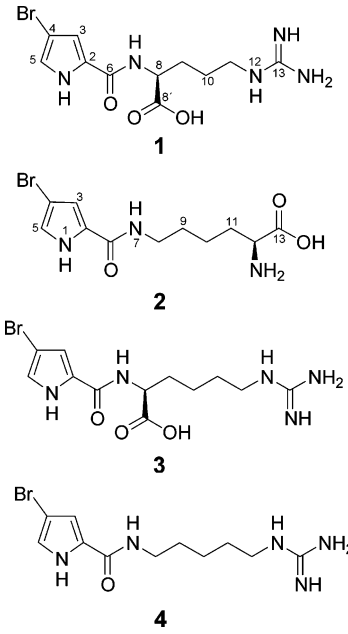
Two new bromopyrrole alkaloids were isolated from the Caribbean sponge *Stylissa caribica*. The new natural products, 4-bromopyrrole-2-carboxyarginine (**1**) and 4-bromopyrrole-2-carboxy-*N*(ϵ)-lysine (**2**), are derivatives of amino acids linked with a 4-bromopyrrole-2-carboxylic acid. The structures were elucidated on the basis of NMR and MS/MS data and their absolute configurations assigned via synthesis.

The common structural motifs of the pyrrole-imidazole alkaloid family are the bromopyrrole and the aminoimidazole rings. The most prominent member of this group is oroidin,[1] which is the biosynthetic precursor of many complex natural products.[2] In a hypothetical biosynthetic pathway of oroidin the last step is the formation of the amide bond between the bromopyrrole-2-carboxylic acid and the aminopropylimidazole moiety.[3] Some years ago, we have isolated the first pyrrole-imidazole alkaloid with a guanidine function instead of the aminoimidazole (**3**) from the sponge *Agelas wiedenmayeri*.[4] This compound and its decarboxylated derivate laughine (**4**)[5] could be an alternative biosynthetic precursor. Here, we describe the isolation and structure elucidation of two new related compounds. They only differ from **3** by replacement of homoarginine by arginine in **1** and by lysine in **2**. In the lysine derivative the central amide bond is formed with the side chain amino group N(ϵ) of lysine.

The sponge material was extracted with CH_2Cl_2/MeOH (1:1), and the resulting crude extract was analyzed by HPLC/HRMS. Comparison of the experimental masses with the literature revealed two unknown substances with an isotopic pattern of a singly brominated molecule. The crude extract was partitioned by liquid/liquid extraction. The resulting *n*-BuOH fraction was purified by Sephadex LH-20 chromatography and reversed-phase HPLC.

The structures of 4-bromopyrrole-2-carboxyarginine (**1**) and 4-bromopyrrole-2-carboxy-*N*(ϵ)-lysine (**2**) were elucidated by 1D and 2D NMR data (Tables 1 and 2) and MS analysis. The positive electrospray mass spectrum of **1** displayed clusters of ion peaks $[M + H]^+$ at *m*/*z* 346/348. The high-resolution mass of *m*/*z* 346.0487 indicated the molecular formula $C_{11}H_{17}N_5O_3Br$ ($[M + H]^+$). Examination of the ^{1}H NMR data revealed the presence of a 4-bromopyrrole-2-carboxamide moiety. The ^{13}C NMR signal at 173.5 ppm and two additional oxygens suggested a carboxyl group. The HMBC correlation from H-8 to C-8′ and the COSY correlation from H-7 to H-8 indicated an *N*-terminal connection between 4-bromopyrrole-2-carboxylic acid and arginine. A positive Sakaguchi reaction[6] and the loss of a guanidine group and ammonia under MS/MS conditions supported the presence of a free guanidine group. The positive electrospray mass spectrum of **2** displayed clusters of ion peaks $[M + H]^+$ at *m*/*z* 318/320. The high-resolution mass of *m*/*z* 318.0445 indicated the molecular formula $C_{11}H_{16}N_3O_3Br$ ($[M + H]^+$). Similar to **1** the ^{1}H NMR data revealed the presence of a 4-bromopyrrole-2-carboxamide moiety. The HMBC correlations from H-7 to C-8 and C-9 as well as a positive ninhydrin reaction[6] on free amino acids proved the connectivity between the side chain amino group of lysine with the 4-bromopyrrole-2-carboxylic acid.

Chart 1. Structural Formulas of 4-Bromopyrrole-2-carboxyarginine (**1**), 4-Bromopyrrole-2-carboxy-*N*(ϵ)-lysine (**2**), 4-Bromopyrrole-2-carboxyhomoarginine (**3**), and Laughine (**4**)



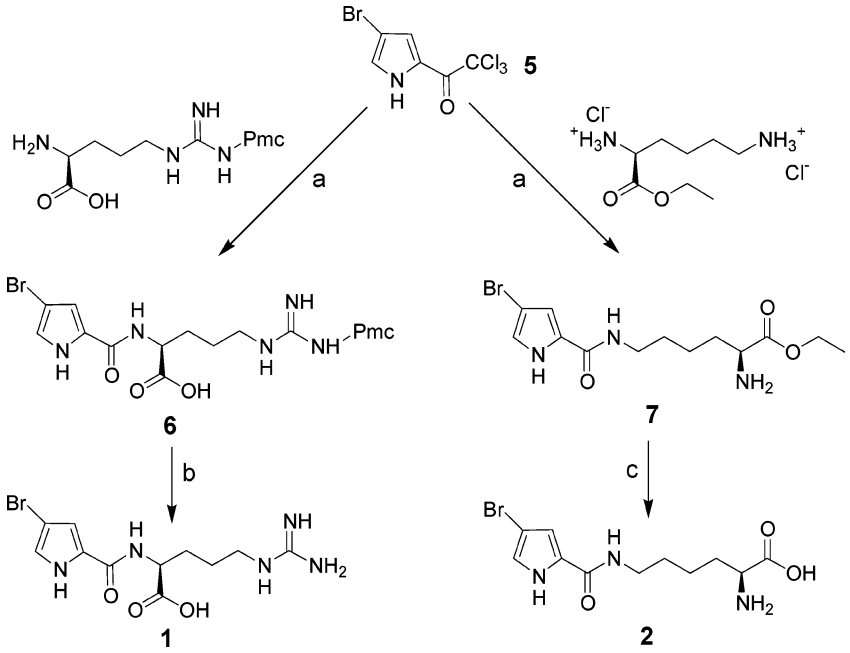
To assign the absolute configuration of **1** and **2**, it was attempted to hydrolyze the compounds in order to apply Marfey's method. Even with HCl (36%, 18 h, 80 °C) no hydrolysis was observed. Therefore, the syntheses of **1** and **2** were carried out. Reaction of N^G-2,2,5,7,8-pentamethylchroman-6-sulfonyl-L-arginine and 4-bromopyrrol-2-yl trichloromethyl ketone (**5**)[8] at room temperature yielded the protected 4-bromopyrrole-2-carboxyarginine (**6**). Hydrolysis with TFA gave **1** (Scheme 1). The configuration of **1** was obtained by measuring the specific rotation. The natural product appears as L-4-bromopyrrole-2-carboxyarginine since the sign of specific rotation of natural and synthetic product is identical. Reaction of L-lysine ethyl ester with 4-bromopyrrol-2-yl trichloromethyl ketone (**5**)[8] and subsequent ester hydrolysis regioselectively gave **2**.[4b] The configuration of **2** was determined by comparison of optical rotation of natural and synthetic product and additionally by HPLC using Marfey's method.[7] Comparison of

[§] Presented at the 4th European Conference on Marine Natural Products, Paris, France, Sept 12−16, 2005 (Book of Abstracts, p P69).

* To whom correspondence should be addressed. Tel: +49-471-48311497. Fax: +49-471-48311425. E-mail: mkoeck@awi-bremerhaven.de.

10.1021/np0502746 CCC: $33.50
Published on Web 12/24/2005

Scheme 1. Solution Phase Synthesis of **1** and **2**[a]



[a] (a) i-Pr_2NEt, MeCN, room temperature, 6 h; (b) TFA (98%), 45 min, room temperature; (c) HCl (32%), 22 h, room temperature.

Table 1. NMR Data for 4-Bromopyrrole-2-carboxyarginine (**1**) Recorded in DMSO-d_6[a]

position	δ (^{13}C)/δ(^{15}N)[b] [ppm]	δ (^{1}H) [ppm]	^{1}H,^{1}H-COSY	^{1}H,^{13}C-HMBC
1	(161)	11.84 (1H, s)	3, 5	3, 4
2	126.3			
3	112.1	6.98 (1H, m)	1	2, 5
4	94.9			
5	121.4	7.00 (1H, m)	1	2, 3, 4
6	159.5			
7	(112)	8.20 (1H, d, J = 8.1 Hz)	8	6, 8, 9
8	51.5	4.35 (1H, m)	7, 9	6, 8′, 9, 10
8’	173.5			
9	28.0	1.85 (1H, m), 1.70 (1H, m)	8, 10	8, 8′, 10, 11
10	25.3	1.55 (2H, m)	9, 11	8, 9, 11
11	40.3	3.12 (2H, m)	10, 12	9, 10, 13
12	(85)	7.61 (1H, t, J = 5.5 Hz)	11	11, 13
13	156.7			

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [b] For positions no. 1, 7, and 12 δ(^{15}N) is given in parentheses.

Table 2. NMR Data for 4-Bromopyrrole-2-carboxy-*N*(ϵ)-lysine (**2**) Recorded in DMSO-d_6[a]

position	δ (^{13}C)/δ(^{15}N)[b] [ppm]	δ (^{1}H) [ppm]	^{1}H,^{1}H-COSY	^{1}H,^{13}C-HMBC
1	(161)	11.80 (1H, s)	3, 5	2, 3, 4, 5
2	127.0			
3	111.3	6.82 (1H, m)	1, 5	2, 5, 6
4	94.8			
5	121.0	6.96 (1H, m)	1, 3	2, 3, 4, 6
6	159.5			
7	(108)	8.09 (1H, t, J = 5.7 Hz)	8	6, 8, 9
8	38.2	3.19 (2H, dd, J = 6.4, 6.2 Hz)	7, 9	6, 9, 10
9	28.8	1.48 (2H, m)	8	8, 10, 11
10	21.9	1.39 (2H, m)	11	8, 9, 11, 12
11	29.9	1.77 (2H, m)	10, 12	9, 10, 12, 13
12	52.3	3.77 (1H, t, J = 6.0 Hz)	11	10, 11, 13
12-NH_2		8.15 (br)		
13	171.1			

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [b] For positions no. 1 and 7 δ(^{15}N) is given in parentheses.

retention times of natural and synthetic product derivatives and the same sign of specific rotation revealed the *S*-configuration for **2**.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 25 °C. The DQF-^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, ^{1}H,^{15}N-HSQC, and ^{1}H,^{15}N-HMBC experiments were carried out using standard parameters. HPLC-MS analyses were performed with an Agilent 1100 HPLC system and a Bruker Daltonics microTOF$_{LC}$ mass spectrometer. Separation was achieved by a Waters XTerra RP$_{18}$ column (3.0 × 150 mm, 3.5 μm) applying a MeCN/H_2O/HCOOH gradient. UV detection was performed with a DAD (Agilent) at a wavelength of 280 nm. ESI-MS/MS spectra were recorded with an Esquire 3000+ ion trap (Bruker Daltonics). Optical rotation was measured with a Perkin-Elmer 214 MC polarimeter at 23 °C.

Animal Material. The sponge *Stylissa caribica* was collected by scuba diving at Little San Salvador in the Bahamas (74 ft depth, July 2000). The samples were immediately frozen after collection and kept at −20 °C until extraction. The sponge material was compared with previously investigated material of *S. caribica*[8] and was found to match closely (Dr. Michael Assmann, personal communication). The specimens form erect wedged-shaped, thick-bladed columns with irregularly corrugated lengthwise grooves and ridges, subdivided in places to form honeycomb-like depressions. The surface in the depressions is shiny smooth, looking fleshy. The color in life is orange-brown, turning rather dark red-brown in EtOH. A detailed taxonomic description of the sponge is given in ref 9.

Extraction and Isolation. The freeze-dried sponge samples of *S. caribica* (94.7 g) were crushed with a mill and extracted at room temperature exhaustively in a 1:1 mixture of CH_2Cl_2/MeOH. The orange-colored crude extract of *S. caribica* was partitioned between *n*-hexane (4 × 400 mL) and MeOH (300 mL). The MeOH extract was

then partitioned between *n*-BuOH (3 × 500 mL) and H_2O (300 mL). The resulting *n*-BuOH (15.9 mg) fraction from the solvent partitioning scheme was purified by gel chromatography on Sephadex LH-20 (Pharmacia) using MeOH as mobile phase. Final purification of the isolated compounds was achieved by preparative RP_{18} HPLC on a Kromasil RP_{18} column (16 × 250 mm, 10 μm) applying a MeCN/TFA (0.1% in H_2O) gradient to afford **1** (8.9 mg, 0.009% of dry weight) and **2** (10.3 mg, 0.011% of dry weight).

1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) Derivatization and Absolute Configuration of 2 (Marfey's method[7]). To 10 μL (130 μg) of amino acid solution were added 100 μL of 0.1 M $NaHCO_3$ and 100 μL of 3 mM 1-fluoro-2,4-dinitrophenyl-5-L-alanine. The solution was heated to 80 °C for 5 min. Then 50 μL of 0.2 M HCl and 40 μL of 50% aqueous MeCN containing 0.1% formic acid were added to the reaction mixture. Separation was achieved by a Waters XTerra RP_{18} column (3.0 × 150 mm, 3.5 μm) applying an MeCN/H_2O/HCOOH gradient (0 min: 10% MeCN/90% HCOOH (0.1% in H_2O), 30 min: 60% MeCN/40% HCOOH (0.1% in H_2O)) with a flow rate of 0.4 mL/min. UV detection was performed with a DAD (Agilent) at a wavelength of 340 nm. Retention times: natural product, 23.82 min; synthetic compound, 23.80 min.

4-Bromopyrrole-2-carboxyarginine (1): light yellow oil; $[\alpha]_D^{23}$ −16 (*c* 0.25, MeOH); UV (DAD) λ_{max} 271 nm; HPLC-HRESI-(+)MS: t_R = 7.1 min, *m*/*z* 346.0487 $[M + H]^+$ (calcd for $C_{11}H_{17}N_5O_3{}^{79}Br$, *m*/*z* 346.0509, Δm = 6.4 ppm).

4-Bromopyrrole-2-carboxy-*N*(ϵ)-lysine (2): light yellow oil; $[\alpha]_D^{23}$ +5.2 (*c* 0.50, MeOH); UV (DAD) λ_{max} 268 nm; HPLC-HRESI-(+)MS: t_R = 8.1 min, *m*/*z* 318.0445 $[M + H]^+$ (calcd for $C_{11}H_{17}N_3O_3{}^{79}Br$, *m*/*z* 318.0448, Δm = 0.9 ppm).

4-Bromopyrrol-2-yl Trichloromethyl Ketone (5). Synthesis was performed according to Kitamura et al.[10c] based on the method of Bailey et al.[10b] A solution of Br_2 (308 μL, 6 mmol) in 20 mL of glacial HOAc was added slowly to a stirred solution of pyrrol-2-yl trichloromethyl ketone (1266 mg, 6 mmol) in 5 mL of glacial HOAc. Pyrrol-2-yl trichloromethyl ketone was synthesized according to Bailey et al.[10a] from pyrrole and trichloroacetyl chloride. After 4 h 30 mL of H_2O was added and the solution was extracted twice with 50 mL of DCM. The combined DCM solutions were dried (Na_2SO_4), and the solvent was evaporated. The crude products were purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **5** as a white powder (754 mg, 44%): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.84 (1H, br s, H-1), 7.54 (1H, dd, H-5), 7.32 (1H, dd, H-3); ^{13}C NMR (DMSO-d_6, 100.7 MHz) δ 171.6 (C-6), 129.0 (C-5), 122.0 (C-2), 121.5 (C-3), 97.6 (C-4), 94.5 (C-7); HRESI-(−)MS *m*/*z* 287.8364 $[M - H]^-$ (calcd for $C_6H_2NO^{35}Cl_3{}^{79}Br$, *m*/*z* 287.8380, Δm = 5.6 ppm).

(2*S*)-2-{[1-(4-Bromo-1*H*-pyrrol-2-yl)methanoyl]amino}-5-[*N*-Pmc-guanidino]pentanoic Acid (6). A 159 mg (0.55 mmol) amount of **5**, 190 mg (0.43 mmol) of N^G-2,2,5,7,8-pentamethylchroman-6-sulfonyl-L-arginine, and 150 μL (0.91 mmol) of *N*,*N*-diisopropylethylamine were suspended in MeCN (3 mL). After stirring for 6 h at room temperature the solvent was evaporated. The crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **6** as a colorless oil (80 mg, 23.7%). For this compound no NMR data were measured. HRESI-(+)MS: *m*/*z* 612.1464 $[M + H]^+$ (calcd for $C_{25}H_{35}N_5O_6S^{79}Br$, *m*/*z* 612.1486, Δm = 3.5 ppm).

(2*S*)-2-{[1-(4-Bromo-1*H*-pyrrol-2-yl)methanoyl]amino}-5-guanidinopentanoic Acid (1). A solution of **6** (60 mg, 0.098 mmol) in TFA (98%, 3 mL) was stirred for 45 min at room temperature. The solvent was evaporated, and the crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **1** as a colorless oil (22 mg, 65.3%). The NMR data were identical to the natural product. $[\alpha]_D^{23}$ −8 (*c* 0.15, MeOH); HRESI-(+)MS *m*/*z* 346.0501 $[M + H]^+$ (calcd for $C_{11}H_{17}N_5O_3{}^{79}Br$, *m*/*z* 346.0509, Δm = 2.3 ppm).

(2*S*)-2-Amino-6-[1-(4-bromo-1*H*-pyrrole-2-ylmethanoyl)amino]-hexanoic Acid Methyl Ester (7). To a solution of L-lysine methyl ester hydrochloride (100 mg, 0.40 mmol) in MeCN (3 mL) were added 4-bromopyrrole-2-yl trichloromethyl ketone (**5**, 159 mg, 0.55 mmol) and *N*,*N*-diisopropylethylamine (150 μL, 0.91 mmol). After 6 h at room temperature the solvent was evaporated. The crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **7** as a colorless oil (55.6 mg, 29.2%). For this compound no NMR data were measured. HRESI-(+)MS: *m*/*z* 346.0745 $[M + H]^+$ (calcd for $C_{13}H_{21}N_3O_3{}^{79}Br$, *m*/*z* 346.0761, Δm = 4.5 ppm).

(2*S*)-2-Amino-6-[1-(4-bromo-1*H*-pyrrole-2-ylmethanoyl)amino]-hexanoic Acid (2). A 55.6 mg portion of **7** was dissolved in HCl (32%). After 22 h at room temperature the solvent was evaporated. The crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **2** as a colorless oil (17.8 mg, 34.9%). The NMR data were identical to the natural product. $[\alpha]_D^{23}$ +5.4 (*c* 0.41, MeOH); HRESI-(+)MS *m*/*z* 318.0435 $[M + H]^+$ (calcd for $C_{11}H_{17}N_3O_3{}^{79}Br$, *m*/*z* 318.0448, Δm = 4.0 ppm).

Acknowledgment. The sponge collection was carried out by Dr. M. Assmann during a scientific expedition to the Bahamas in 2000. During this time the project was sponsored by the DFG (Ko1314/3-1 to 3-4). We would like to acknowledge the support of Prof. Dr. J. R. Pawlik (University of North Carolina at Wilmington, NC), who gave members of the Köck research group the opportunity to participate in the scientific sojourns to the Bahamas in the years 1998, 1999, 2000, 2001, and 2003. We further thank Dr. H.-O. Burmeister (TU Braunschweig) for measuring the optical rotation.

References and Notes

(1) (a) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc. D* **1971**, 1129−1130. (b) Garcia, E. E.; Benjamin, L. E.; Fryer, R. I. *J. Chem. Soc., Chem. Commun.* **1973**, 78−79. (c) Walker, R. P.; Faulkner, D. J.; van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 6772−6773.
(2) Al Mourabit, A.; Potier, P. *Eur. J. Org. Chem.* **2001**, 237−243.
(3) Andrade, P.; Kerr, R. G.; Willoughby, R.; Pomponi, S. A. *Tetrahedron Lett.* **1999**, *40*, 4775−4778.
(4) (a) Assmann, M.; Lichte, E.; Köck, M.; van Soest, R. W. M. *Org. Lett.* **1999**, *1*, 455−457. (b) Lindel, T.; Hochgürtel, M.; Assmann, M.; Köck, M. *J. Nat. Prod.* **2000**, *63*, 1566−1569.
(5) Williams, D. E.; Patrick, B. O.; Behrisch, H. W.; van Soest, R.; Roberge, M.; Andersen, R. J. *J. Nat. Prod.* **2005**, *68*, 327−330.
(6) Auterhoff, H.; Kovar, K.-A. *Identifizierung von Arzneistoffen*; Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, 1977.
(7) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(8) (a) Lehnert, H.; van Soest, R. W. M. *Beaufortia* **1998**, *48*, 71−103. (b) Alvarez, B.; van Soest, R. W. M.; Rützler, K. *Smithson. Contrib. Zool.* **1998**, *598*, 1−47
(9) Assmann, M.; van Soest, R. W. M.; Köck, M. *J. Nat. Prod.* **2001**, *64*, 1345−1347.
(10) (a) Bailey, D. M.; Johnson, R. E.; Albertson, N. F. *Org. Synth.* **1971**, *51*, 100−102. (b) Bailey, D. M.; Johnson, R. E. *J. Med. Chem.* **1973**, *16*, 1300−1302. (c) Kitamura, C.; Yamashita, Y. *J. Chem. Soc., Perkin Trans. 1* **1997**, *10*, 1443−1448.

NP0502746