Synthesis of the Marine Natural Product

No-(4-Bromopyrrolyl-2-carbonyl) L-homoarginine, a Putative Biogenetic Precursor of the Pyrrole–Imidazole Alkaloids

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Received April 12, 2000

Lysine is proposed as an alternative biosynthetic precursor of the pyrrole–imidazole alkaloids frequently found in marine sponges. As a putative key intermediate, the natural product No-(4-bromopyrrolyl-2-carbonyl) L-homoarginine (1) from the sponge Agelas wiedenmayeri was synthesized in the solid phase starting from Fmoc/Pmc-protected L-homoarginine and in solution starting from readily available L-lysine methyl ester.

Pyrrole–imidazole alkaloids continue to be isolated from marine sponges and represent one of the most prominent groups of natural products exclusive to the marine environment. The underlying C11N2 building block consists of a pyrrolyl-2-carbonyl unit being connected via an amide linkage to a 2-amino-5-(3-amino)propylimidazole partial structure. Recently, No-(4-bromopyrrolyl-2-carbonyl)-L-homoarginine (1) was isolated from the marine sponge Agelas wiedenmayeri, together with known pyrrole-imidazole alkaloids such as oroidin (2). In this paper, we describe solid- and solution-phase syntheses of 1 and the determination of its absolute configuration. After the discovery of the aplysinamisines I and II from Aplysina cauliformis, 1 and 2 constitute a new pair of natural products pointing at a biogenetic relationship between lysine and 2-amino-5-(3-amino)propylimidazoles in marine sponges. Oroidin (2) contributes to the survival of the genus Agelas by protecting the sponge against predation by the reef fish Thalassoma bifasciatum.

The solid-phase synthesis of 1 (Scheme 1) was achieved starting from (Fmoc/Pmc)-protected L-homoarginine (3), which was coupled to 2-chlorotrityl chloride polystyrene resin (4), forming compound 5. After removal of the Fmoc-protecting group with 40% piperidine in DMF, 4-bromopyrrole-2-carboxylic acid (6) was attached using DIC/HOBt as coupling reagents. Finally, treatment of the product 7 with 98% TFA led to the removal of the Pmc group and to the cleavage of No-(4-bromopyrrolyl-2-carbonyl)-L-homoarginine (1) from the resin. The overall yield was 72%. Alternatively, reaction of L-lysine methyl ester (8, Scheme 2) with the bis-Boc-protected pyrazole-1-carboxamidine (9) regioselectively gave the protected homoarginine 12, which was further converted to the methyl ester 14 via reaction with the pyrrolotrichloromethyl ketone 13. Treatment of 14 with HCl(Et2O) led to the selective removal of the Boc groups. Hydrolysis with 8 N HCl provided the natural product 1 as the hydrochloride in 60% overall yield. Reaction of 8 with 9 in acetonitrile/N,N-diisopropylmethyamine led to the selective acylation of the ε-amino group to 10.

The UV maxima of 1 in aqueous phosphate buffer, pH 7, were observed at 272 (ε 3.99) and 202 (ε 3.89) nm, while the CD spectrum (Figure 1) exhibited only one significant minimum. The still undetermined absolute configuration of the natural product 1 was based on the basis of negative Cotton effects at 216 nm (Δε - 1.6) for the synthetic L-1 and at 214 nm (Δε - 1.6) for the natural product 1. At 270 nm, the CD is not intense enough to be useful for the assignment of the absolute stereochemistry of the No-pyrrolotrichloromethyl amino acid 1. L-1 shows a negative optical rotation.

Several alternatives have been discussed with regard to the biosynthesis of the pyrrole–imidazole alkaloids. While the pyrrole part is generally expected to derive from...
CH3CN, room temperature, 24 h, 80%; (c) 8 N HCl, room temperature, 4 h, 85%.

Figure 1. CD spectrum of synthesized natural product L-1 (2,2,2-trifluoroethanol, c = 1.5 mM).

Figure 2. Proposal of the lysine-derived δ-hydroxyhomogarginine (15) as an alternative biogenetic precursor of the 2-amino-5-(3-aminopropylimidazoles).

proline/ornithine, different proposals have been put forward for the 2-amino-5-(3-aminopropylimidazoles part. Recently, the first experimental study on the biosynthesis of the pyrrole-imidazole alkaloid stevensine was performed by Pomponi, Kerr, and co-workers.9 The incorporation of histidine might proceed via an analogue of clathramide A10 (Figure 2) isolated from the oroidin source Agelas clathrodes. The C-methylation of the imidazole ring of 17 could provide the carbon atom required for chain elongation, for example, via a cyclopropanoid intermediate. Kitagawa et al.11 and Braekman et al.12 proposed ornithine as a biogenetic precursor, with the carboxyl carbon atom being incorporated into the imidazole ring and the α- and δ-amino groups incorporated into the imidazole ring and the side chain, respectively.

The discovery of the aplysinamisines and of the homogarginine derivative 1, together with the co-occurrence of lysine and bromopyrroles in marine sponges,13 points to the existence of an alternative biosynthesis (Figure 2). In analogy to the role of 4-hydroxyarginine as a biosynthetic precursor of the 2-aminimidazolone anatoxin-a(s),14 δ-hydroxyhomogarginine (15) is postulated as an intermediate in the biosynthesis of 5-(3-aminopropyl)-2-aminimidazoles. Intramolecular cyclization of 15 would lead to a 2-aminimidazoline, which would be further oxidized and undergo decarboxylation. The aminohomohistamine 16 with its saturated side chain is a partial structure of the marine natural product aerophobin-2 from Aplysia aerophoba.15 Horne et al. have shown that by oxidation of 16 (R = dibromopyrrolyl-2-carboxyl) the vinyl double bond of oridin (2) can be introduced.16 The formation of the putative intermediate 15 could occur via the guanidination of lysine to an analogue of the natural product 1, followed by δ-hydroxylation.

Experimental Section

General Experimental Procedures. The melting points are uncorrected. NMR chemical shifts refer to those of residual solvent signals based on δHMS = 0. FABMS were obtained with nitrobenzyl alcohol as matrix. Solvents were purified and dried according to standard procedures. Column chromatography was carried out on Si gel 60 (60–200 mesh, Merck) on Sephadex LH-20 (Pharmacia). HPLC separation columns were pre-filled with Kromasil RP18 (Knaufi Gmmer). CD spectra were obtained using the JASCO spectropolarimeter J-710. Thin-layer chromatography (TLC) was performed on Si gel plates (precoated Si gel plate F254 Merck).

4-Bromopyrrole-2-carboxylic Acid (6). Ethyl pyrrole-2-carboxylate18 was brominated and then hydrolyzed according to Anderson and Lee.19 After purification by preparative reversed-phase HPLC (gradient: 5 min A, 0–40% B in 45 min; A: 10% MeCN/H2O + 0.1% TFA; B: 100% MeCN + 0.1% TFA; flow rate 10 mL/min) 6 was frozen in liquid nitrogen and freeze-dried to afford 1.19 g (33%) of a white-gray powder; mp 142 °C (dec); UV (MeOH) λmax (log ε) 264 (4.05) nm; IR (KBr) νmax (cm−1) 2930, 2853, 1737, 1615, 1550, 1432, 1366, 1206, 1121 cm−1; 1H NMR (DMSO-d6, 250 MHz) δ = 8.45 (1H, NH), 7.10 (1H, CH), 6.75 (1H, CH); 13C NMR (DMSO-d6, 63 MHz) δ = 162.5 (1C, COOH), 123.8 (1C, NHCCO), 123.3 (1C, NHCHBr), 115.8 (1C, CBrCHC), 95.7 (1C, CBr); ESIMS (neg) m/z 188 (100), 199 (99); HRFABMS m/z 188.9420 [M + H]+ (calcd for C9H9BrNO2, 188.9426).
shaken 3 × 20 min each time with a solution of piperidine in DMF (40%, 5 mL). Afterward the resin was washed 4 × 5 min with DMF (10 mL). Then a solution of 4-bromopyrrole-2-carboxylic acid (42 mg, 0.20 mL, 0.22 mmol) and 4-dimethylaminopyridine (450 mg, 3.33 mmol) in DMF (5 mL) was added, and the mixture was shaken for 2 min at room temperature. After the addition of N,N-diisopropylcarbodiimide (404 µL, 2.59 mmol), the mixture was allowed to shake for 5 h at room temperature. After removing the solvent using air pressure, and the remaining resin was washed twice for 5 min with CH2Cl2 (10 mL), i-PrOH (10 mL), and Et2O (10 mL). Finally, the resin was treated with 98% TFA (5 mL) and shaken for 30 min at room temperature. The liquid phase was collected, and the resin was washed (2 × 5 min each) with H2O (2 × 10 mL) and MeCN (2 × 10 mL). Both the acid reaction solution and washings were combined, frozen with liquid nitrogen, and freeze-dried. The obtained crude white powder was purified by preparative reversed-phase HPLC (gradient: 5 min A, 0–40% B in 45 min; A: 10% MeCN/H2O + 0.1% TFA, B: 100% MeCN – 0.1% TFA; flow rate 10 mL/min). The purified HPLC fraction was frozen and freeze-dried yielding 100 mg (72%) of l-1 as white powder; tR 7.85 min (gradient: 20–60% MeCN/H2O + 0.1% TFA in 40 min; flow rate 1 mL/min); UV (H2O) λmax (log ε) 271 (4.06), 360 (4.37), 272 (4.01) nm; IR (KBr) νmax 3369, 3300, 3116, 2939, 2857, 1734, 1628, 1560; 1H NMR (DMSO-d6, 250 MHz) δ 11.87 (1H, NH), 8.14 (1H, NH), 7.41 (1H, NH), 6.96 (1H, CH), 6.91 (1H, CH), 4.25 (d, J = 4.9 Hz, CH2), 3.05 (d, J = 5.3 Hz, 2H, CH2NH), 1.73 (2H, CH2), 1.47 (m, 2H, CH2CH2), 1.37 (m, 2H, CH2CH2), 13C NMR (DMSO-d6, 63 MHz) δ 174.9 (IC, COOH), 159.2 (IC, CONH), 157.0 (1C, NCNHCO), 126.9 (1C, NCCH), 121.1 (1C, NCCHBCB), 111.9 (1C, CBRCB), 95.0 (1C, CB), 52.8 (1C, 406.1, CH), 403.6 (1C, CBCH), 28.3 (IC, CHCH3), 22.9 (IC, CHCH3), ESIMS (neg) m/z 358 (82), 360 (62); HRFABMS m/z 3600.0781 (calcd for C18H34N4O6, C 53.42%, H 8.43%, N 13.62%, calcd for C18H34N4O6, C 53.42%, H 8.43%, N 13.62%); HRFABMS m/z 3600.0781 (calcd for C18H34N4O6, C 53.42%, H 8.43%, N 13.62%); HRFABMS m/z 374.0877 (calcd for C18H34N4O6, C 53.42%, H 8.43%, N 13.62%).

The residue of l-1 was purified by preparative reversed-phase HPLC (gradient: 5 min A, 0–40% B in 45 min; A: 10% MeCN/H2O + 0.1% TFA, B: 100% MeCN – 0.1% TFA; flow rate 10 mL/min). The purified HPLC fraction was frozen and freeze-dried yielding 100 mg (72%) of l-1 as white powder; tR 7.85 min (gradient: 20–60% MeCN/H2O + 0.1% TFA in 40 min; flow rate 1 mL/min); UV (H2O) λmax (log ε) 271 (4.06), 360 (4.37), 272 (4.01) nm; IR (KBr) νmax 3369, 3300, 3116, 2939, 2857, 1734, 1628, 1560; 1H NMR (DMSO-d6, 250 MHz) δ 11.87 (1H, NH), 8.14 (1H, NH), 7.41 (1H, NH), 6.96 (1H, CH), 6.91 (1H, CH), 4.25 (d, J = 4.9 Hz, CH2), 3.05 (d, J = 5.3 Hz, 2H, CH2NH), 1.73 (2H, CH2), 1.47 (m, 2H, CH2CH2), 1.37 (m, 2H, CH2CH2), 13C NMR (DMSO-d6, 63 MHz) δ 174.9 (IC, COOH), 159.2 (IC, CONH), 157.0 (1C, NCNHCO), 126.9 (1C, NCCH), 121.1 (1C, NCCHBCB), 111.9 (1C, CBRCB), 95.0 (1C, CB), 52.8 (1C, 406.1, CH), 403.6 (1C, CBCH), 28.3 (IC, CHCH3), 22.9 (IC, CHCH3), ESIMS (neg) m/z 358 (82), 360 (62); HRFABMS m/z 3600.0781 (calcd for C18H34N4O6, C 53.42%, H 8.43%, N 13.62%).

(25)-2-Amino-6-[1-(4,5-dibromo-1-pyrrol-2-yl)-2-methanoyl]amino][hexanoido]Acid Methyl Ether (10). To a solution of l-lysine methyl ester hydrochloride (8, 200 mg, 0.86 mmol) in acetonitrile (3 mL) was added 2,2,2-trichloro-1-(4,5- dibromo-1-pyrrol-2-yl)ethanone (9, 318 mg, 0.86 mmol) and N,N-diisopropylethylamine (0.3 mL, 1.81 mmol). After 3 h at room temperature, the solution was evaporated under vacuum, and the crude residue was purified by column chromatography (Si gel, CHCl3/MeOH 10:1) to yield 10 as colorless foam (265 mg, 75%); [α]D +10° (c 0.02, MeOH); UV (MeOH) λmax (log ε) 208 (4.26), 234 (4.37), 270 (4.01) nm; IR (KBr) νmax 3369, 3300, 3116, 2939, 2857, 1734, 1628, 1560; 1H NMR (MeOH-d4, 250 MHz) δ 11.48 (1H, br, s, NH), 10.64 (1H, br, s, NH), 8.33 (1H, br, s, NH), 6.89 (1H, m, CBRCB), 6.84 (1H, m, NH), 6.74 (1H, m, CBRCB), 3.09 (2H, m, CH2), 3.38 (2H, m, CH2NH), 1.56 (18H, s, CH3), 2.00–3.60 (6H, m, CH2CH2); HRFABMS m/z 543.2570 (calcd for C23H37N5O779Br, 543.2570); C 53.42%, H 8.43%, N 13.62%.

(25)-2-[1-(4-Bromo-1-phenyl-2-pyrrol-2-yl)phenylmethanoylamino][6-N,N-bis-(ter-butoxy carbonyl)]guanidine Hexanoic Methyl Ester (14). To a solution of 12 (400 mg, 1.00 mmol) in MeCN (3 mL) was added 2,2,2-trichloro-1-(4-bromo-1-phenyl-2-pyrrol-2-yl)ethanone (13, 270 mg, 1.00 mmol) and N,N-diisopropylethylamine (0.20 mL, 1.10 mmol). After 2 h at room temperature, the solvent was evaporated, and the crude residue was purified by column chromatography (Si gel, CHCl3/MeOH 50:1) to yield 14 as colorless foam (511 mg, 89%); [α]D +0.6 (c 1.05, MeOH); UV (MeOH) λmax (log ε) 208 (4.26), 234 (4.37), 270 (4.01) nm; IR (KBr) νmax 3369, 3300, 3116, 2939, 2857, 1734, 1628, 1560; 1H NMR (MeOH-d4, 250 MHz) δ 11.48 (1H, br, s, NH), 10.64 (1H, br, s, NH), 8.33 (1H, br, s, NH), 6.89 (1H, m, CBRCB), 6.84 (1H, m, NH), 6.74 (1H, m, CBRCB), 3.09 (2H, m, CH2), 3.38 (2H, m, CH2NH), 1.56 (18H, s, CH3), 2.00–3.60 (6H, m, CH2CH2); HRFABMS m/z 543.2570 (calcd for C23H37N5O779Br, 543.2570); C 53.42%, H 8.43%, N 13.62%.
29.3 (1C, CH2CH2NH), 24.3 (1C, CHCH2C\textsubscript{2}H); FABMS m/z 382/384 (84/86) [M + Na]+, 360/362 (80/80) [M]+; HRFABMS m/z 382.0486 (calcld for C\textsubscript{12}H\textsubscript{18}N\textsubscript{5}O\textsubscript{3}79BrNa, 382.0491).

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (Li 597/2-2 and Ko 1314/3-1 to 3-4). T.L. and M.H. thank Professor Dr. Richard Neidlein for generous support. Damian Kokot is thanked for technical assistance. M.A. and M.K. are grateful to Ellen Lichte for performing HPLC analyses and acknowledge the support of Professor Dr. Christian Griesinger.

References and Notes


NP0001600