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Isolation and Synthesis of Caprolactins A and B, New Caprolactams from a Marine Bacterium

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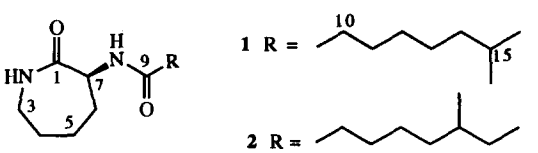
Abstract: Two new caprolactams have been isolated from an unidentified Gram-positive bacterium obtained from a deep-ocean sediment sample. Caprolactins A (**1**) and B (**2**), which were obtained as an inseparable mixture, are composed of cyclic-L-lysine linked to 7-methyloctanoic acid and 6-methyloctanoic acid, respectively. The structures were proposed using spectroscopic methods and confirmed by synthesis. Both caprolactins A and B are cytotoxic towards human epidermoid carcinoma (KB) cells and human colorectal adenocarcinoma (LoVo) cells and exhibit antiviral activity towards *Herpes simplex* type II virus.

Marine bacteria have been the subject of a growing number of natural products studies. Recent investigations have demonstrated that, like their terrestrial counterparts, these organisms are prolific producers of unique secondary metabolites. Interesting compounds have been isolated from bacteria cultured from a variety of marine habitats. Examples include the tetracyclic alkaloid alteramide A, isolated from a bacterium *Alteromonas* sp. found in association with the marine sponge *Halichondria okadai*;[1] the octalactins, which are cytotoxic eight-membered-ring lactones isolated from a *Streptomyces* sp. bacterium obtained from the surface of a gorgonian octacoral;[2] and the macrolactins, cytotoxic and antiviral macrolides isolated from an unidentified Gram-positive bacterium cultured from a deep-sea sediment sample.[3] We would now like to report the isolation and syntheses of caprolactins A (**1**) and B (**2**), new caprolactams produced by an unidentified Gram-positive marine bacterium cultured from deep ocean sediments. Both caprolactins A and B are mildly cytotoxic towards human epidermoid carcinoma (KB) cells and human colorectal adenocarcinoma (LoVo) cells with MIC values of 10 and 5 μg/mL, respectively, and exhibit antiviral activity towards *Herpes simplex* type II virus at a concentration of 100 μg/mL.



The bacterium[4] was grown in liquid culture and the whole culture broth was extracted with ethyl acetate. Chromatography over Sephadex LH-20, followed by reverse-phase HPLC, yielded what appeared to be a single pure compound, but was later determined to be a mixture of **1** and **2**. The EI mass spectrum of the sample showed a single molecular ion at *m/z* 268 requiring a molecular formula of $C_{15}H_{28}N_2O_2$ (268.2161, Δ -1.0 mmu); however, the ^{13}C NMR spectrum contained 21 discrete signals, indicating that the sample consisted of a mixture of two isomeric compounds. Characteristic ^{13}C NMR signals (see Table 1) included those assigned to two amide carbonyl carbons (175.7 and 172.3 ppm) and a heteroatom-substituted carbon at δ 52.0. The presence of the two amides was supported by IR peaks at 3335, 3272, 1635, and 1617 cm^{-1} and by ^1H NMR signals at δ 6.86 (d, J = 5.2 Hz) and δ 6.21 (t, J = 4.6 Hz). The ^1H NMR spectrum also contained a one-proton signal at δ

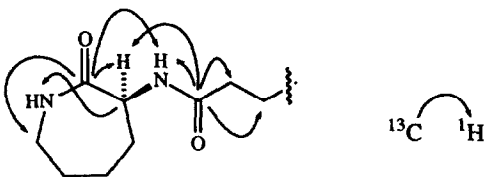
4.52 (ddd, J = 11.1, 5.9, 2.0 Hz), which could be assigned to H7, and two two-proton signals at δ 3.26 and 2.21, assigned to protons H3 and H10, respectively, the latter of which appeared as a doublet of triplets (J = 7.6, ~3 Hz).

Table 1. ^{13}C NMR Assignments for **1** and **2**.

	Compound	
Atom no.	**1**	**2**
1	172.3	172.3
3	42.1	42.1
4	28.9	28.9
5	27.9	27.9
6	31.7	31.7
7	52.0	52.0
9	175.7	175.7
10	36.6	36.6
11	25.7	26.0
12	29.5	26.7
13	27.1	34.2
14	38.8	36.2
15	27.9	29.4
16	22.6	11.3
17	22.6	19.1

[a]All data were recorded at 125 MHz in $CDCl_3$. Chemical Shifts were referenced to the solvent (77.0 ppm).

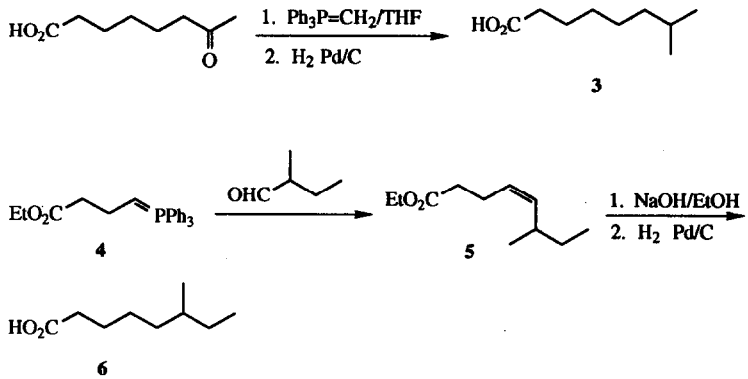
An HMBC experiment[5] allowed the construction of the primary cyclic structural unit found in both **1** and **2**. The most important correlations are shown below. A COSY experiment confirmed the acylated cyclo-lysine subunit; however, significant signal overlap did not allow the complete assignment of the acyl side-chains. The mass spectrum showed an ion at m/z 170, formed by the loss of a C_7H_{14} side-chain, presumably through a McLafferty-type rearrangement, and a base peak at m/z 127, generated by cleavage of the N8-C9 bond.



Acid hydrolysis (6 N HCl, 120 °C for 16 hr) of the mixture of caprolactins A and B yielded a single water-soluble product, which by TLC (silica gel, n-propanol/NH_4OH 7:3) matched an authentic sample of lysine. Also, the hydrolysis yielded a 3:2 ratio of two organic-soluble hydrolysis products, as determined by gas chromatography, confirming that the sample did, in fact, contain two closely related compounds. The absolute configuration of the lysine moiety was unambiguously determined to be L, both by optical rotation and by derivatization of the aqueous sample with Marfey's reagent,[6] followed by TLC analysis.[7]

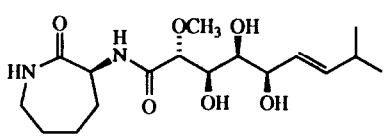
Based on the ^{13}C NMR chemical shifts (see Table 1), together with the MS data, caprolactin A was proposed to contain a 7-methyloctanoic acid unit, while the acyl side-chain in caprolactin B was proposed to be 6-methyloctanoic acid. In order to confirm that caprolactins A and B corresponded to structures **1** and **2**, respectively, the acyl units were synthesized as outlined in Scheme I, and coupled with (±)-2-aminocaprolactam[8] using pivalic anhydride as a coupling agent.[9,10]

7-Methyloctanoic acid (**3**) was prepared in two steps from 7-oxooctanoic acid by a Wittig methylenation, followed by hydrogenation over palladium/carbon. 6-Methyloctanoic acid (**6**) was synthesized using a Wittig coupling of (±)-2-methylbutanal and ylide **4**, formed by treating the appropriate phosphonium iodide salt with NaOEt in DMF.[11,12] Saponification of the resulting ester **5**, followed by hydrogenation, produced the desired acid (**6**).



The NMR spectra recorded for both synthetic **1** and **2** matched perfectly the corresponding peaks observed in the spectra obtained for the mixture of natural products, thus confirming the proposed structures. The signals assigned to the H10 methylene protons in the spectra recorded for both synthetic products were observed as clean triplets, indicating that the doublet of triplets which occurred in the spectrum of the natural products resulted from a small chemical shift difference (~3 Hz) between the H10 signals for **1** and **2**. The absence of separate sets of ^{13}C NMR signals in the spectrum obtained for synthetic **2**, arising from the diastereomeric products necessarily formed when (±)-2-aminocaprolactam and (±)-6-methyloctanoic acid are coupled, is consistant with having chiral centers which are far apart within a molecule and, therefore, do not influence one another. The configuration of the C14 center in caprolactin B remains undetermined.

Natural products containing a cyclized lysine are uncommon; in fact, the only reported examples are the bengamides,[13,14] isolated from a *Jaspis* sp. sponge, four sesquiterpene caprolactams from the sponges *Pachastrella* sp.[15] and *Poecillastra sollasi*,[16] and the fungal metabolite circinatin.[17] Compared to the caprolactins,



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these metabolites all incorporate more highly functionalized acyl side-chains. Circinatin was reported to be nontoxic, while the bengamides exhibited anthelmintic activity. Recently, there has been considerable speculation that compounds isolated from sponges may be, in some cases, products of symbiotic bacterial secondary metabolism. The obvious similarities between the caprolactins and the bengamides [e.g., bengamide E (**4**)] may lend additional circumstantial support for a suggested bacterial origin for the bengamides.[13]

EXPERIMENTAL SECTION

Culture Conditions: The bacterium, designated PC12/1000-B4, was obtained from a sample of sediments taken at a 6.67 m depth along a deep ocean sediment sample (# PCOD012 in University of Hawaii Corelab) which was drilled at 11° 26.4' N latitude 168° 14.4' W longitude at a depth of 5065 m on October 10, 1978. A sample of sediment was diluted 1000:1 with sterile artificial seawater and plated onto marine agar 2216 (Difco) petri plates. The isolated culture was grown in 16 L of marine broth 2216 (Difco) for 13 days at 23-25 °C with aeration.

Extraction and Purification: The liquid culture was extracted with ethyl acetate yielding 1.1 g of organic soluble material. Chromatography of 484.0 mg of extract over Sephadex LH-20 (110 x 2 cm) using CH_2Cl_2/MeOH (1:1) as eluent, yielded aliquots which were combined into three fractions. The middle fraction (278.7 mg) was further purified by reverse-phase HPLC (Rainin Microsorb C18, 25 cm x 10 mm, CH_3CN/H_2O, 2.0 mL/min) to give 246.9 mg of a mixture of caprolactins A (**1**) and B (**2**).

Natural Caprolactins A and B (1 + 2): $[\alpha]^{22}_D$ +5.4° (c 1.03, CH_2Cl_2); UV (MeOH) λ_{max} 219, 274 (sh) nm; IR (film) ν 3335, 3272, 2926, 2854, 1635, 1617, 1526, 1478 cm^{-1}; ^1H NMR δ ($CDCl_3$) 6.86 (1H, d, J = 5.2 Hz), 6.21 (1H, t, J = 4.6 Hz), 4.52 (1H, ddd, J = 11.1, 5.9, 2.0 Hz), 3.26 (2H, m), 2.21 (2H, dt, J = 7.6, 3.0 Hz), 2.07 (1H, dm, J = 13.7 Hz), 1.98 (1H, m), 1.88 - 1.78 (3H, m), 1.62 (2H, m), 1.50 (1H, sept, J = 6.7 Hz), 1.5 - 1.3 (3H, m), 1.3 - 1.25 (6H, m), 1.17 - 1.08 (2H, m), 0.84 (6H, d, J = 6.7 Hz), 0.83 (t, J = 7.4 Hz), 0.82 (d, J = 6.3); ^{13}C NMR see Table 1; EIMS *m/z* (rel int) 268 (15), 183 (14), 170 (65), 155 (10), 129 (37), 128 (34), 127 (100); HREIMS 268.2161 ($C_{15}H_{28}N_2O_2$, Δ -1.0 mmu), 170.1057 ($C_8H_{14}N_2O_2$, Δ -0.2 mmu), 127.0873 ($C_6H_{11}N_2O$, Δ -0.1 mmu).

Determination of Lysine Absolute Configuration: To a mixture of **1** and **2** (10 mg) in a pressure tube, was added 6 N HCl (1 mL) and the tube was purged with argon and sealed. After heating for 16 h at 120 °C, the aqueous solution was extracted with diethyl ether and aqueous solution was evaporated under a stream of argon. An authetic sample of L-lysine was likewise dissolved in 6 N HCl and then concentrated under a stream of argon. The optical rotation for the hydrolysis product ($[\alpha]^{25}_D$ +10.8° (c 0.833, H_2O)) matched the value obtained for authentic L-lysine ($[\alpha]^{25}_D$ +10.9° (c 0.834, H_2O)). Furthermore, the hydrolysis product, L-lysine, and DL-lysine were all treated with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, FDAA)[5] and subjected to TLC for analysis.[7] To 100 μL of an aqueous sample of amino acid (5 mg/mL) was added 1% FDAA in acetone (200 μL) and $NaHCO_3$ (40 μL, 1.0 M) and the solution was stirred at 45 °C for 45 minutes. The reaction was allowed to cool to room temperature and and was then quenched with 6 N HCl. TLC analyses were performed on reversed-phase C18 plates with methanol/0.3 M sodium acetate (pH 4) in a ratio of 0.43/0.57 as the eluting solvent. Under these conditions the derivatized hydrolysis product clearly matched derivatized L-lysine.

7-Methyl-7-octenoic acid: To a solution of methyltriphenylphosphonium bromide (2.31 g, 6.48 mmol) in dry THF (25 mL) under argon at -78 °C was added *n*-butyllithium (6.64 mmol, 1.6 M in hexane). The solution was stirred for 45 min and a solution of 7-oxooctanoic acid (0.5 g, 3.16 mmol) in THF was added dropwise. The reaction mixture was then allowed to warm to room temperature and stirring was continued for another 1.5 h. The reaction was quenched with H_2O and diluted with diethyl ether. The organic solution was extracted with 0.1 M NaOH (x3) and the combined aqueous layers were acidified to pH 2 and extracted again with diethyl ether (x3). The combined ether layers were dried ($MgSO_4$) and concentrated to give an organic residue, which was purified by flash chromatography over silica gel (9:1->6:4 hexane/ethyl acetate). 7-Methyl-7-octenoic acid (280 mg, 1.79 mmol, 57%) was obtained as a colorless oil: IR (film) ν 3074, 2934, 2860, 2675, 2380, 2341, 1715, 1651, 1414, 1374, 1283, 1225, 1087, 938, 887 cm^{-1}; ^1H NMR δ ($CDCl_3$) 10.80 (1H, bs), 5.3 (1H, m), 5.19 (1H, dd, J = 10.0, 10.4 Hz), 2.4 (4H, m), 1.25 (3H, m), 0.93 (3H, d, J = 6.7 Hz), 0.83 (3H, t,

J = 7.4 Hz); ^{13}C NMR δ ($CDCl_3$) 179.9, 137.9, 125.6, 34.4, 33.4, 30.1, 22.7, 20.9, 11.9; EIMS *m/z* (rel int) 156 (7), 138 (19), 101 (15), 81 (16), 69 (59), 56 (100); HREIMS 156.1136 ($C_9H_{16}O_2$, Δ 1.4 mmu).

7-Methyloctanoic acid (3): To a solution of 7-methyl-7-octenoic acid (100 mg, 0.64 mmol) in ethanol (2-3 mL.) was added 10% Pd/C (6 mg). The mixture was stirred under an atomosphere of hydrogen for 1 h, at which time the reaction mixture was filtered through Celite, and concentrated to give 7-methyloctanoic acid (101 mg, 0.64 mmol, 100%) as a colorless oil: IR (film) ν 3425, 2961, 2874, 2363, 1710, 1654, 1460, 1413, 1378, 1286, 1236 cm^{-1}; ^1H NMR δ ($CDCl_3$) 10.5 (1H, br s), 2.35 (2H, t, J = 7.5), 1.61 (2H, m), 1.3 (5H, m), 1.12 (2H, m), 0.85 (3H, t, J = 7.2 Hz), 0.84 (3H, d, J = 6.3 Hz); ^{13}C NMR δ ($CDCl_3$) 180.5, 36.1, 34.2, 29.4, 26.6, 25.0, 19.1, 11.3; EIMS *m/z* (rel int) 158 (2), 115 (25), 99 (19), 97 (16), 87 (18), 83 (20), 73 (100); HREIMS 158.1306 ($C_9H_{18}O_2$, Δ 0.1 mmu).

Ethyl (Z)-6-methyl-4-octenoate (5): To a solution of NaOEt (0.64 g, 7.86 mmol) in DMF (35 mL) was added (4-carbethoxybutyl)triphenylphosphonium iodide[11,12] (3.7 g, 7.3 mmol). After stirring the reaction for one hour at 0 °C under an atmosphere of argon, (±)-2-methylbutanal (0.48 g, 5.61 mmol) was added dropwise. Upon warming to room temperature, the reaction was quenched with H_2O and extracted with diethyl ether. The organic extract was dried ($MgSO_4$) and concentrated to give the desired product (306 mg, 1.66 mmol, 30% based on starting aldehyde), as a colorless oil. IR (film) 3420, 2963, 2090, 1738, 1645, 1457, 1372, 1349, 1173, 1048 cm^{-1}; ^1H NMR δ ($CDCl_3$) 5.26 (1H, m), 5.12 (1H dd, J = 9.8, 10.6 Hz), 4.1 (2H, q, J = 7.1 Hz), 2.25 (4H, m), 1.23 (3H, t, J = 7.1 Hz.), 1.25 (1H, m), 0.90 (3H, d, J = 6.6 Hz), 0.85 (2H, m), 0.80 (3H t, J = 7.4 Hz); ^{13}C NMR δ ($CDCl_3$) 173.2, 137.6, 126.0, 60.2, 34.6, 33.4, 30.1, 23.1, 20.1, 14.2, 11.9; EIMS *m/z* (rel int) 184 (4), 110 (24), 96 (100); HREIMS 184.1453 ($C_{11}H_{20}O_2$, Δ 1.1 mmu).

(Z)-6-Methyl-4-octenoic acid: To a solution of ethyl (Z)-6-methyl-4-octenoate (80 mg, 0.43 mmol) in methanol (6.4 mL) was added 2 N NaOH (1.6 mL) and the solution was stirred at room temperature for 12 h. The solution was then acidified to pH 2 and extracted with diethyl ether (x3). The organic layer was dried ($MgSO_4$) and concentrated, giving (Z)-6-methyl-4-octenoic acid (64 mg, 0.41 mmol, 95%) as a colorless oil. The reaction was monitored by silica gel TLC using bromophenol indicator for detection: IR (film) ν 2959, 2943, 1712, 1458, 1281, 1211, 937 cm^{-1}; ^1H NMR δ ($CDCl_3$) 10.80 (1H, bs), 5.18 (1H, m), 5.08 (1H, dd, J = 10.0, 10.4 Hz), 2.29 (4H, m), 1.15 (3H, m), 0.82 (3H, d, J = 6.7 Hz), 0.73 (3H, t, J = 7.4 Hz); ^{13}C NMR δ ($CDCl_3$) 179.9, 137.9, 125.6, 34.4, 33.4, 30.1, 22.7, 20.9, 11.9; EIMS *m/z* (rel int) 156 (7), 127 (15), 109 (18), 96 (81), 85 (29), 83 (22), 81 (100); HREIMS 156.1139 ($C_9H_{16}O_2$, Δ 1.1 mmu).

6-Methyloctanoic acid (6): To a solution of (Z)-6-methyl-4-octenoic acid (60 mg, 0.38 mmol) in ethanol (2-3 mL.) was added 10% Pd/C (4 mg). The mixture was stirred under an atmosphere of hydrogen for 2 h, at which time the reaction mixture was filtered through Celite and concentrated to give 6-methyloctanoic acid (55 mg, 0.35 mmol, 92%) as a colorless oil: IR (film) ν 3425, 2961, 2874, 2362, 1710, 1654, 1460, 1412, 1378, 1286, 1236 cm^{-1}; ^1H NMR δ ($CDCl_3$) 10.30 (1H, br s), 2.35 (2H, t, J = 7.5 Hz), 1.63 (2H, m), 1.31 (5H, m), 1.12 (2H, m), 0.85 (6H, m, J = 7.2, 6.3 Hz); ^{13}C NMR δ ($CDCl_3$) 180.5, 36.1, 34.2, 29.4, 26.6, 25.0, 19.1, 11.3; EIMS *m/z* (rel int) 129 (9), 111 (28), 96 (97), 83 (59), 74 (100); HREIMS 158.1305 ($C_9H_{18}O_2$, Δ 0.2 mmu).

Pivalic anhydride coupling: To a solution of acid, 7-methyloctanoic acid (50 mg, 316 mmol) or 6-methyloctanoic acid (12 mg, 0.076 mmol), in dichloromethane (5 ml) was added triethylamine (2 eq) and pivaloyl chloride (1 eq). The mixture was stirred at 0 °C and monitored by TLC. To this mixture was added 2-aminocaprolactam[7] (1 eq) and the reaction was stirred at 0 °C for 4 hours. The reaction mixture was washed with saturated NH_4Cl , $NaHCO_3$, water, and brine. The organic layer was dried ($MgSO_4$) and concentrated to give the desired amides, caprolactams A (12.0 mg, 15%) and B (11.5 mg, 50%), as a white powders.

Synthetic Caprolactin A (1): IR (film) ν 3314, 3226, 3085, 2955, 2926, 2858, 1670, 1634, 1534, 1484, 1434, 1377, 1333, 1288, 965 cm^{-1}; ^1H NMR δ ($CDCl_3$) 6.85 (1H, d, J = 4.15 Hz), 6.01 (1H, br t), 4.53 (1H, ddd, J = 11.3, 5.9, 1.7 Hz), 3.26 (2H, m), 2.21 (2H, t, J = 7.5 Hz), 2.08 (dm, J = 13.5 Hz), 1.99 (1H, m), 1.88 - 1.76 (2H, m), 1.63 (2H, m), 1.50 (1H, sept, J = 6.5 Hz), 1.48 - 1.34 (2H, m), 1.23 (4H, m), 1.15 (2H, m), 0.85 (3H, d, J = 6.8 Hz); ^{13}C NMR δ 175.6 (C9), 172.4 (C1), 52.0 (C7), 42.2 (C3), 38.8 (C14), 36.7 (C10), 31.7 (C6), 29.5 (C12), 28.9 (C4), 27.9 (C5), 27.1 (C13), 25.7 (C11), 22.6 (C16/C17); HREIMS 268.2135 ($C_{15}H_{28}N_2O_2$, Δ 1.6 mmu).

Synthetic Caprolactin B (2): IR (film) ν 3314, 3226, 3085, 2955, 2926, 2858, 1670, 1634, 1534, 1484, 1434, 1377, 1333, 1288, 965 cm^{-1}; ^1H NMR δ ($CDCl_3$) 6.87 (1H, d, J = 5.3 Hz), 6.35 (1H, br t), 4.52 (1H, ddd, J = 11.3, 6.0, 1.8 Hz), 3.26 (2H, m), 2.20 (2H, t, J = 7.6 Hz), 2.06 (1H, dm, J = 13.0 Hz), 1.98 (1H, m), 1.87 - 1.77 (2H, m), 1.60 (2H, m), 1.5 - 1.32 (2H, m), 1.32 - 1.25 (5H, m), 1.10 (2H, m), 0.83 (3H, t, J = 7.4 Hz), 0.82 (3H, d, J = 6.4 Hz); ^{13}C NMR δ ($CDCl_3$) 175.8 (C9), 172.3 (C1), 52.0 (C7), 42.1 (C3), 36.6 (C10), 36.2 (C14), 34.2 (C13), 31.7 (C6), 29.4 (C15), 28.8 (C4), 27.9 (C5), 26.7 (C12), 25.9 (C11), 19.1 (C17), 11.3 (16); HREIMS 268.2187 ($C_{15}H_{28}N_2O_2$, Δ -3.6 mmu).

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