Cyclic Peptides of the Nocardamine Class from a Marine-Derived Bacterium of the Genus *Streptomyces*

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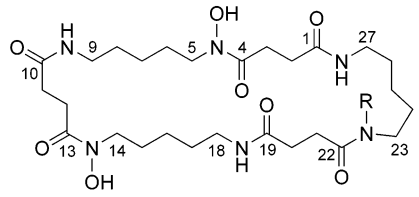
Two new cyclic peptides (**2** and **3**) along with the previously reported nocardamine (**1**) were isolated from the culture broth of an actinomycete of the genus *Streptomyces* isolated from an unidentified marine sponge. On the basis of the results of combined spectral analyses, the structures of the new compounds were defined to be the dehydroxy and desmethylenyl derivatives of nocardamine, respectively. The new compounds exhibited weak inhibition against the enzyme sortase B.

Marine microorganisms are widely recognized as rich sources of secondary metabolites.[1] These organisms, flourishing in diverse marine environments, have produced a wide variety of structurally unique and biologically active compounds that have attracted considerable attention for biomedical studies.[1−4] During the course of our search for novel natural products from marine bacteria, we collected an actinomycete of the genus *Streptomyces* from an unidentified sponge collected from Jaeju Island, Korea. The culture broth of this strain contained several nitrogenous compounds in its moderately polar chromatographic fractions. We describe herein the isolation and structure determination of two new cyclic peptides of the nocardamine class.

The strain M1087 was inoculated in SYP medium and fermented in a 3 L Fernbach flask for 10 days. The culture broth was filtered through a membrane and subjected to HP20 adsorption chromatography. Guided by the results of ^{1}H NMR analysis, separation of the moderately polar fractions using vacuum flash chromatography on silica gel followed by ODS HPLC led to the isolation of compounds **1**−**3** as colorless amorphous solids.

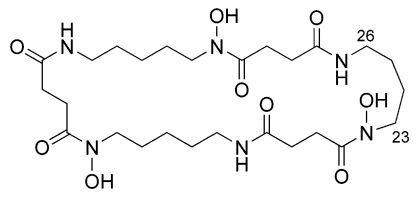
The spectral data for compound **1**, including those obtained from NMR and mass spectrometry, were in good agreement with reported data for nocardamine previously isolated from various strains of bacteria such as *Nocardia* sp., *Pseudomonas stutzeri*, and *Streptomyces hygroscopicus* var. *geldanus*.[5−10] In particular, the 3-fold symmetry of the molecule, a characteristic feature of this compound, was evident from both ^{1}H and ^{13}C NMR data, in which signals of only seven methylene protons and nine carbons were observed.[9]

The molecular formula of compound **2** was deduced as $C_{27}H_{48}N_6O_8$ on the basis of HRFABMS analysis. However, the ^{13}C NMR spectra for this compound showed only highly disproportionate signals of 20 carbons (6 × C, 14 × CH_2). Although the chemical shifts of most of the carbons were very similar to those of **1**, the presence of disproportionate signals of methylene carbons suggested that this compound lost the characteristic 3-fold symmetry of nocardamine. The



1 R = OH

2 R = H



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^{1}H NMR data of **2** also showed proton signals at chemical shifts similar to those of **1** with the appearance of a singlet peak at δ 2.46 as the most noticeable change. These spectral differences, coupled with the loss of an oxygen atom in the molecular formula of **2** from **1**, revealed that an oxygenated functionality of **1** was changed to a different group in **2**.

With the aid of this information, the structure of **2** was determined by 2-D NMR analyses. A combination of ^{1}H COSY, TOCSY, and g(gradient)HSQC experiments revealed the presence of two succinyl ($COCH_2CH_2CO$) and three cadaverine ($NCH_2CH_2CH_2CH_2CH_2N$) moieties in the molecule. Careful examination of the ^{1}H NMR data revealed that chemical shifts of the terminal methylene protons of each cadaverine unit were either δ 3.60 or 3.16. Comparison of the NMR data with **1**, combined with the molecular formula, showed that two of the cadaverine units had NOH (terminal methylene at δ 3.60) and NH (δ 3.16) at each terminal, while the remaining unit had NH at both ends. The alternate connection among the succinyl and cadaverine units was determined by gHMBC data in which carbonyl carbons showed long-range correlations with neighboring methylene protons.

For the remaining unit, the gHSQC data showed a direct coupling between the methylene carbon at δ 32.6 and a singlet proton peak at δ 2.46 corresponding to four protons

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by integration in the ^{1}H NMR spectra. The singlet mode of these protons as well as the long-range correlations between the methylene protons and neighboring carbonyl carbons in the gHMBC data revealed that one of the succinyl groups was indeed a succinyl amide ($NHCOCH_2$-CH_2CONH). This interpretation was in accordance with the presence of a cadaverine unit with an NH group at both ends. Thus, the structure of compound **2** was determined to be the dehydroxy derivative of nocardamine (**1**).

The molecular formula of compound **3** was assigned as $C_{26}H_{46}N_6O_9$ on the basis of combined ^{13}C NMR and HRFABMS spectrometry. The spectral data of this compound were highly compatible with those obtained for **1** and **2**, with a decrease of signal intensity of protons at δ ~1.3 in the ^{1}H NMR spectra as the most noticeable difference. A combination of 2-D NMR analyses showed that **3** contained the same succinyl amides as **1**. In addition to two C_5 cadaverine units, however, detailed interpretation of NMR data showed the appearance of a C_4 putrescine ($NHCH_2CH_2CH_2CH_2N$) unit that was supported by long-range correlations of the newly appearing carbons at δ 27.4 (C-25) and 25.0 (C-24) with neighboring methylene protons in the gHMBC data. Thus the structure of compound **3** was defined as a desmethylenyl derivative of nocardamine (**1**).

Nocardamine and bisucaberine, a dimeric cyclic peptide of the same structural class, are related to the ferrichrome antibiotics and exhibit significant binding affinity to ferric ion.[10−12] Nocardamine is also reported to display antibacterial activity against mycobacteria.[15] However, in our measurement of antimicrobial activity, compounds **1**−**3** were not active against various strains of bacteria (Gram-positive: *Bacillus subtilis* ATCC 6633, *Micrococcus leuteus* ATCC 10240, *Staphylococcus aureus* ATCC 6538, Gram-negative: *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 10031, *Salmonella typhimurium* ATCC 14028) and fungi (*Candida albicans* ATCC 10231, *Trichophyton mentagrophytes* IFO 40996, *Trichophyton rubrum* IFO 6204) including the mycobacterium *Proteus vulgaris* ATCC 3851 at a concentration of 200 μg/mL. Contrarily, the new compounds exhibited weak inhibition against the recombinant enzyme sortase B with EC_{50} values of 88.3 and 126.4 μg/mL for **2** and **3**, respectively, while nocardamine was inactive (EC_{50} >200 μg/mL).[16,17]

Experimental Section

General Experimental Procedures. Melting points were measured on a Büchi B-540 apparatus. IR spectra were recorded on a Mattson GALAXY spectrophotometer. NMR spectra were recorded in CD_3OD solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectra were obtained by using a JEOL JMS-HX 110 high-resolution mass spectrometer and provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Collection and Taxonomic Identification. The bacterial strain M1087 was isolated using a modified Bennett agar plate (0.1% yeast extract, 0.1% beef extract, 0.2% tryptone, 1% dextrose, 1.5% agar) from an unidentified sponge collected using scuba (15 m depth) off the shore of Jaeju Island, Korea, in October 1999. The strain was maintained on a yeast extract-malt extract agar containing 70% natural seawater. The colony morphology of the strain M1087 grown on an ISP-5 agar plate (1% glycerol, 0.1% L-asparagine, 0.1% K_2HPO_4, 0.01% $FeSO_4 \cdot 7H_2O$, 0.01% $MnCl_2 \cdot 4H_2O$, 0.01% $ZnSO_4 \cdot 7H_2O$, 2% agar) at 30 °C for 3 days was round, regular, entire, and matt type with brownish gray vegetative, having a white mycelium with conidia sporopore.

For taxonomic identification, this strain was analyzed following the 16S rDNA partial sequence method.[13,14] The sequence of 576 base pairs was in good agreement with several type strains of *Streptomyces*; 99.65% similarity to *S. albidoflavus* DSM 40455, *S. canescens* DSM 40001, *S. coelicolor* DSM 40233, *S. felleus* DSM 40130, *S, odorifer* DAM 40347, and *S. sampsonii* ATCC25495 and 98.25% similarity to *S. intermedius* DSM 40372 and *S. rutgersensis* ssp. *rutgersensis* DSM 40077, respectively, while similarity with other type strains was much lower. The strain is currently on deposit in the Microbial Collection, KORDI, under the curatorship of H.J.S.

Fermentation. The slant culture of M1087 was inoculated in a 500 mL Erlenmeyer flask containing 100 mL of SYP medium (1% soluble starch, 0.4% yeast extract, 0.2% bactopeptone, 10 mM TrisHCl buffer, pH 7.5) in 75% aged seawater and incubated at 27 °C for 4 days on a rotary shaker (150 rpm). Fermentation was carried out by transfer of 5 mL of seed culture to a 3 L Fernbach flask containing 0.8 L of the same medium and incubation for 10 days under similar conditions.

Extraction and Isolation. The combined fermentation broth (30 L) was filtered using a membrane (pore size 0.45 μm) in a tangential filter system. The filtered broth was subjected to Diaion HP20 adsorption chromatography sequentially using H_2O, 50% aqueous MeOH, 50% aqueous acetone, MeOH, acetone, and EtOAc as eluents. The fractions eluted with MeOH and acetone were combined, dried in vacuo (13.3 g), and separated by silica vacuum flash chromatography using a 10% gradient mixture of CH_2Cl_2 and MeOH. The fraction eluted with 60% CH_2Cl_2 in MeOH (680.7 mg) was filtered through a ODS Sep-Pak column (60% aqueous MeOH), and the residue (575.4 mg) was separated by reversed-phase HPLC (YMC-ODS-A column, 60% aqueous MeOH) to yield 39.0, 17.6, and 15.5 mg of **1**−**3**, respectively.

Nocardamine (1): colorless solid, mp 185−188 °C (dec) [lit. 181−183 °C];[9] IR (KBr) ν_{max} 3420 (br), 1635, 1480, 1245 cm^{-1}; HRFABMS *m/z* 601.3561 $[M + H]^+$ (calcd for $C_{27}H_{49}N_6O_9$, 601.3564).

Dehydroxynocardamine (2): colorless solid, mp 179−182 °C (dec); IR (KBr) ν_{max} 3420 (br), 1640, 1480, 1250 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.60 (4H, t, J = 6.3 Hz, H-5, H-14), 3.16 (4H, t, J = 6.3 Hz, H-23, H-27), 3.15 (4H, t, J = 6.3 Hz, H-9, H-18), 2.77 (4H, t, J = 6.8 Hz, H-3, H-12), 2.46 (4H, s, H-20, H-21), 2.46 (4H, t, J = 6.8 Hz, H-2, H-11), 1.62 (4H, tt, J = 6.8, 6.3 Hz, H-6, H-15), 1.50 (8H, m, H-8, H-17, H-24, H-26), 1.34 (2H, p, J = 6.8 Hz, H-25), 1.32 (4H, p, J = 6.8 Hz, H-7, H-16); ^{13}C NMR (CD_3OD) δ 175.0 (C, C-1, C-10), 174.5 (C, C-4, C-13, C-19, C-22), 48.6 (CH_2, C-5, C-14), 40.2 (CH_2, C-9, C-18, C-23, C-27), 32.6 (CH_2, C-20, C-21), 31.6 (CH_2, C-2, C-11), 29.9 (CH_2, C-8, C-17), 29.8 (CH_2, C-24, C-26), 28.9 (CH_2, C-3, C-12), 27.2 (CH_2, C-6, C-15), 24.9 (CH_2, C-25), 24.6 (CH_2, C-7, C-16); HRFABMS *m/z* 585.3613 $[M + H]^+$ (calcd for $C_{27}H_{49}N_6O_8$, 585.3612).

Desmethylenylnocardamine (3): colorless solid, mp 179−181 °C; IR (KBr) ν_{max} 3420 (br), 1635, 1480, 1230 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.60 (6H, t, J = 6.3 Hz, H-5, H-14, H-23), 3.17 (6H, t, J = 6.3 Hz, H-9, H-18, H-26), 2.77 (6H, t, J = 6.8 Hz, H-3, H-12, H-21), 2.46 (6H, t, J = 6.8 Hz, H-2, H-11, H-20), 1.63 (6H, tt, J = 7.3, 6.3 Hz, H-6, H-15, H-24), 1.51 (6H, m, H-8, H-17, H-25), 1.32 (4H, m, H-7, H-16); ^{13}C NMR (CD_3OD) δ 175.0 (C, C-1, C-10, C-19), 174.6 (C, C-4, C-13, C-22), 48.6 (CH_2, C-5, C-14, C-23), 40.1 (CH_2, C-9, C-18), 40.0 (CH_2, C-26), 31.7 (CH_2, C-2, C-11, C-20), 29.7 (CH_2, C-8, C-17), 29.0 (CH_2, C-3, C-12, C-21), 27.4 (CH_2, C-25), 27.2 (CH_2, C-6, C-15), 25.0 (CH_2, C-24), 24.5 (CH_2, C-7, C-16); HRFABMS *m/z* 587.3408 $[M + H]^+$ (calcd for $C_{26}H_{47}N_6O_9$, 587.3405).

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