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Article

Production of a New Cyclic Depsipeptide by the Culture Broth of *Staphylococcus* sp. Isolated from *Corallina officinalis* L.

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Abstract: A new cyclic depsipeptide (**1**) has been isolated from culture broth of *Staphylococcus* sp. (No. P-100826-4-6) derived from *Corallina officinalis* L., together with the known compounds indol-3-carboxylic acid (**2**), 1,5-dideoxy-3-C-methyl arabinitol (**3**), thymine (**4**), uracil (**5**), cyclo (L-pro-L-omet) (**6**) and macrolactin B (**7**). The structure of (**1**) was established to be cyclo (2 α , 3-diaminopropionic acid-L-Asn-3- β -hydroxy-5-methyl-tetradecanoic acid-L-Leu¹-L-Asp-L-Val-L-Leu²-L-Leu³) by extensive spectroscopic techniques including ¹H NMR, ¹³C NMR, ¹H-¹H COSY, HMBC, HSQC, NOESY, and HRFABMS. The antimicrobial activities of compounds **1–7** were evaluated. Compounds **1–5**, and **7** showed moderate antimicrobial activity while compound **6** exhibited a potent antimicrobial and antifungal activities.

Keywords: *Corallina officinalis*; *Staphylococcus* sp.; cyclic depsipeptide; antimicrobial assay

1. Introduction

Bioprospecting studies of endophytic microorganisms play a principal part in the discovery of lead compounds for the improvement of drugs for the management of humanity's diseases [1–3]. Marine microorganisms are widely recognized as promising sources of secondary metabolites [2,4–6]. These organisms, prosperous in diverse marine environments, have produced a wide variety of structurally exclusive and biologically active compounds that have attracted significant interest for biomedical researches [4,7–9].

In the last decade, a significantly increased interest in the isolation of bioactive secondary metabolites from marine microbes has been reported [10]. Peptides, which were derived from different organisms including marine microbes, represent an important chemical class with diverse structures

and significant biological activities. The biological activity of marine-derived peptides has been shown to depend on the composition and sequence of amino acids, their structural properties, as well as on the environmental habitat for producer bacteria [11,12]. These substances are actively synthesized by marine microorganisms during their life cycle [13]. To date, lots of peptide metabolites, which are consisting of 20–40 amino acids, have been separated from various marine microorganisms [11,12,14]. Most of them are capable of quick inhibition or killing of a wide range of microbes. Other antimicrobial peptides (proteins consisting of 100 or more amino acids) disrupt the function or the structure of microbial cell membranes by binding to specific targets [15,16].

In our pursuit of isolation of natural compounds from marine sources [17–20], chemical investigation for the antimicrobial extract of *Staphylococcus* sp. No. [P-100826-4-6] derived, was carried out. This study led to discovery of a new natural cyclic depsipeptide (1) along with six known compounds; indol-3-carboxylic acid (2) [21], 1,5-dideoxy-3-C-methyl arabinitol (3) [22], thymine (4) [21], uracil (5) [23], cyclo (L-pro-L-omet) (6) [24], and macrolactin B (7) [25] (Figure 1). Therefore, structure identification of the isolated pure compounds 1–7 and their antimicrobial activity will be discussed.

2. Results and Discussion

2.1. Isolation Method of Compounds 1–7

The fermented broth of the marine bacterium *Staphylococcus* sp. No. [P-100826-4-6] was extracted with different organic solvents. Successive fractionation of the combined extracts was done using silica gel column chromatography based on increasing polarity, Sephadex LH-20, Diaion HP-20, reversed-phase C18 silica gel column; subsequently, final purification on a C18 RP-HPLC column gave seven compounds 1–7 (Figure 1).

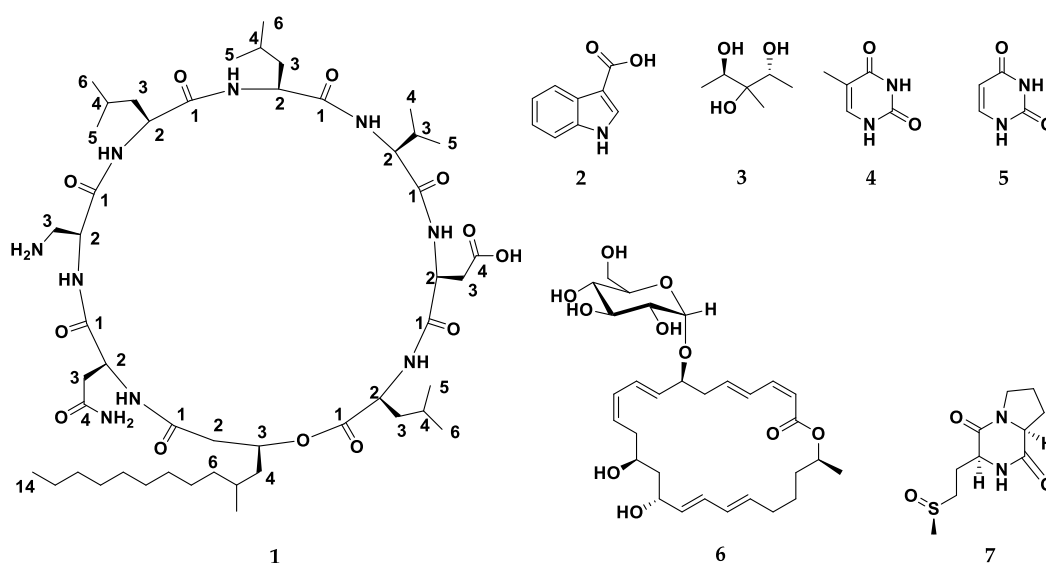


Figure 1. Structure of compounds 1–7.

2.2. Characterization of Compounds 1–7 Structures

Compound 1 (Figure 1) was obtained as a white amorphous powder by several chromatographic procedures obtained from the fermented broth of *Staphylococcus* species. It gave a $[M + H]^+$ peak in the (FABMS) at m/z 994.7 and (HRFABMS) elemental composition $[M + Na]^+$ at m/z 1016.6396 (calcd for $C_{49}H_{87}N_9NaO_{12}$, 1016.6372, $\Delta+2.5$ mmu) (Figures S1–S3) indicating 11 degrees of unsaturation. The 1H NMR spectrum of 1 (Table 1, Figure S4) with HSQC and HMBC spectral data confirmed the existence of 8 methine protons (C-H) [δ_H 5.64, 5.64, 5.05, 4.98, 4.90, 4.83, 4.70, 4.62, 1H each], 7 amide protons NH-C=O [δ_H 9.58, 9.48, 9.20, 8.91, 8.83, 8.75, 8.41, partially overlapped, 1H each], 10 methyl groups, among them 9 methyl doublets [δ_H 1.17, 1.13, 1.02, 0.96, 0.95, 0.93, 0.92, 0.85, 3H

each, d, $J = 6.6$ Hz], one methyl triplet [δ_{H} 0.81, 3H, t, $J = 6.9$ Hz] and long methylene chain centered at δ_{H} 1.22 (12H, brs). The ^{13}C NMR and DEPT spectra of **1** (Table 1, Figure S5) with HSQC and HMBC spectral data exhibited 49 signals, attributable to 10 carbonyl carbons [δ_{C} 175.6, 175.5, 174.8, 173.8, 173.6, 173.4, 172.5, 172.4, 172.0, and 171.7], 7 bearing nitrogen methines [δ_{C} 61.0, 55.0, 53.5, 52.7, 52.5, 52.5, and 51.5], one oxymethine [δ_{C} 72.5], 5 methines [δ_{C} 28.5, 25.4, 25.3, 25.1, and 24.9] 10 methyls [δ_{C} 23.6, 23.4, 23.2, 22.8, 21.8, 21.5, 21.5, 19.5, 18.7, and 14.4], 16 methylenes (Table 1). We could easily deduce that **1** was depsipeptide composed of 1 Val, 3 Leu, 1 Asn, 1 Asp, and 1 A₂Pr residues interlinked with 3-hydroxy-5-methyl fatty acid by analysis of HSQC, HMBC, COSY and NOESY (Figures S6–S9) correlations as shown in Figure 2. The sequence of amino acids in **1** was identified by NOESY and HMBC data analysis for **1** as in Figure 2. NOESY correlations found in **1** between NH protons and adjacent amino acids methine protons clearly identified the following amide bonds: Asp-CO/Val-NH (δ_{H} 4.83/9.48), HMTDA-CO/Asn-NH (δ_{H} 5.64/8.91), HMTDA-CO/Leu¹-NH (δ_{H} 5.64/8.41). The connectivity between HMFA and Leu¹ was also identified by NOESY correlation between α -methine proton HMFA and NH proton of Leu¹ (δ_{H} 5.64/8.41) and Asn (δ_{H} 5.64/8.91). These data, together with HMBC correlations as shown in Figure 2, finally enable us to set up the structure of **1** as cyclo (-A₂Pr-Asn-HMTDA-Leu¹-Asp-Val-Leu²-Leu³-). FABMS data of **1** (Figures S2 and S3) supported the amino acid sequence of **1** as shown in Table 2. The amino acids absolute configuration of **1** was recognized by the reaction of Marfey's reagent [26,27] with the crude hydrolysate followed by co-injection of standard amino acids using HPLC analysis. The hydrolysate was recognized to possess 3 L-Leu, 1 L-Val, and 1 L-Asp. The configuration of A₂Pr and C-3 of HMTDA were proposed by cautious analysis of NOESY correlations, as shown in Figure 2. The NOESY correlation between β -proton (δ_{H} 5.64) of HMTDA and amide proton (δ_{H} 8.91) of Asn, between amide proton (δ_{H} 8.91) of Asn and α -proton (δ_{H} 4.90) of Asn, between amide proton (δ_{H} 9.20) of A₂Pr and α -proton (δ_{H} 5.64) of A₂Pr, between amide proton (δ_{H} 9.58) of Leu³ and α -proton (δ_{H} 4.62) of Leu³, between amide proton (δ_{H} 8.75) of Leu² and α -proton (δ_{H} 4.98) of Leu², between amide proton (δ_{H} 9.48) of Val and α -proton (δ_{H} 4.70) of Val, revealed that the configuration at A₂Pr and C-3 of HMTDA have the configuration of L-Leu, L-Val, and L-Asp. Therefore, the final structure of **1** was lastly characterized as cyclo (2 α , 3-diamino-propionic acid-L-Asn-3- β -hydroxy-5-methyl-tetradecanoic acid-L-Leu¹-L-Asp-L-Val-L-Leu²-L-Leu³).

Table 1. ^1H and ^{13}C NMR data of compound **1** (pyridine- d_5)^a.

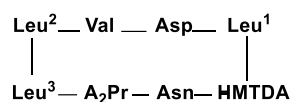
Position	δ_{H} (m, J in Hz)	δ_{C}	Position	δ_{H} (m, J in Hz)	δ_{C}
L-Leu¹			2	4.62 m	52.5 (CH)
1	-	173.4 (C)	3	2.10 m	40.0 (CH ₂)
2	5.05 m	52.7 (CH)	4	1.97 m	25.1 (CH)
3	1.97, 2.10 m	39.5 (CH ₂)	5	1.02 d (6.6)	23.4 (CH ₃)
4	1.97 m	24.9 (CH)	6	0.95 d (6.6)	21.5 (CH ₃)
5	0.91 d (6.6)	21.3 (CH ₃)	NH	9.58 br s	-
6	0.85 d (6.6)	22.8 (CH ₃)	A₂Pr		
NH	8.41 br s		1	-	173.8 (C)
L-Asp			2	5.64 m	51.5 (CH)
1	-	172 (C)	3	3.62 dd (15.8, 8.8) 3.40 dd (15.8, 4.8)	37.3 (CH ₂)
2	4.83 m	53.5 (CH)	NH	9.20 br s	-
3	1.97, 2.67 m	33.7 (CH ₂)	NH ₂	Not observed	-
4		175.6 (C)	L-Asn		
NH	8.83 (1H, brs)	-	1	-	173.6 (C)
L-Val			2	4.90 m	55.0 (CH)
1	-	172.5 (C)	3	2.67, 2.90 m	35.0 (CH ₂)
2	4.70 t (6.6)	61.0 (CH)	4	-	175.5 (C)
3	2.67 m	28.5 (CH)	NH	8.91 br s	-
4	1.17 d (6.6)	19.5 (CH ₃)	NH ₂	Not observed	-

Table 1. Cont.

Position	δ_H (m, J in Hz)	δ_C	Position	δ_H (m, J in Hz)	δ_C
5	1.13 d (6.6)	18.7 (CH ₃)	HMTDA		
NH	9.48 br s	-	1	-	171.7 (C)
L-Leu²			2	2.94, 2.90 m	43.0 (CH ₂)
1	-	172.4 (C)	3	5.64 m	72.5 (CH)
2	4.98 q (7.9)	52.5 (CH)	4	1.76 m	42.6 (CH ₂)
3	2.10 m	39.6 (CH ₂)	5	1.40 m	25.4 (CH)
4	1.97 m	25.3 (CH)	5Me	0.93 d (6.6)	23.6 (CH ₃)
5	0.96 d (6.6)	23.2 (CH ₃)	6	1.29 (overlapped)	32.0 (CH ₂)
6	0.92 d (6.6)	21.8 (CH ₃)	7–12	1.22 m	28.7–29.0 (CH ₂)
NH	8.75 br s	-	13	1.33 (overlapped)	22.9 (CH ₂)
L-Leu³			14	0.81 t (6.9)	14.4 (CH ₃)
1	-	174.8 (C)			

^a Spectra were acquired at 23 °C. Chemical shifts were given in δ (ppm).

Table 2. (+) FABMS parent and fragment ions of compound 1.



Fragments	<i>m/z</i>
[Leu ³ -A ₂ Pr-Asn-HMTDA-Leu ¹ -Asp-Val-Leu ²] ⁺	994.7
[Leu ³ -A ₂ Pr-Asn-HMTDA-Leu ¹ -Asp-Val] ⁺	881.6
[A ₂ Pr-Asn-HMTDA-Leu ¹ -Asp] ⁺	669.6
[A ₂ Pr-Asn-HMTDA-Leu ¹] ⁺	554.6
[A ₂ Pr-Asn-HMTDA] ⁺	441.4
[A ₂ Pr-Asn] ⁺	201.2

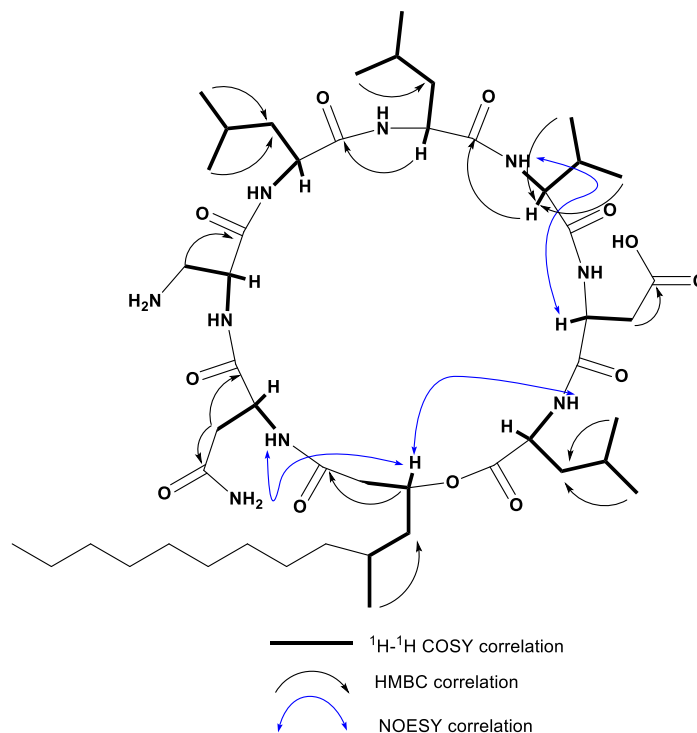


Figure 2. Selected correlations of COSY, HMBC, and NOESY observed for compound 1.

The other known compounds 2–7, were established by analysis of their spectroscopic data (MS and NMR), and with comparison of these data with those mentioned in the literature to be: indol-3-carboxylic acid (2) [21], 1,5-dideoxy-3-C-methyl arabinitol (3) [22], thymine (4) [21], uracil (5) [23], cyclo (L-pro-L-omet) (6) [24], and macrolactin B (7) [25] as shown in Figure 1.

2.3. Biological Activities of the Pure Compounds 1–7

The antimicrobial activities (Table 3) of compounds 1–7 were examined for their growth inhibition of 6 microorganisms including Gram-negative, Gram-positive bacteria and fungi using paper disk method with replication ($n = 2$). The antimicrobial activities were studied in a concentration of 100 $\mu\text{g}/\text{disc}$. As a result, compounds 1–5, and 7 showed moderate activity against *Schizophyllum commune*, *Staphylococcus aureus* subsp. *aureus*, and *Escherichia coli*, with inhibition zones between 9 and 13, while compound 6 exhibited a significant antifungal activity against *Aspergillus niger*, *Penicillium crustosum*, and *Schizophyllum commune*, with inhibition zones of 16, 18 and 23. Furthermore, compound 6 showed antibacterial activity against *Staphylococcus aureus* subsp. *aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, with inhibition zones of 20, 25 and 21 as shown in Table 3.

Table 3. Antimicrobial activities of compounds 1–7.

Compound	Inhibition Zone (mm, 100 $\mu\text{g}/\text{disc}$)					
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. commune</i>	<i>P. crustosum</i>	<i>A. niger</i>
1	13	11	NA	11	NA	NA
2	11	9	NA	13	NA	NA
3	10	13	NA	12	NA	NA
4	10	12	NA	11	NA	NA
5	12	11	NA	10	NA	NA
6	20	21	25	23	18	16
7	13	10	NA	13	NA	NA
Ciprofloxacin ^a	23	24	29			
Nystatin ^b				23	20	19

^a positive antibacterial control (50 $\mu\text{g}/\text{disc}$); ^b positive antifungal control (100 $\mu\text{g}/\text{disc}$).

The minimum inhibitory concentration (MIC) of compound 6 were evaluated. As a result, compound 6 exhibited a potent antifungal activity against *Aspergillus niger*, *Penicillium crustosum*, and *Schizophyllum commune* with MIC value of 50 $\mu\text{g}/\text{mL}$. Additionally, compound 6 showed antibacterial activity against *Staphylococcus aureus* subsp. *aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, with MIC value of 100 $\mu\text{g}/\text{mL}$.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were considered using JASCO DIP-370 digital polarimeter. IR spectra were obtained with JASCO FT/IR-410 spectrophotometers. ¹H and ¹³C NMR, HSQC, ¹H-¹H COSY, HMBC, and NOESY spectra were obtained using Unity plus 500 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 125 MHz for ¹³C and 500 MHz for ¹H. Chemical shifts of ¹H-NMR and ¹³C NMR are expressed in δ values referring to the solvent peak δ_{H} 7.19, 7.55 and 8.71, δ_{C} 123.5, 135.5 and 149.9 for pyridine-*d*₅, and coupling constants are expressed in Hz. TLC was carried out on aluminum-backed plates (Merck, Kieselgel 60 F₂₅₄, 0.25 mm) and RP-18 F_{254s} plates (Merck). Si-gel F254 with a particle size of 0.0045–0.075 mm mesh (Wako Pure Chemical Industries Ltd., Osaka, Japan), Cosmosil 5C18-140 PREP (Nacalai tesque, No.379-34), and Sephadex LH-20 (Sigma-Aldrich, Darmstadt, Germany) were used as stationary phases for column chromatography. High resolution FABMS were obtained using JMS DX-303 spectrometer (JEOL Ltd., Tokyo, Japan). Preparative HPLC was utilized using a Develosil

C-30-UG-5 (250 × 4.6 mm i.d Nomura Chemical Co., Aichi, Japan) adjusting the rate of flow at 1.5 mL/min, and a TOSOH RI-8020 detector.

3.2. Biological Materials

The isolated strain *Staphylococcus* sp. P-100826-4-6 (Figure 3) was obtained from *Corallina officinalis*, collected in Nagasaki Shitsu coast, Japan, in 2010. The voucher specimen was maintained at Garden for Medicinal Plants, Graduated School of Biomedical Sciences, Nagasaki University. *Staphylococcus* sp. was cultured on a medium based on sea water composed of (0.1% MgSO₄, 0.3% KH₂PO₄, 0.3% yeast extract, 0.5% polypeptone, 1% glucose, 25% distilled water, 75% sea water) for 28 days at 25 °C on a rotary shaker. After culturing, sonication and filtration of the broth (32L) was conducted.



Figure 3. *Staphylococcus* sp. (No. P-100826-4-6) derived from *Corallina officinalis* L.

3.3. Purification of Compounds 1–7

After filtration, the broth was extracted three times with 10 L EtOAc. The EtOAc extract was completely dried to produce EtOAc extract (5.2 g). The water layer was subjected to Diaion HP-20 column eluting with water, 60% MeOH, 100% MeOH, and acetone correspondingly to yield eluted fractions of 60% MeOH (28.3 g) and 100% MeOH (8.0 g), and acetone (3.9 g). The EtOAc extract was chromatographed on silica gel column using CHCl₃:MeOH; 10:0~0:10 to yield 25 fractions.

The 15th fraction (96 mg) was subjected to Sephadex LH-20 using CHCl₃:MeOH; 1:1 as eluent to yield four sub-fractions (fractions A–D). Fraction D (10 mg) was purified on ODS column using 50% MeOH:H₂O to give compound 2 (7 mg).

The 18th fraction (198 mg) was subjected to Sephadex LH-20 CHCl₃:MeOH; 1:1 to give three fractions (fractions A–C). Fraction B (125 mg) was chromatographed on ODS column using 50% MeOH:H₂O followed by final purification on Sephadex LH-20 eluted with CHCl₃:MeOH; 1:1 to obtain compound 3 (86 mg). Fraction C (15 mg) was subjected to RP-HPLC Develosil C-30 using 55% MeOH:H₂O to give compound 4 (8 mg).

The 20th fraction (476 mg) was subjected to Sephadex LH-20 eluted with CHCl₃:MeOH; 1:1 to give three sub-fractions (fractions A–C). Fraction A (290 mg) was chromatographed on silica gel column with CHCl₃:MeOH; 10:0 ~ 0:10 as eluent to afford three fractions. Fraction A-1 (50 mg) was finally purified on RP-HPLC Wakosil 5C-18 using 70% MeOH-H₂O to afford compound 1 (8 mg). Fraction C (35mg) was recrystallized from MeOH to yield compound 5 (31 mg). Dissolution of the 100% MeOH fraction (8.0 g) was done using CHCl₃:MeOH:H₂O; 5:5:1 to afford soluble fraction (4.7 g). The soluble fraction was subjected to Sephadex LH-20 using CHCl₃:MeOH; 1:1 to obtain seven sub-fractions (fractions A–G). Fraction D (587 mg) was chromatographed on ODS column using MeOH:H₂O gradient elution to give seven fractions. Fraction D-1 (45 mg) was chromatographed on Sephadex LH-20 using CHCl₃:MeOH; 1:1 isocratic elution to obtain two fractions (fractions D-1a and D-3b). Medium

pressure liquid chromatography (MPLC) used for final purification of fraction D-3b (17 mg) using CHCl_3 :MeOH; 98:2 as eluent to give compound **6** (5.5 mg). Fraction D-6 (95 mg) was subjected to silica gel column using gradient elution of CHCl_3 :MeOH to give four fractions (fractions D-6a–D-6d). Fraction D-6c (40 mg) was finally isolated on Sephadex LH-20 using CHCl_3 :MeOH; 1:1 to obtain compound **7** (26.7 mg).

Compound 1: Cyclo (2 α , 3-diamino-propionic acid-L-Asn-3- β -hydroxy-5-methyl-tetradecanoic acid-L-Leu¹-L-Asp-L-Val-L-Leu²-L-Leu³): White amorphous powder [α]_D³⁰ - 29.8° (*c* = 0.01, pyridine); IR ν_{max} (dry film) 3318, 2965, 2862, 1733, 1684 cm^{-1} ; ¹H and ¹³C NMR data (see Table 1); (HRFABMS) elemental composition [M + Na]⁺ at *m/z* 1016.6396 (calcd for C₄₉H₈₇N₉NaO₁₂, 1016.6372, Δ +2.5 mmu), (+) FABMS *m/z*: 994.7 [M + H]⁺ (Figures S1–S3).

3.4. Configuration of Amino Acids

Hydrolysis of 1.5 mg of compound **1** was achieved using 1 mL of 6 N HCl for 16 h at 110 °C. Concentration for the resulting hydrolysate was followed by complete dryness under a vacuum to afford a residue. Dissolution of the solid residue was achieved in 50 μL of pure H₂O and 40 μL of 1 M NaHCO₃ aq. and 100 μL of 1% of (2S)-2-(5-fluoro-2,4-dinitroanilino)-4-methylpentanamide (FDLA) dissolved in acetone. Heating of the formed mixture was performed for 1 h at 37 °C, followed by adding 20 μL of 1 N HCl. A yellow solid was formed after complete drying of the previously prepared solution. The resulting solid was dissolved in 40% MeCN:H₂O (500 μL) and co-injected with standard D- and L- amino acids using RP-HPLC (UV detector: 340 nm, flow rate: 1 mL/min, mobile phase: 40% MeCN:H₂O).

3.5. Antimicrobial Activity of Compounds 1–7

Antimicrobial activities of the pure compounds were checked using paper disk methods [28,29] against *Staphylococcus aureus* subsp. *aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Schizophyllum commune*, and *Penicillium crustosum*, with concentration 100 and 50 $\mu\text{g/disk}$. Determination of the MIC for compound **6** was achieved by using tube-dilution method [30].

4. Conclusions

Chemical study of the antimicrobial extracts obtained from *Staphylococcus* sp. derived from *Corallina officinalis* L., yielded a new cyclic depsipeptide (**1**) along with the known compounds indol-3-carboxylic acid (**2**), 1,5-dideoxy-3-C-methyl arabinitol (**3**), thymine (**4**), uracil (**5**), cyclo (L-pro-L-omet) (**6**), and macrolactin B (**7**). The structure of the isolated compounds were elucidated by extensive spectroscopic methods including (¹H NMR, ¹³C NMR, COSY, HMBC, HSQC, NOESY, HRFABMS, and IR). The antimicrobial activities of the isolated compounds were evaluated. Compounds **1–5**, and **7** slightly showed an inhibition zone against *Schizophyllum commune*, *Escherichia coli*, and *Staphylococcus aureus* subsp. *aureus*, while compound **6** exhibited a potent antifungal activity against *Aspergillus niger*, *Penicillium crustosum*, and *Schizophyllum commune*, with MIC value 50 $\mu\text{g/mL}$. Additionally, compound **6** showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* subsp. *aureus*, and *Pseudomonas aeruginosa* with MIC 100 $\mu\text{g/mL}$.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2218-1989/9/11/273/s1>, Figure S1: Elemental composition of compound **1**, Figure S2: FABMS spectrum of compound **1**, Figure S3: FABMS fragments of compound **1**, Figure S4: ¹H-NMR spectrum of compound **1** (pyridine-d₅), Figure S5: ¹³C-NMR spectrum of compound **1** (pyridine-d₅), Figure S6: HSQC spectrum of compound **1** (pyridine-d₅), Figure S7: HMBC spectrum of compound **1** (pyridine-d₅), Figure S8: ¹H-¹H COSY spectrum of compound **1** (pyridine-d₅), Figure S9: NOESY spectrum of compound **1** (pyridine-d₅).

Author Contributions: R.F.A.A. and K.Y. designed the experiments. A.I.M.K. and K.Y. collected the marine specimen. A.I.M.K. and R.F.A.A. performed the experiments. A.I.M.K., A.O.N. and D.M.A. performed the biological activity study. A.I.M.K., A.A.B., S.S.E. and G.T.M. analyzed the data. S.S.E. and A.I.M.K. wrote and edited the manuscript.

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References

1. Newman, D.J.; Cragg, G.M.; Snader, K.M. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* **2003**, *66*, 1022–1037. [[CrossRef](#)] [[PubMed](#)]
2. Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* **2012**, *75*, 311–335. [[CrossRef](#)] [[PubMed](#)]
3. Harvey, A.L. Natural products in drug discovery. *Drug Discov. Today* **2008**, *13*, 894–901. [[CrossRef](#)] [[PubMed](#)]
4. Fenical, W. Chemical studies of marine bacteria: Developing a new resource. *Chem. Rev.* **1993**, *93*, 1673–1683. [[CrossRef](#)]
5. Blunt, J.W.; Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2018**, *35*, 8–53. [[CrossRef](#)] [[PubMed](#)]
6. Blunt, J.W.; Copp, B.R.; Munro, M.H.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2010**, *27*, 165–237. [[CrossRef](#)] [[PubMed](#)]
7. Faulkner, D.J. Marine natural products. *Nat. Prod. Rep.* **2002**, *19*, 1–48. [[PubMed](#)]
8. Fenical, W.; Jensen, P.R. Marine microorganisms: A new biomedical resource. In *Pharmaceutical and Bioactive Natural Products*; Springer: Berlin/Heidelberg, Germany, 1993; pp. 419–457.
9. Lu, X.; Cao, X.; Liu, X.; Jiao, B. Marine microbes-derived anti-bacterial agents. *Mini Rev. Med. Chem.* **2010**, *10*, 1077–1090. [[CrossRef](#)] [[PubMed](#)]
10. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2017**, *34*, 235–294. [[CrossRef](#)] [[PubMed](#)]
11. Andryukov, B.G.; Mikhaylov, V.V.; Besednova, N.N.; Zaporozhets, T.S.; Bynina, M.P.; Matosova, E.V. The Bacteriocinogenic Potential of Marine Microorganisms. *Russ. J. Mar. Biol.* **2018**, *44*, 433–441. [[CrossRef](#)]
12. Chen, E.; Chen, Q.; Chen, S.; Xu, B.; Ju, J.; Wang, H. Mathermycin, a Lantibiotic from the Marine Actinomycete *Marinactinospora thermotolerans* SCSIO 00652. *Appl. Environ. Microbiol.* **2017**, *83*, e00926-17. [[CrossRef](#)] [[PubMed](#)]
13. Andryukov, B.; Mikhailov, V.; Besednova, N. The Biotechnological Potential of Secondary Metabolites from Marine Bacteria. *J. Mar. Sci. Eng.* **2019**, *7*, 176. [[CrossRef](#)]
14. Bohringer, N.; Fisch, K.M.; Schillo, D.; Bara, R.; Hertzner, C.; Grein, F.; Eisenbarth, J.H.; Kaligis, F.; Schneider, T.; Wagele, H.; et al. Antimicrobial Potential of Bacteria Associated with Marine Sea Slugs from North Sulawesi, Indonesia. *Front. Microbiol.* **2017**, *8*, 1092. [[CrossRef](#)] [[PubMed](#)]
15. Felnagle, E.A.; Jackson, E.E.; Chan, Y.A.; Podevels, A.M.; Berti, A.D.; McMahon, M.D.; Thomas, M.G. Nonribosomal peptide synthetases involved in the production of medically relevant natural products. *Mol. Pharm.* **2008**, *5*, 191–211. [[CrossRef](#)] [[PubMed](#)]
16. Miller, B.R.; Gulick, A.M. Structural Biology of Nonribosomal Peptide Synthetases. *Methods Mol. Biol.* **2016**, *1401*, 3–29. [[PubMed](#)]
17. Elhady, S.S.; Al-Abd, A.M.; El-Halawany, A.M.; Alahdal, A.M.; Hassanean, H.A.; Ahmed, S.A. Antiproliferative Scalarane-Based Metabolites from the Red Sea Sponge *Hyrtios erectus*. *Mar. Drugs* **2016**, *14*, 130. [[CrossRef](#)] [[PubMed](#)]
18. Alahdal, A.; Asfour, H.; Ahmed, S.; Noor, A.; Al-Abd, A.; Elfaky, M.; Elhady, S. Anti-*Helicobacter*, Antitubercular and Cytotoxic Activities of Scalaranes from the Red Sea Sponge *Hyrtios erectus*. *Molecules* **2018**, *23*, 978. [[CrossRef](#)] [[PubMed](#)]
19. Asfour, H.Z.; Awan, Z.A.; Bagalagel, A.A.; Elfaky, M.A.; Abdelhameed, R.F.A.; Elhady, S.S. Large-Scale Production of Bioactive Terrein by *Aspergillus terreus* Strain S020 Isolated from the Saudi Coast of the Red Sea. *Biomolecules* **2019**, *9*, 480. [[CrossRef](#)] [[PubMed](#)]

20. Khedr, A.I.M.; Mohamed, G.A.; Orabi, M.A.A.; Ibrahim, S.R.M.; Yamada, K. Staphylopeptide A, a new cyclic tetrapeptide from culture broth of *Staphylococcus* sp. *Phytochem. Lett.* **2015**, *13*, 11–14. [[CrossRef](#)]
21. Li, G.-Q.; Deng, Z.-W.; Li, J.; Fu, H.-Z.; Lin, W.-H. Chemical constituents from starfish *Asterias rollestoni*. *J. Chin. Pharm. Sci.* **2004**, *13*, 81–86.
22. Tang, J.; Gao, H.; Hong, K.; Jiang, M.; Zhou, G.; Wang, N.; Yao, X. Secondary metabolites from a mangrove bacterium *Bacillus* sp. *Chin. J. Med. Chem.* **2008**, *18*, 206–209.
23. Kitajima, J.; Ishikawa, T.; TANAKA, T.; IDA, Y. Water-soluble constituents of fennel. IX. glucides and nucleosides. *Chem. Pharm. Bull.* **1999**, *47*, 988–992. [[CrossRef](#)]
24. Yang, X.-Q.; Yang, Y.-B.; Zhou, H.; He, G.-W.; Zhao, L.-X.; Xu, L.-H.; Ding, Z.-T. New megastigmane glycoside and alkaloids from *Streptomyces* sp. YIM 63342. *Nat. Prod. Res.* **2013**, *27*, 1191–1196. [[CrossRef](#)] [[PubMed](#)]
25. Zheng, C.-J.; Lee, S.; Lee, C.-H.; Kim, W.-G. Macrolactins O–R, glycosylated 24-membered lactones from *Bacillus* sp. AH159-1. *J. Nat. Prod.* **2007**, *70*, 1632–1635. [[CrossRef](#)] [[PubMed](#)]
26. Ibrahim, S.R.M.; Min, C.C.; Teuscher, F.; Ebel, R.; Kakoschke, C.; Lin, W.; Wray, V.; Edrada-Ebel, R.; Proksch, P. Callyaerins A–F and H, new cytotoxic cyclic peptides from the Indonesian marine sponge *Callyspongia aerizusa*. *Biorg. Med. Chem.* **2010**, *18*, 4947–4956. [[CrossRef](#)] [[PubMed](#)]
27. Ibrahim, S.R.M.; Edrada-Ebel, R.; Mohamed, G.A.; Youssef, D.T.A.; Wray, V.; Proksch, P. Callyaerin G, a new cytotoxic cyclic peptide from the marine sponge *Callyspongia aerizusa*. *Arkivoc* **2008**, 164–171. [[CrossRef](#)]
28. Kiehlbauch, J.A.; Hannett, G.E.; Salfinger, M.; Archinal, W.; Monserrat, C.; Carlyn, C. Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. *J. Clin. Microbiol.* **2000**, *38*, 3341–3348. [[PubMed](#)]
29. Bonev, B.; Hooper, J.; Parisot, J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *J. Antimicrob. Chemother.* **2008**, *61*, 1295–1301. [[CrossRef](#)] [[PubMed](#)]
30. Jorgensen, J.H.; Turnidge, J.D. Susceptibility test methods: Dilution and disk diffusion methods. In *Manual of Clinical Microbiology*, 11th ed.; American Society of Microbiology: Washington, DC, USA, 2015; pp. 1253–1273.



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