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# Structural Analysis of a New Cytotoxic Demethylated Analogue of Neo-*N*-methylsansalvamide with a Different Peptide Sequence Produced by *Fusarium solani* Isolated from Potato

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**ABSTRACT:** A novel cytotoxic cyclic pentadepsipeptide, neosansalvamide, was produced by *Fusarium solani* KCCM90040 isolated from *Fusarium*-contaminated potato in Korea. The molecular formula of neosansalvamide was analyzed as $C_{32}H_{50}N_4O_6$ by electrospray ionization tandem mass spectrometry and combined structural analysis. The one- and two-dimensional nuclear magnetic resonance and absolute configuration of amino acid spectral data allowed for the resolution of cyclic five subunits linked in the following order: (*S*)-leucic acid, two L-leucine, L-valine, and L-phenylalanine, and this sequence shows a molecular structure as a new demethylated analogue of neo-*N*-methylsansalvamide but having a different peptide sequence. The cytotoxic effects of neosansalvamide were investigated by sulforhodamine B bioassay on four human cancer cell lines. The $IC_{50}$ value of neosansalvamide required to inhibit cell growth *in vitro* by 50% for A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (skin melanoma), and MES-SA (uterine sarcoma) cell lines were 11.70 ± 0.55, 10.38 ± 0.64, 13.99 ± 1.32, and 11.75 ± 0.13 $\mu$M, respectively (mean ± standard error).

**KEYWORDS:** *Neosansalvamide, cyclic pentadepsipeptide, Fusarium solani, cytotoxic activity*

## INTRODUCTION

The fungal genus *Fusarium* contains several species that produce several minor mycotoxins, including the cytotoxic cyclic depsipeptides, such as beauvericin and enniatins, which can be found in *Fusarium*-infected plants.[1] Cytotoxic cyclic hexadepsipeptide, beauvericin, and enniatin analogues consist of alternating residues of D-2-hydroxyisovaleric acid and a branched-chain *N*-methyl-L-amino acid linked by peptide and ester bonds; the ionophoric properties that arise from their molecular structures are related to their biological activities,[2,3] such as cytotoxic,[4,5] antifungal,[6] and antibiotic[1] effects, as well as calcium channel antagonism.[7] Recently, new cytotoxic cyclic pentadepsipeptides, sansalvamide and its analogue, *N*-methylsansalvamide, were produced by *Fusarium* strains.[8,9] Sansalvamide A was first reported from marine microorganism *Halodule wrightii*,[8] and it is composed of four hydrophobic amino acids [phenylalanine (Phe), two leucine (Leu), and valine (Val)] and one hydroxy acid [leucic acid (*O*Leu)]. Interestingly, some *Fusarium* strains isolated from green algae produced its *N*-methylated analogue, a *N*-methylsansalvamide composed of a hydroxy acid (*O*Leu) and four amino acids [Phe, Leu, *N*-methylleucine (*N*-MeLeu), and Val]. This *N*-methylated sansalvamide exhibits cytotoxic activity against various human tumor cell lines [concentration causing 50% growth inhibition ($GI_{50}$) = 8.3 $\mu$M].[9] Sansalvamide and *N*-methylsansalvamide exhibited a strong cytotoxic effect against a human pancreatic cancer cell, suggesting that they could be valuable therapeutic agents.[10]

Recently, we isolated several *Fusarium* strains in Korea and investigated their secondary metabolites.[4,5,11−13] Some *Fusarium* isolates have been shown to produce cytotoxic cyclic hexadepsipeptides (enniatin H, I, and MK1688), together with beauvericin.[11] They also exhibited a potent inhibitory activity against human cancer cell lines and immunodeficiency virus type-1 integrase, as shown in our previous reports.[4,5,14] We also found that one *Fusarium* isolate (*Fusarium solani* KCCM90040) produced a novel cyclic pentadepsipeptide, named neo-*N*-methylsansalvamide, exhibiting *in vitro* cytotoxic effects on various human cancer cell lines.[15] In the cyclic structure of neo-*N*-methylsansalvamide, five subunits are linked in the following order: (*S*)-2-hydroxy-4-methylpentanoic acid, *N*-methyl-L-leucine, L-valine, L-leucine, and L-phenylalanine.

Interestingly, a novel demethylated analogue of neo-*N*-methylsansalvamide was found in the culture extract of *F. solani* KCCM90040, which shows a different peptide sequence in the cyclic structure compared to those of neo-*N*-methylsansalvamide. Therefore, the aims of the present research are to elucidate the chemical structure of this new analogue with several analytical methods and to compare its *in vitro* cytotoxic effect on human cancer cell lines to the cytotoxicity of cyclic depsipeptides in our previous results.[5,15]

## MATERIALS AND METHODS

**Isolation and Identification of *Fusarium* Strain.** The *Fusarium* strain was isolated from *Fusarium*-contaminated potato in Korea using acidified potato dextrose agar (APDA; 4.0 g/L potato extracts, 20.0 g/L dextrose, and 15.0 g/L agar; pH was adjusted to 3.5 with 10% tartaric acid solution), as shown previously.[12] The isolate was cultured on potato dextrose agar (PDA) and stored at −75 °C for further studies. A *Fusarium* isolate producing new cyclic pentadepsipeptide was identified by morphological characteristics and ITS-5.8rDNA sequence analysis in our previous study.[15] DNA sequences of polymerase chain reaction (PCR) products were analyzed and compared to the database using the Basic Local Alignment Search

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Tool (BLAST), searching sequences against the National Center for Biotechnology Information sequence database. The identified isolate was deposited in the Korean Culture Center of Microorganisms (KCCM) with the assigned number of 90040.

**Production and Purification of Cyclic Pentadepsipeptide.** A total of 50 g of rice was autoclaved for 30 min at 121 °C, and afterward, sterilized water was added to produce a moisture content of about 40%, in a 500 mL Erlenmeyer flask. After cooling, the spores of *Fusarium* isolate were inoculated (approximately $10^5$ spores) and the culture was maintained at 25 °C for 14 days. The culture was extracted with solvent [acetonitrile/methanol/water = 16:3:1 (v/v/v)],[16] and the extract was defatted twice with 25 mL of *n*-heptane. After the bottom layer was evaporated to dryness, the residue was dissolved in 50 mL of solvent [methanol/water = 55:45 (v/v)] and extracted twice with 25 mL of dichloromethane. After the dichloromethane phase was evaporated to dryness, the residue was resolved in high-performance liquid chromatography (HPLC)-grade methanol. Neosansalvamide was purified according to the method by Moretti et al., with minor modifications.[17] A GROM-sil pack octadecylsilane preparative column (1.0 × 25 cm; Alltech Grom, Rottenburg-Hailfingen, Germany) was used for the initial purification step, for which the mobile phase was an acetonitrile/water solution (65:35, v/v) at a flow rate of 4 mL/min for 50 min. The compound was further purified with a Shiseido pack C18 column (0.46 × 25 cm; Shiseido, Tokyo, Japan). The second HPLC was performed for 45 min at a constant flow rate (1 mL/min) with a mixture of acetonitrile and water (70:30, v/v). The compound was detected at 210 nm.

**Structural Analysis of Cyclic Pentadepsipeptide.** Ultraviolet (UV) spectra were obtained with a UV−vis spectrophotometer (IMPLEN, Munchen, Germany), and infrared (IR) analysis was performed using a potassium bromide pellet with a Fourier transform (FT) IR-8400S IR spectrophotometer (Shimadzu, Kyoto, Japan). The melting point of the purified compound was measured with a melting-point apparatus obtained from Thermo Fisher Scientific (Waltham, MA). The chemical structure was elucidated using one-dimensional (1D) nuclear magnetic resonance (NMR) [$^1$H NMR, $^{13}$C NMR, and distortionless enhancement by polarization transfer (DEPT)] data obtained from a Bruker DMX 600 spectrometer system (Bruker, Karlsruhe, Germany). Correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond coherence (HMBC) analyses were performed on a Bruker AVANCE 800 spectrometer system (Bruker, Karlsruhe, Germany), and a Varian VNS 600 spectrometer system (Palo Alto, CA) was applied to obtain nuclear Overhauser effect spectroscopy (NOESY) data. All 1D and two-dimensional (2D) NMR spectra were collected in deuterated methanol ($CD_3OD$) (Figure 1 and Table 1).

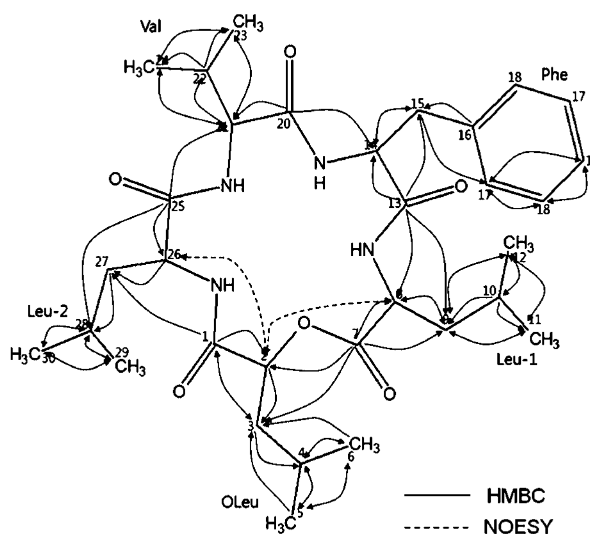


Figure 1. HMBC and NOESY correlations of neosansalvamide.

Table 1. NMR Spectral Data of a Novel Cyclic Pentadepsipeptide in $CDOD_3$

| position | $^{13}$C | $^1$H (*J* in parentheses) | HMBCs |
|---|---|---|---|
| *O*Leu | | | |
| 1 | 172.254 | | H2, H3, H27 |
| 2 | 75.407 | 5.191 q (4.59, 4.81) | H3 |
| 3 | 42.436 | 1.950 m, 1.695 m | H4 |
| 4 | 25.939 | 1.695 m | H5, H6 |
| 5 | 23.253 | 0.997 t (6.62) | H3, H4, H6 |
| 6 | 23.205 | 0.997 t (6.62) | H3, H4, H5 |
| Leu1 | | | |
| 7 | 171.175 | | H2, H3, H8, H9 |
| 8 | 52.895 | 4.743 q (6.01, 5.90) | H9 |
| 9 | 42.740 | 1.876 m, 1.630 m | H8, H11, H12 |
| 10 | 26.065 | 1.379 t (7.09) | H9, H11, H12 |
| 11 | 23.436 | 0.854 d (6.59) | H9, H12 |
| 12 | 22.049 | 0.787 d (6.51) | H9, H10, H11 |
| Phe | | | |
| 13 | 173.887 | | H8, H9, H14, H15 |
| 14 | 57.905 | 4.685 q (4.85, 4.78) | H15 |
| 15 | 39.514 | 3.157 m | H14, H17 |
| 16 | 138.803 | | H19, H15 |
| 17 | 130.696 | 7.345 d (7.38) | H18, H19 |
| 18 | 127.811 | 7.185 t (7.28) | H17 |
| 19 | 129.528 | 7.257 t (7.55) | H17, H18 |
| Val | | | |
| 20 | 173.392 | | H14, H21 |
| 21 | 62.408 | 4.151 d (10.17) | H22, H23, H24 |
| 22 | 31.968 | 2.289 m | H23, H24 |
| 23 | 20.028 | 0.956 t (6.86) | H24 |
| 24 | 19.528 | 0.956 t (6.86) | H23 |
| Leu2 | | | |
| 25 | 174.247 | | H21, H26, H28, H29 |
| 26 | 55.289 | 4.115 d (10.11) | H27 |
| 27 | 30.903 | 1.290 s | H28 |
| 28 | 26.065 | 1.630 m | H29, H30 |
| 29 | 22.317 | 0.905 q (7.14, 6.06) | H28, H30 |
| 30 | 22.411 | 0.956 t (6.86) | H28, H29 |

The molecular structure of this cyclic pentadepsipeptide was further analyzed by electrospray ionization−liquid chromatography−tandem mass spectrometry (ESI−LC−MS/MS) after a chromatographic separation, and their fragment patterns were pointed out in Figure 2. ESI was performed using a LC−MS detection trap VL mass spectrometer (Agilent, Santa Clara, CA). The positive ion (ESI$^+$) of the mass spectrometry (MS) mode was used for MS analysis, and the positive alternant scan mode was selected to determine $[M + H]^+$. The ESI−MS conditions were as follows: capillary voltage, 71.6 V; source voltage, 4.5 kV; capillary temperature, 325 °C; and sheath gas and auxiliary gas, 89.7 and 14.6 arbitrary units, respectively. The mass spectrometer was initially programmed to perform full scans as *m/z* 100−900 for a novel cyclic pentadepsipeptide to investigate the protonated molecular ion signal of this compound, as well as possible fragment ions and adducts.

**Determination of the Absolute Amino Acid Configuration.** The absolute amino acid configurations in cyclic pentadepsipeptide were determined according to the method by Cueto et al.[9] A novel cyclic pentadepsipeptide was dissolved in 6 N HCl (0.5 mL), hydrolyzed for 18 h at 110 °C, and then evaporated to dryness. After evaporation, amino acid derivation was conducted with Marfey's reagent [1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-FDAA)]. A total of 50 mL of Marfey's reagent (L-FDAA; 10 mg in 1 mL of acetone) and 1 M $NaHCO_3$ (100 mL) were added to the acid hydrolysate. After the mixture was maintained at 80 °C for 3 min, 2 N HCl (50 mL) and 50% aqueous methyl cyanide (300 mL) were added

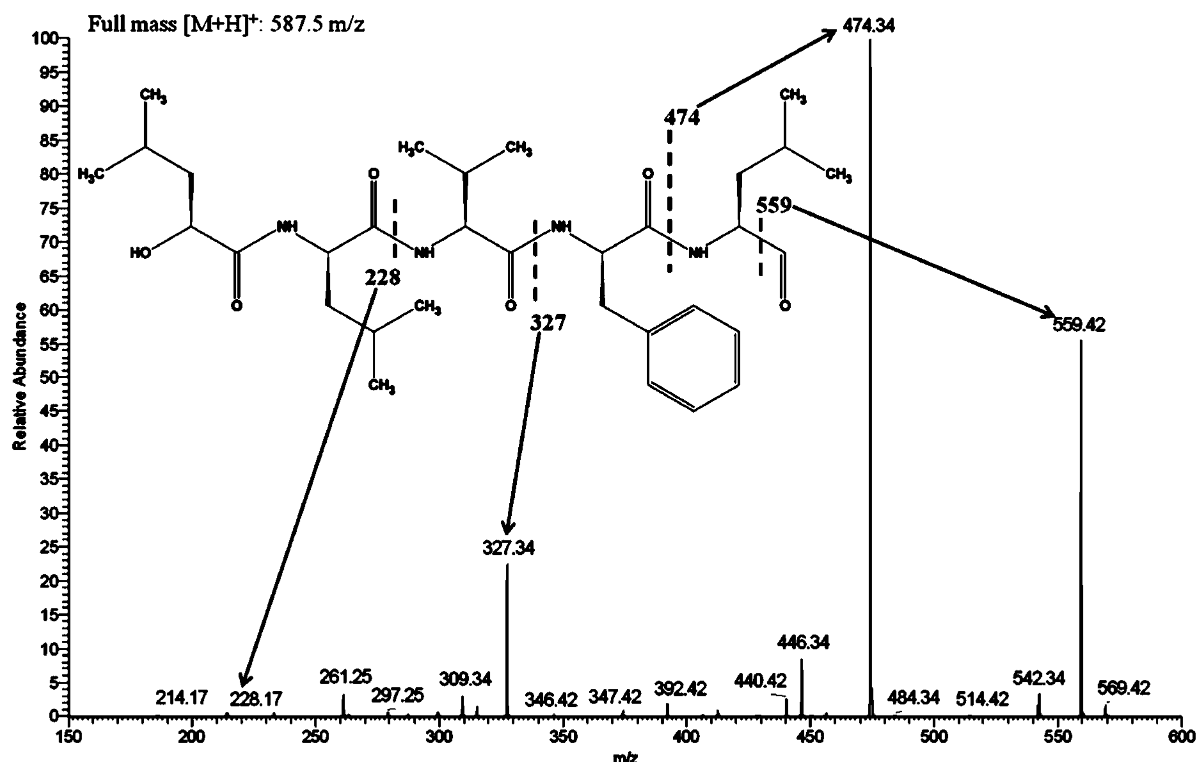


**Figure 2.** Cleavage sites and spectrum of neosansalvamide by LC−ESI−MS/MS analysis.

sequentially. An aliquot of the resulting mixture was analyzed with a reversed-phase column (0.46 × 25 cm; Shiseido, Tokyo, Japan) on HPLC. Hydrolyzed amino acids were eluted with a linear gradient of 10−50% aqueous acetonitrile (0.01% phosphoric acid) over 60 min at 340 nm. The amino acids were identified by co-injection with authentic amino acid standards. The retention times of the amino acid standards were as follows: L-Leu, 46.74 min; D-Leu, 54.01 min; L-Phe, 46.64 min; D-Phe, 51.81 min; L-Val, 40.12 min; and D-Val, 47.52 min.

**Cell Culture.** Four different cancer cell lines (A549, human non-small-cell lung cancer cell line; SK-OV-3, ovarian cancer cell line; SK-MEL-2, skin melanoma cell line; and MES-SA, uterine sarcoma cell line) were purchased from the American Type Culture Collection (Rockville, MD). The cells were cultured in flasks (Falcon T-25, Becton Dickinson, Lincoln Park, NJ) containing 10 mL of RPMI 1640 medium with glutamine, sodium bicarbonate, gentamicin, amphotericin, and 5% fetal bovine serum. Cultured cells were dissociated with 0.25% trypsin and 3 mM 1,2-cyclohexylene dinitrilotetraacetic acid solution for passaging. The cells were maintained in an incubator at 37 °C in a humidified atmosphere of 5% $CO_2$ in air.

**Cytotoxicity *in Vitro*.** The cytotoxicity of the cyclic pentadepsipeptide was investigated by the sulforhodamine B (SRB) bioassay.[18] Cells were inoculated over a series of standard 96-well flat-bottom microplates and then pre-incubated for 24 h to allow for attachment to the microtiter plate. The purified compound (0.1, 0.3, 1, 3, 10, and 30 $\mu$M) and positive control (doxorubicin; $10^{-4}$, $10^{-3}$, $10^{-2}$, $10^{-1}$, 1, and 10 $\mu$M) were treated on cancer cells and then incubated for 72 h.[19,20] After incubation, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4 °C for 1 h. After washing with distilled water, the cells were incubated with 0.4% SRB solution for 30 min at room temperature. The cells were washed again and solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 and 690 nm with a microtiter plate reader (E-max, Molecular Devices, Sunnyvale, CA). To eliminate the effects of non-specific absorbance, the absorbance at 690 nm was subtracted from that at 520 nm. All of the data represent the average values from four wells in each experiment and are expressed as the mean ± standard error (SE) values for at least four independent experiments.

## RESULTS

In our previous report, 31 *Fusarium* isolates from potato in Korea were identified by morphological characterization and DNA sequence analysis.[12,15] Among the isolated *Fusarium* strains, one isolate (*F. solani* KCCM90040) was shown to produce a new cyclic depsipeptide analogue along with cytotoxic cyclic pentadepsipeptide, neo-*N*-methylsansalvamide.

**Structural Analysis of Cyclic Pentadepsipeptide.** The cyclic pentadepsipeptide was eluted (retention time of 9.7 min) with acetonitrile/water solution (70:30, v/v) at a flow rate of 1 mL/min in reverse-phase HPLC analysis. The molecular weight of this compound was determined to be $m/z$ 586.5 on the basis of the ESI−MS measurement. The IR analysis data revealed amide (1654 $cm^{-1}$) and ester (1745 $cm^{-1}$) bonds in the molecular structure. The wavelength of maximum absorbance in the UV spectra and the melting point of this cytotoxic cyclic pentadepsipeptide were about 217 nm and 82 °C, respectively. The $^{1}H$ and $^{13}C$ NMR spectral data of a purified compound are showed in Table 1. The presence of five carbonyl resonances at $\delta$ values of 171.157, 172.254, 173.392, 173.887, and 174.247 suggests that the molecule is a pentapeptide. Four $^{13}C$ NMR signals observed among $\delta$ 52.289, 52.895, 57.905, and 62.408 were characteristics of the $\alpha$ carbons of amino acid residues. One additional signal at $\delta$ 75.407 was highly suggestive of the $\alpha$ carbon of the $\alpha$-hydroxy acid moiety. The signals in the aromatic region of the $^{13}C$ NMR spectrum between $\delta$ 127.811, 129.528, 130.696, and 138.803 were characteristic of a monosubstituted phenyl group and suggested the presence of a Phe residue, which accounted for an additional four unsaturations and left one remaining unsaturation, requiring that this compound possessed one ring. Further analysis of DEPT and 2D NMR spectral data (COSY, HMQC, and HMBC) allowed for the resolution of five subunits: *O*Leu, two Leu, Val, and Phe (Figure 1 and Table 1). The sequence of the

**Table 2. $EC_{50}$ Values of Neosansalvamide against Four Human Cancer Cell Lines (Mean ± SE Values)**

| | $EC_{50}$ ($\mu$M) | | | |
|---|---|---|---|---|
| sample | A549 | SK-OV-3 | SK-MEL-2 | MES-SA |
| neosansalvamide | 11.70 ± 0.55 | 10.38 ± 0.64 | 13.99 ± 1.32 | 11.75 ± 0.13 |
| doxorubicin | 0.03 ± 0.01 | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.01 ± 0.0001 |

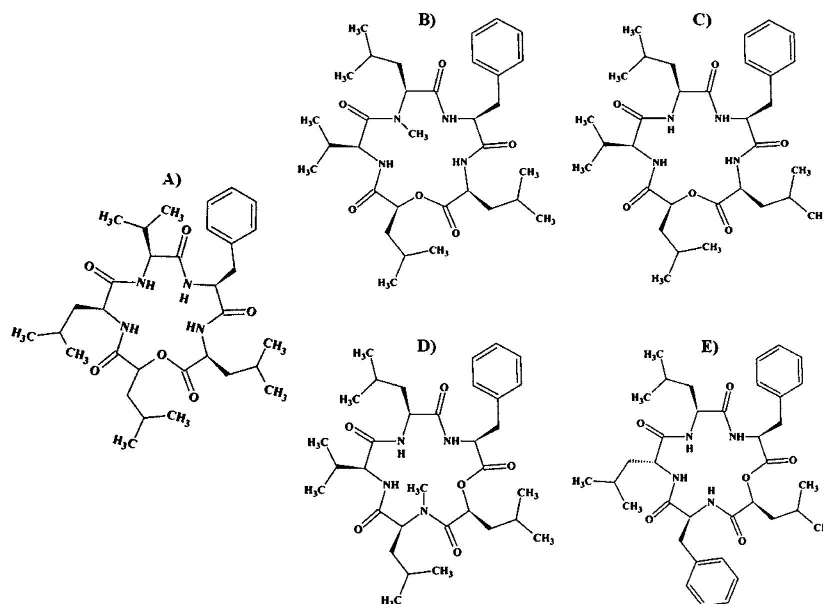


**Figure 3.** Chemical structures of neosansalvamide and other cyclic pentadepsipeptides: (A) neosansalvamide, (B) *N*-methylsansalvamide, (C) sansalvamide, (D) neo-*N*-methylsansalvamide, and (E) zygosporamide.

five fragments in a novel compound was determined by analysis of HMBC data, which generally showed correlations of carbonyl carbons of each amino acid (Table 1). HMBCs from H-2 (*O*Leu) and H-8 (Leu) to C-7 ($\delta$ 171.175) revealed that this carbonyl carbon is a linkage between Leu and *O*Leu by an ester bond. Correlations of the $\alpha$ proton in Phe ($\delta$ 4.685) adjunct to the carbonyl carbon at $\delta$ 173.887 with the proton in Leu ($\delta$ 4.743) led to the following sequence of *O*Leu-Leu-Phe. From correlations of the carbonyl carbon at $\delta$ 173.392 (Val) with $\alpha$ protons at $\delta$ 4.115 of Leu2 ($\delta$ 174.247), a partial sequence of this cyclic peptide was established as Val-Leu2. Finally, the linkage of ring closure was confirmed by HMBC between H-26 ($\delta$ 4.115) of Leu2 and the carbonyl carbon (C-1, $\delta$ 172.254) of *O*Leu. Structural analysis revealed the molecular formula of the cyclic pentadepsipeptide as $C_{32}H_{50}N_4O_6$. The absolute configurations of amino acids in the molecular structure were established with Marfey's reagent.[21] A comparison of retention times in HPLC analysis between hydrolyzed amino acids with authentic L and D forms of amino acid standards revealed that all component amino acids (two Leu, Val, and Phe) are L form in configuration. The stereochemistry of the hydroxy acid (*O*Leu) was elucidated by analysis of NOESY data. The H-2 proton of the *O*Leu residue showed NOESY correlations with H-8 of Leu-1 and H-23 of Leu-2. These results suggest that these protons are on the same side and that the stereochemistry of the hydroxy acid (*O*Leu) of neo-*N*-methylsansalvamide can be assigned a "*S*" configuration. The fragmentation of this cyclic pentadepsipeptide in ESI−MS/MS analysis showed clear serial loss of the subunits through cleavage across the ester and amide bonds (Figure 2). The spectrum exhibited $[M + H]^+$ ions following $[M + H - 28]^+$ ions in MS because of the loss of −CO after opening the cyclic molecule to the carbonyl carbon. The next fragment ions in the spectra were created by the loss of Leu-Phe-Val. This cyclic pentadepsipeptide is one of the new sansalvamiede analogues, and it was named as neosansalvamide.

**Cytotoxic Effects of Cyclic Pentadepsipeptide.** The inhibitory activities were expressed as the concentration required for *in vitro* inhibitition of cell growth by 50% ($EC_{50}$) with purified neosansalvamide. The $EC_{50}$ values of neosansalvamide on the growth of A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (skin melanoma), and MES-SA (uterine sarcoma) cell lines were 11.70 ± 0.55, 10.38 ± 0.64, 13.99 ± 1.32, and 11.75 ± 0.13 $\mu$M, respectively (Table 2).

## DISCUSSION

In previous reports related to cyclic pentadepsipeptides, which showed a similar cyclic pentadepsipeptide structure, sansalvamide, *N*-methylsansalvamide, and zygosporamide could be produced by different fungi.[8,9,22] However, *F. solani* KCCM90040 showed co-production of the demethylated new cytotoxic pentadepsipeptide analogue of neo-*N*-methylsansalvamide, which shows a different peptide sequence in the cyclic depsipeptide structure.

The neosansalvamide exhibited a molecular weight of $m/z$ 586.5 on the basis of ESI−MS analysis, which is the same as sansalvamide.[8] This molecular weight is similar to those of other cyclic pentadepsipeptides, such as *N*-methylsansalvamide, neo-*N*-methylsansalvamide ($m/z$ 600.5),[9,15] and zygosporamide ($m/z$ 634.5).[22] The NMR analysis ($^1$H NMR, $^{13}$C NMR, DEPT, COSY, HMQC, and HMBC in $CD_3DH$) of a neosansalvamide in association with the molecular mass data revealed that the only possible structure of this compound is that of a cyclic depsipeptide. The amino acids (two Leu, Phe,

and Val) are connected via amide bonds, and cyclization of this cyclic peptide is achieved via ester bonding of *O*Leu and Leu, as shown Figure 3. This chemical structure is similar to those of a previously reported cytotoxic cyclic pentadepsipeptide[8,9,15,22] (Figure 3). Especially, sansalvamide is composed of the same four amino acids and one hydroxy acid; however, the peptide sequence in sansalvamide is quite different from that of a neosansalvamide. A sansalvamide consisted of -Leu-Phe-Leu-Val-*O*Leu-, and all amino acids in sansalvamide possess the L configuration.[8] The further structural analysis of a neosansalvamide was investigated by ESI–MS/MS analysis. The cyclic depsipeptides afforded $[M + H - 28]^+$ ions in MS because of the loss of −CO after opening of the cyclic molecule to carbonyl carbon.[13,23,24] Uhlig and Ivanova also detected the loss of −CO after opening of cyclic depsipeptides, and they regarded it as one of the important keys for identification of cyclic depsipeptides.[25]

Interestingly, although neo-*N*-methylsansalvamide and its demethylated analogue, neosansalvamide, and are co-produced by *F. solani* KCCM90040, the sequences of hydroxy acid and amino acids in their cyclic molecular structures are different. The sequence of neo-*N*-methylsansalvamide is -*O*Leu-*N*-MeLeu-Val-Leu-Phe-.[15] However, *O*Leu is linked with Leu by an ester bond in the cyclic peptide structure of neosansalvamide (-*O*Leu-Leu-Val-Phe-Leu-).

Sansalvamide, first defined cytotoxic cyclic pentadepsipeptide produced by *Fusarium* species, showed the cytototoxic potency against HCT116 (colon carcinoma), COLO205 (colon), and SK-MEL-2 (melanoma) cancer cell lines with $EC_{50}$ values of 9.8, 3.5, and 5.9 $\mu$g/mL, respectively.[8] *N*-Methylsansalvamide, a demethylated analogue of sansalvamide, exhibited *in vitro* cytotoxicity in the National Cancer Institute (NCI) human tumor cell line screen ($EC_{50}$ = 8.3 $\mu$M).[9] These activities of the sansalvamide analogues can be attributed to their inhibition of topoisomerase I, and their cytotoxicity may be mediated by this mechanism.[10] A recently defined cyclic pentadepsipeptide, zygosporamide, showed significant cytotoxic activities in the NCI's 60 cell line panel ($EC_{50}$ = 9.1 $\mu$M).[22] Neo-*N*-methylsansalvamide showed a cytotoxic effect against A549, SK-OV-3, SK-MEL-2, and MES-SA cell lines with the $EC_{50}$ values of 10.73 ± 0.15, 11.24 ± 1.23, 10.02 ± 0.53, and 13.96 ± 0.74 $\mu$M, respectively.[15] *In vitro* cytotoxic effects of neosansalvamide against the tested cancer cell cultures were similar to those of neo-*N*-methylsansalvamide. Cyclic hexadepsipeptide mycotoxins, such as beauvericin and enniatin analogues, were reported as stronger cytotoxic compounds than cyclic pentadepsipeptides. The range of $EC_{50}$ values of beauvericin and enniatins H, I, and MK1688 against the above cancer cell lines was between 0.5 and 2.0 $\mu$M,[11] and they induced apoptosis on the human colorectal cancer cell line by DNA fragmentation.[4] Their ionophoric effects can also be attributed to the cytotoxic activities against cancer cell lines because altering ion transport across membranes may lead to disruption of the cationic selectivity of the cell wall.[26] Recently, Qadri et al. reported that beauvericin stimulated $Ca^{2+}$ entry with subsequent cell membrane scrambling and inhibited $Ca^{2+}$-activated $K^+$ channels with blunting of cell shrinkage.[27] A further study is needed to demonstrate the cytotoxic mechanism of new cyclic pentadepsipeptides, neo-*N*-methylsansalvamide and its demethylated analogue, neosansalvamide, produced by *F. solani* KCCM90040 based on the above previous reports.

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### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Fairlie, D.; Abbenante, P. G.; March, D. R. Macrocyclic peptidomimetics forcing peptides into bioactive conformations. *Curr. Med. Chem.* **1995**, *2*, 654−686.

(2) Lee, C.; Gorisch, H.; Kleinkauf, H.; Zocher, R. A highly specific D-hydroxyisovalerate dehydrogenase from the enniatin producer *Fusarium sambucinum*. *J. Biol. Chem.* **1992**, *267*, 11741−11744.

(3) Shemyakin, M. M.; Ovchinnikov, Y. A.; Ivanov, N. T.; Antonov, V. K.; Vinogradova, E. I.; Shkrob, A. M.; Malenkov, G. G.; Evstratov, A. V.; Laine, I. A.; Melnik, E. I.; Ryabova, I. D. Cyclodepsipeptides as chemical tools for studying ionic transport through membranes. *J. Membr. Biol.* **1969**, *1*, 402−430.

(4) Hyun, U.; Lee, D. H.; Lee, C.; Shin, C. G. Apoptosis induced by enniatins H and MK1688 isolated from *Fusarium oxysporum* FB1501. *Toxicon* **2008**, *53*, 723−728.

(5) Lee, H. S.; Song, H. H.; Jeong, J. H.; Shin, C. G.; Choi, S. U.; Lee, C. Cytotoxicities of enniatins H, I, and MK1688 from *Fusarium oxysporum* KFCC 11363P. *Toxicon* **2008**, *51*, 1178−1185.

(6) Fukuda, T.; Arai, M.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Omura, S. New beruvericins, ptoentiators of antifungal micnazole activity, produced by *Beauveria* sp. FKI-1366. Structure elucidation. *J. Antibiot.* **2004**, *57*, 117−124.

(7) Nakamura, T.; Ono, A.; Tanzawa, K.; Hamano, K.; Ochi, R. Voltage- and use-dependent block of L-type calcium current by leualacin, a peptide isolated from *Hapsidospora irregularis*. *J. Mol. Cell. Cardiol.* **1992**, *24*, 107.

(8) Belofsky, G. N.; Jensen, P. R.; Fenical, W. Sansalvamide: A new cytotoxic cyclic depsipeptide produced by a marine fungus of the genus *Fusarium*. *Tetrahedron Lett.* **1999**, *40*, 2913−2916.

(9) Cueto, M.; Jensen, P. R.; Fenical, W. *N*-Methylsansalvamide, a cytotoxic cyclic depsipeptide from a marine fungus of the genus *Fusarium*. *Phytochemistry* **2000**, *55*, 223−226.

(10) Ujiki, M. B.; Milam, B.; Ding, X. Z.; Roginsky, A. B.; Salabat, M. R.; Talamonti, M. S.; Bell, R. H.; Gu, W.; Silverman, R. B.; Adrian, T. E. A novel peptide sansalvamide analogue inhibits pancreatic cancer cell growth through G0/G1 cell-cycle arrest. *Biochem. Biophys. Res. Commun.* **2006**, *340*, 1224−1228.

(11) Song, H. H.; Ahn, J. H.; Lim, Y. H.; Lee, C. Analysis of beauvericin and unusual enniatins co-produced by *Fusarium oxysporum* FB1501 (KFCC 11363P). *J. Microbiol. Biotechnol.* **2006**, *16*, 1111−1119.

(12) Song, H. H.; Lee, H. S.; Jeong, J. H.; Park, H. S.; Lee, C. Diversity in beauvericin and enniatins H, I, and MK1688 by *Fusarium oxysporum* isolated from potato. *Int. J. Food Microbiol.* **2008**, *122*, 296−301.

(13) Song, H. H.; Lee, H. S.; Lee, G. P.; Ha, S. D.; Lee, C. Structural analysis of enniatin H, I, and MK1688 and beauvericin by liquid chromatography−tandem mass spectrometry (LC−MS/MS) and their production by *Fusarium oxysporum* KFCC 11363P. *Food Addit. Contam.* **2009**, *26*, 518−526.

(14) Shin, C. G.; An, D. G.; Song, H. H.; Lee, C. Beauvericin and enniatins H, I and MK1688 are new potent inhibitors of human immunodeficiency virus type-1 integrase. *J. Antibiot.* **2009**, *62*, 687−690.

(15) Song, H. H.; Lee, H. S.; Lee, C. A new cytotoxic cyclic pentadepsipeptide, neo-*N*-methylsansalvamide produced by *Fusarium solani* KCCM90040, isolated from potato. *Food Chem.* **2011**, *126*, 472−478.

(16) Kostecki, M.; Wisniewska, H.; Perrone, G.; Ritieni, A.; Golinski, P.; Chelkowski, J.; Logrieco, A. The effects of cereal substrate and temperature on production of beauvericin, moniliformin and fusaproliferin by *Fusarium subglutinans* ITEM-1434. *Food Addit. Contam.* **1999**, *16*, 361−365.

(17) Moretti, A.; Logrieco, A.; Bottalico, A.; Randazzo, G. Production of beauvericin by *Fusarium proliferatum* from maize in Italy. *Mycotoxin Res.* **1994**, *10*, 73−78.

(18) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

(19) Cores, E. P.; Holland, J. F.; Wang, J. J.; Sinks, L. F. Doxorubicin in disseminated osteosarcoma. *J. Am. Med. Assoc.* **1972**, *221*, 1132−1138.

(20) Kenis, Y.; Michel, J.; Rimoldi, R.; Levy, P.; Israel, L. Results of a clinical trial with intermittent doses of adriamycin in lung cancer. *Eur. J. Cancer* **1972**, *8*, 485−489.

(21) Marfey, P. Determination of D-amino acids. II. Use of a bifunctional reagent, 1,5-diflouoro-2,4-dinitrobenzene. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(22) Oh, D. C.; Jensen, P. R.; Fenical, W. Zygosporamide, a cytotoxic cyclic depsipeptide from the marine-derived fungus *Zygosporium masonii*. *Tetrahedron Lett.* **2006**, *47*, 8625−8628.

(23) Ngoka, L. C. M.; Gross, M. L.; Toogood, P. L. Sodium-directed selective cleavage of lactones: A mothod for structure determination of cyclodepsipeptides. *Int. J. Mass Spectrom.* **1999**, *182*, 289−298.

(24) Sewram, V.; Nieuwoudt, T. W.; Marasas, W. F. O.; Shephard, G. S.; Ritieni, A. Determination of the *Fusarium* mycotoxins, fusaproliferin and beauvericin by high-performance liquid chromatography−electrospray ionization mass spectrometry. *J. Chromatogr., A* **1999**, *858*, 175−185.

(25) Uhlig, S.; Ivanova, L. Determination of beauvericin and four other enniatins in grain by liquid chromatography mass spectrometry. *J. Chromatogr., A* **2004**, *1050*, 173−178.

(26) Ojcius, D. M.; Zychlinsky, A.; Li, M. Z.; Young, J. D. E. Ionophore-induced apoptosis: Role of DNA fragmentation and calcium fluxes. *Exp. Cell Res.* **1991**, *197*, 43−49.

(27) Qadri, S. M.; Kucherenko, Y.; Lang, F. Beauvericin induced erythrocyte cell membrane scrambling. *Toxicology* **2011**, *283*, 24−31.