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FR209602 and Related Compounds, Novel Antifungal Lipopeptides from *Coleophoma crateriformis* No.738

I. Taxonomy, Fermentation, Isolation and Physico-chemical Properties

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Abstract Novel antifungal lipopeptides, FR209602, FR209603 and FR209604, were isolated from the fermentation broth of a fungal strain No. 738 which was identified as *Coleophoma crateriformis* from morphological and physiological characteristics. The antibiotics were purified by solvent extraction, HP-20, YMC-ODS and silica gel column chromatography and lyophilization. These compounds were structurally similar to FR901379 previously reported by ourselves which had a sulfate residue in the cyclic peptide portion.

Keywords FR209602, FR209603, FR209604, antifungal, 1,3-β-glucan synthesis

Introduction

The fungal cell wall, which is composed of glucan, chitin and mannan, is an ideal target for antifungal drugs, because it is essential to fungi and fundamentally different compared to mammalian cells. In particular, 1,3-β-glucan is a promising target for antifungal agents [1]. In recent years, two new antifungal drugs, micafungin [2~4] and caspofungin [5], which inhibit fungal 1,3-β-glucan synthase, have been launched.

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In the course of searching for new fungal 1,3-β-glucan synthesis inhibitors from microorganisms, the novel lipopeptides FR209602-4 were isolated from the cultured broth of *Coleophoma crateriformis* No. 738. These compounds have similar structures to FR901379, the points of difference being the amino acid constituents of the cyclic peptide portion of their structures. It is known that amino acid composition affects both the antifungal spectrum as well as the chemical stability of lipopeptides [6, 7]. Therefore, the attainment of diversity in the cyclic peptide nucleus by screening for new natural products is important for further research on antifungals of the echinocandin family.

In this paper, we describe the taxonomy, fermentation, isolation, physico-chemical properties and structure elucidation.

Materials and Methods

General Procedures

Melting points were taken on a Yanagimoto micro melting point apparatus. IR spectra were measured on a Perkin-Elmer 16PC FT-IR. UV spectra were recorded on a 220A spectrophotometer. Optical rotations were determined on a Jasco Dip-140 polarimeter, using a 10 cm-micro cell. ^{1}H (500 MHz), ^{13}C (125 MHz) and all 2D NMR spectra were obtained with a Bruker DRX500 spectrometer. ESI-MS and HRESI-MS spectra were measured on a Micromass-LCT.

Taxonomic Studies

The producing fungus, strain No. 738, was originally

isolated from a leaf sample collected at Mt. Tateyama, Toyama Prefecture, Japan. The cultural characteristics on various agar media were observed after 14 days of incubation at 25°C: malt extract agar, potato dextrose agar (Difco 0013), Czapek's solution agar, Sabouraud dextrose agar (Difco 0190), Emerson Yp Ss agar (Difco 0739), corn meal agar (Difco 0386), MY20 agar. The compositions of malt extract agar, Czapek's solution agar and MY20 agar were based on the JCM Catalogue of Strains [8]. The color descriptions used in this study were taken from the Methuen Handbook of Colour [9]. The temperature range of growth was determined on potato dextrose agar (NISSUI). The morphological characteristics were examined from the cultures of sterile leaf segments affixed on a Miura's LCA plate [10].

Fermentation

An aqueous seed medium (60 ml) containing sucrose 4.0%, glucose 1.0%, soluble starch 2.0%, Pharmamedia 3.0%, soybean flour 1.5%, KH_2PO_4 1.0%, $CaCO_3$ 0.2%, Adekanol LG-109 (defoaming agent, Asahi Denka Co., Ltd.) 0.05% and Silicone KM-70 (defoaming agent, Shin-Etsu Chemical Co., Ltd.) 0.05% was placed in a 225-ml Erlenmeyer flask and was sterilized at 120°C for 30 minutes. A loopful of *C. crateriformis* No. 738 was used to inoculate the seed flask. The inoculated flask was shaken on a rotary shaker (220 rpm, 5.1 cm throw) at 25°C for 7 days, and 8 ml of the seed culture was transferred to 160 ml of the same sterile seed medium in the 500-ml Erlenmeyer flasks. The flasks were shaken on a rotary shaker (220 rpm, 5.1 cm throw) at 25°C for 2 days, and 640 ml (four flasks) of the second seed culture was used to inoculate 20 liters of sterile production medium consisting of modified starch 3.0%, starch acid hydrolysates 6.0%, Pharmamedia 1.0%, chicken meat bone meal 1.0%, dried yeast 2.0%, $(NH_4)_2SO_4$ 0.05%, $Na_2HPO_4 \cdot 12H_2O$, β-cyclodextrin 1.0%, Adekanol LG-109 0.05% and Silicone KM-70 0.05% in a 30-liter jar fermentor. The fermentation was conducted at 25°C for 4 days under aeration of 20 liters/minute and agitation of 250 rpm.

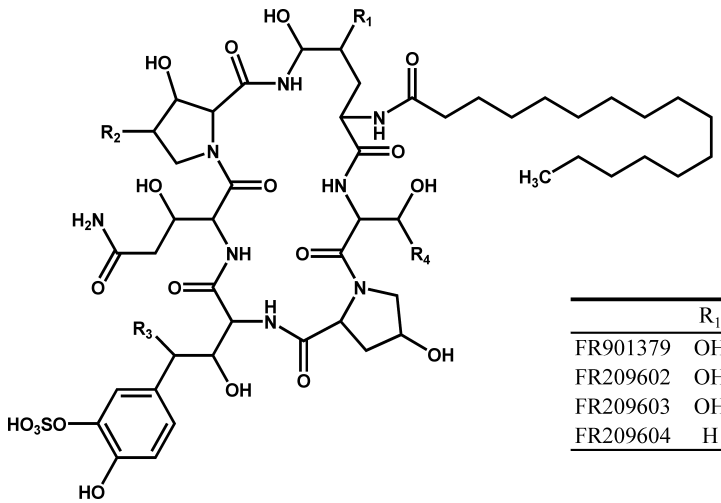
HPLC Analysis

Detection of FR209602, FR209603 and FR209604 in the fermentation broth and fractions during purification was performed by HPLC using a reverse phase column YMC Pack ODS-AM 303, S-5 120A (250×4.6 mm I.D., YMC Co., Ltd.). The mobile phase was 55% aqueous acetonitrile containing 0.5% $NaH_2PO_4 \cdot 2H_2O$. The flow rate was 1.0 ml/minute. The detection wavelength was set at 210 nm.

Results

Characteristics of the Producing Strain

The cultural characteristics are summarized in Table 1. The growth of the microorganism was rather restricted on various agar media and grayish colonies were formed. Growth on potato dextrose agar was rather rapid, reaching 3～4 cm in diameter after two weeks at 25°C. The colony surface was convex to raised, cottony and partly fasciate, sectoring, and showed several colors: pale gray to olive at the center; white to yellowish white at the margin; and olive brown at the sectors. The colony margin was hygroscopic and lustrous. The reverse was yellowish gray; olive gray at the sectors. Growth on corn meal agar was rather restricted, with colonies attaining 2.5～3.5 cm in diameter under the



	R_1	R_2	R_3	R_4
FR901379	OH	CH_3	OH	CH_3
FR209602	OH	CH_3	H	H
FR209603	OH	H	H	H
FR209604	H	CH_3	H	H

Fig. 1 Structures of FR901379, FR209602, FR209603 and FR209604.

Table 1 Cultural characteristics of strain No. 738

Media		Cultural characteristics
Malt extract agar	G:	Rather restricted, 3.0~3.5 cm
	S:	Circular, flat, velvety, exudate at the center, not formed reproductive structures, dark gray (1F1) to violet gray (16F2) at the center and yellowish white (4A2) to yellowish gray (4B2) at the margin
	R:	Dark gray (1F1) at the center and yellowish gray (4B2) to olive gray (4D3) at the margin
Potato dextrose agar (Difco 0013)	G:	Rather rapidly, 3.0~4.0 cm
	S:	Circular to irregular, convex to raised, cottony and partly fasciate, hygroscopic and lustrous at the margin, sectoring, not formed reproductive structures, pale gray (1B1) to olive (1F3) at the center, white to yellowish white (4A2) at the margin and olive brown (4F3) at the sectors
	R:	Yellowish gray (2B-C2), and olive gray (2F2) at the sectors
Czapek's solution agar	G:	Very restricted, 0.5 cm
	S:	Irregular, scanty, flat, not formed reproductive structures, grayish brown (5F3)
	R:	Brownish gray (5F2)
Sabouraud dextrose agar (Difco 0190)	G:	Rather restricted, 3.0~3.5 cm
	S:	Circular, convex, velvety and partly fasciate, hygroscopic, lustrous, sectoring, not formed reproductive structures, yellowish white (4A2), and grayish brown (5D-E3) at the center and sectors
	R:	Pale yellow (4A3), and olive brown (4D3) at the sectors
Emerson Yp Ss agar (Difco 0739)	G:	restricted, 1.5~2.5 cm
	S:	Circular to irregular, flat to raised, velvety, sulcate, sectoring, not formed reproductive structures, pale gray (1B1) to light gray (1D1), produced dark green soluble pigment
	R:	Olive (1-2F3), and yellowish gray (2D2) at the center
Corn meal agar (Difco 0386)	G:	Rather restricted, 2.5~3.5 cm
	S:	Circular, flat, velvety, submerged at the margin, lustrous, not formed reproductive structures, dark gray (1F1) to olive gray (2F2), and olive (1E-F4) at the margin
	R:	Black, and olive gray (2E2) at the margin
MY20 agar	G:	Rather restricted, 2.5~3.5 cm
	S:	Circular to irregular, flat, velvety, hygroscopic, sectoring, not formed reproductive structures, olive brown (4D4-4F3), and grayish orange (6B3) at the center
	R:	Olive brown (4E4), and pale orange (5A3) at the center

Abbreviations: G, growth, measuring colony size in diameter; S, colony surface; R, reverse.

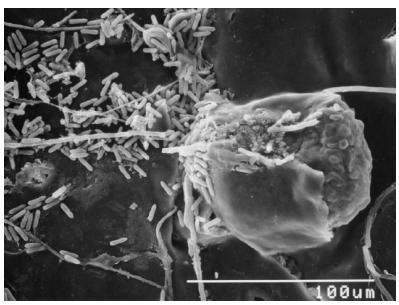


Fig. 2 Electron micrograph of strain No. 738.

same conditions. The surface was flat, velvety and dark gray to olive gray. The colony margin was subimmersed, lustrous and olive. The reverse was black; olive gray at the margin. Strain No. 738 was able to grow at a temperature range from 3 to 30°C, with the growth optimum at 20~24°C.

Strain No.738 produced pycnidial conidiomata in the autoclaved leaf segments affixed on LCA media (Fig. 2), while it formed neither teleomorph nor anamorph on/in agar media. The pycnidia were superficial, separate, and dark brown to black. Their shape was discoid or sometimes papillate, flattened at the base, non-ostiolate or indistinctly ostiolate, unilocular, 90~160(~400) μm in diameter and 50~90 μm high. Pycnidial walls were composed of 1~2 cells layer. Their cells were thick, but thin at the upper part, brown, and composed of textura angularis. The lower cells of inner walls were hyaline, subglobose, 4~6(~7.5) μm in diameter, and formed conidiophores. The conidiophores

were hyaline, smooth, septate, simple to sparingly branched, and 3～13.5×3～4 μm. They formed discrete conidiogenous cells at the apex. The conidiogenous cells were ampulliform to lageniform or cylindrical, and 4～8.5×2.5～4 μm. Conidia were hyaline, smooth, amerosporous, cylindrical, rounded at the apical end, with a small projection at the base, and 10～13×2～3 μm. Both conidia and conidiogenous cells were covered with large sheaths. The sheaths were hyaline, thin-walled campanulate to cylindrical and 14～21.5×3～5 μm. Vegetative hyphae were smooth, septate, brown and branched. The hyphal cells were cylindrical, and 2～7 μm in width. Chlamydospores were not observed.

Comparison of the morphological characteristics of strain No. 738 with fungal taxonomic criteria of von Arx [11] and Sutton [12] indicated that it resembled the coelomycete species *Coleophoma crateriformis* (Dur. & Mont.) Höhn. 1907. There were a few differences between the above characteristics and Sutton's description of *C. crateriformis*: superficial, non-ostiolate or indistinctly ostiolate conidiomata, and sheaths that wrapped conidiogenous cells and conidia. The former discrepancy may be an artifact of pure culture and the sheaths were described as paraphyses by Sutton. Thus, we identified the strain as belonging to *C. crateriformis*, and named it *Coleophoma crateriformis* No. 738. This strain has been deposited to the International Patent Organism Depositary in the National Institute of Advanced Industrial Science and Technology, Japan, as FERM BP-5796.

Isolation and Purification of FR209602, FR209603 and FR209604

The culture broth (20 liters) was extracted with an equal volume of acetone by stirring for 1 hour at room temperature. The mixture was filtered with the aid of diatomaceous earth. The filtrate was concentrated *in vacuo* to remove acetone and adsorbed onto a column (1.5 liters) of DIAION HP-20 (Mitsubishi Chemical Co., Ltd.) packed with water. The column was washed with water (4.5 liters) and 50% aqueous methanol (4.5 liters), and then eluted with methanol (3 liters). The active fraction was concentrated *in vacuo* to an aqueous solution and an equal volume of ethyl acetate was added. The aqueous layer was passed through a column (100 ml) of YMC-GEL (ODS-AM 120-S50, YMC Co., Ltd.) packed with water. The column was washed with 20% and 40% aqueous acetonitrile containing 0.5% $NaH_2PO_4 \cdot 2H_2O$ (300 ml), respectively, and then eluted with 50% aqueous acetonitrile containing 0.5% $NaH_2PO_4 \cdot 2H_2O$ (200 ml). The active fraction was concentrated *in vacuo* to an aqueous solution and adsorbed onto a column (20 ml) of DIAION HP-20 packed with water. The column was washed with water (200 ml) and eluted with methanol (60 ml). The active fraction was concentrated *in vacuo* to an aqueous solution and passed through a column (175 ml) of YMC-GEL (ODS-AM 120-S50) packed with water. The column was eluted with 45% aqueous acetonitrile containing 0.5% $NaH_2PO_4 \cdot 2H_2O$. The active fraction was desalted using a column (20 ml) of DIAION HP-20 and further purified by preparative HPLC, using a YMC-packed column (ODS-AM SH-343-5AM S-5, 250×20 mm i.d., YMC Co., Ltd.) with 55% aqueous acetonitrile containing 0.5% $NaH_2PO_4 \cdot 2H_2O$ as a mobile phase with a flow rate of 9.9 ml/minute. Fractions containing FR209602, FR209603 and FR209604 were collected and desalted using columns (20 ml) of DIAION HP-20, respectively. Active fractions containing the FR209602 and FR209604 substance were concentrated *in vacuo* and lyophilized to give 22.3 mg of FR209602 substance and 4.2 mg of FR209694 substance as white powders, respectively. Active fractions containing the FR209603 substance were lyophilized and dissolved in methanol (100 μl), and then applied to a column (2.5 ml) of Silica gel 60 (200～230 mesh, Merck Co., Ltd.) packed with acetone. The column was washed with acetone - methanol (10 : 1, 7.5 ml) and acetone - methanol (5 : 1, 7.5 ml), and then eluted with acetone - methanol (3 : 1, 5 ml). The eluate was dried, dissolved in distilled water and lyophilized to give 1.2 mg of FR209603 as white powder.

Physico-chemical Properties

Physico-chemical properties of FR209602, FR209603 and FR209604 are summarized in Table 2. All of them were soluble in water, methanol and dimethyl sulfoxide but insoluble in *n*-hexane and chloroform. They displayed positive color reactions to iodine vapor and ceric sulfate though they were negative against Molish, Dragendorff, and $FeCl_3$. The ESI-MS spectra showed molecular ion peaks at *m/z* 1145 $(M+H)^+$, 1131 $(M+H)^+$ and 1129 $(M+H)^+$, respectively.

Structure Elucidation of FR209602

For convenience, the same numbering systems and abbreviations for rare amino acids as those used for echinocandin-like lipopeptides by researchers at Merck laboratories have been adopted. The amino acid abbreviations are shown in Table 3.

The molecular formula $C_{50}H_{80}N_8O_{20}S$ was determined by HR-ESIMS and the elemental analysis (Table 2). At first, the structure of FR209602 was elucidated by comparison of the ^1H and ^{13}C NMR data with those of FR901379 [13, 14] and it was finally established by application of a series of 2-D NMR techniques. In the ^1H NMR spectrum of

Table 2 Physico-chemical properties of FR209602, FR209603 and FR209604

	FR209602	FR209603	FR209604
Appearance	White powder	White powder	White powder
Melting point	173～177°C	160～164°C	155～160°C
$[\alpha]_D^{23}$	−10° (*c* 0.5, MeOH)	− 7.6° (*c* 0.5, MeOH)	−2° (*c* 0.35, MeOH)
ESI-MS (*m*/*z*)	1145 $(M+H)^+$	1131 $(M+H)^+$	1129 $(M+H)^+$
HRESI-MS (*m*/*z*)			
Found	1145.5287	1131.5128	1129.5331
Calcd for M+H	1145.5288	1131.5131	1129.5338
Molecular formula	$C_{50}H_{80}N_8O_{20}S$	$C_{49}H_{78}N_8O_{20}S$	$C_{50}H_{80}N_8O_{19}S$
Elemental analysis			
Calcd as hexahydrate	C 47.09, H 7.19, N 8.79, S 2.51	Not determined	Not determined
Found:	C 47.23, H 7.35, N 8.56, S 2.18	Not determined	Not determined
UV λ_{max}^{MeOH} nm (ε)	278 (1500)	275 (1900)	277 (1600)
Color test			
Positive	I_2, $Ce(SO_4)_2$-H_2SO_4	I_2, $Ce(SO_4)_2$-H_2SO_4	I_2, $Ce(SO_4)_2$-H_2SO_4
Negative	Molish, Dragendorff, $FeCl_3$	Molish, Dragendorff, $FeCl_3$	Molish, Dragendorff, $FeCl_3$
Solubility			
Soluble	H_2O, methanol, DMSO	H_2O, methanol, DMSO	H_2O, methanol, DMSO
Insoluble	*n*-hexane, chloroform	*n*-hexane, chloroform	*n*-hexane, chloroform
IR λ_{max} (KBr) cm^{-1}	3350, 2930, 2850, 1650, 1640, 1520, 1460, 1270, 1250, 1050	3360, 2930, 2850, 1650, 1630, 1540, 1520, 1440, 1280, 1250, 1050	3350, 2920, 2850, 1650, 1640, 1540, 1520, 1450, 1270, 1050
TLC (Rf value)*	0.42	0.34	0.40

* Silica gel 60 F_{254} (E. Merck Co.): *n*-BuOH - acetic acid - H_2O (4 : 1 : 2).

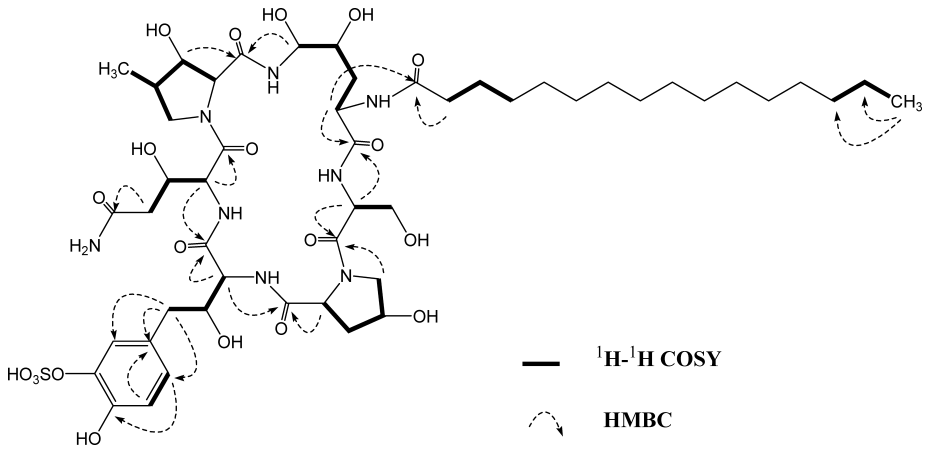


Fig. 3 ^1H-^1H COSY and key HMBC correlations of FR209602.

FR901379, two secondary methyl signals were particularly diagnostic: a doublet methyl signal resonating at 1.18 ppm was characteristic for the Thr methyl and a doublet at 1.05 ppm for the 4-methyl of 3-hydroxy-4-methylproline (OHMePro; see FR209603 below). The absence of a 1.18 ppm doublet in the ^1H spectrum of FR209602 implied the lack of a Thr residue. By a combined analysis of ^1H-^1H COSY and HSQC, the following spin units were deduced. The presence of Ser was inferred from the spin system (methylene (δ_H 4.23, 3.73/δ_C 63.6) and methine (δ_H 4.91/δ_C 56.0)). *Meta*-coupled benzene protons (δ_H 7.16 and 6.88) showed long-range ^1H-^1H correlations to methylene (δ_H 2.58/δ_C 40.7). These key observations indicated the replacement of Thr by Ser and deoxygenation at C-4 in the

Table 3 ^{13}C NMR data for FR209602, FR209603, FR209604 and FR901379 (125 MHz, CD_3OD)

Position	FR209602	FR209603	FR209604	FR901379
4,5-Dihydroxyornithine (DiOHOrn)				
C-1	173.8 s	173.8 s	174.5 s	174.4 s
C-2	51.3 d	51.2 d	52.0 d	51.5 d
C-3	35.9 t	35.5 t	27.3 t	35.0 t
C-4	70.7 d	70.7 d	30.9 t	70.7 d
C-5	74.3 d	74.2 d	71.9 d	74.4 d
Serine (Ser)				
C-1	171.9 s	172.0 s	171.8 s	172.6 s
C-2	56.0 d	56.0 d	56.0 d	58.4 d
C-3	63.6 t	63.6 t	63.8 t	68.2 d
C-4				19.8 q
4-Hydroxyproline (OHPro)				
C-1	174.1 s	174.2 s	174.1 s	173.5 s
C-2	62.3 d	62.4 d	62.5 d	62.4 d
C-3	39.0 t	39.0 t	39.0 t	38.4 t
C-4	71.2 d	71.3 d	71.3 d	71.3 d
C-5	57.0 t	57.0 t	57.0 t	57.1 t
3,4-Dihydroxyhomotyrosine (DiOHTyr)				
C-1	172.5 s	172.7 s	172.8 s	172.5 s
C-2	58.4 d	58.3 d	58.2 d	57.1 d
C-3	73.7 d	73.8 d	73.9 d	76.3 d
C-4	40.7 t	40.8 t	40.8 t	75.5 d
C-1′	130.9 s	131.0 s	131.1 s	134.6 s
C-2′	125.1 d	125.3 d	125.3 d	123.2 d
C-3′	141.0 s	141.1 s	141.1 s	141.1 s
C-4′	149.1 s	149.1 s	149.1 s	150.3 s
C-5′	118.2 d	118.2 d	118.2 d	118.3 d
C-6′	127.9 d	128.0 d	128.0 d	125.5 d
3-Hydroxyglutamine (OHGln)				
C-1	169.4 s	169.5 s	169.1 s	169.4 s
C-2	55.4 d	55.4 d	55.4 d	55.5 d
C-3	70.6 d	70.6 d	70.6 d	70.7 d
C-4	39.6 t	39.5 t	39.5 t	39.7 t
C-5	176.7 s	176.7 s	176.9 s	176.8 s
3-Hydroxy-4-methylproline (OHMePro)				
C-1	172.4 s	172.6 s	172.5 s	172.7 s
C-2	70.1 d	69.7 d	70.3 d	70.1 d
C-3	75.6 d	73.9 d	76.0 d	75.7 d
C-4	39.0 d	34.6 t	39.1 d	39.1 d
C-5	52.8 t	46.9 t	52.9 t	53.0 t
4-Me	11.0 q		11.1 q	11.1 q
Palmitoyl				
C-1	176.1 s	176.2 s	176.3 s	175.8 s
C-2	36.7 t	36.8 t	36.8 t	36.7 t
C-3	26.7 t	26.9 t	27.0 t	26.9 t
C-4	30.3 t	30.3 t	30.3 t	30.3 t
C-5	30.4 t	30.4 t	30.4 t	30.4 t
C-6	30.5 t	30.5 t	30.5 t	30.5 t
C-7	30.7 t	30.7 t	30.7 t	30.7 t
C-8	30.8 t	30.8 t	30.8 t	30.8 t
C-9	30.8 t	30.8 t	30.8 t	30.8 t
C-10	30.8 t	30.8 t	30.8 t	30.8 t
C-11	30.8 t	30.8 t	30.8 t	30.8 t
C-12	30.8 t	30.8 t	30.8 t	30.8 t
C-13	30.6 t	30.6 t	30.6 t	30.7 t
C-14	32.9 t	33.1 t	33.0 t	33.1 t
C-15	23.6 t	23.7 t	23.7 t	23.7 t
C-16	14.4 q	14.4 q	14.4 q	14.4 q

3,4-dihydroxyhomotyrosine residue. A combined analysis of ^{1}H-^{1}H COSY, HSQC and HMBC confirmed the structure as shown in Fig. 3. The ^{13}C NMR data are presented in Table 3.

Structure Elucidation of FR209603

HR-ESIMS revealed the molecular formula $C_{49}H_{78}N_8O_{20}S$ (CH_2 less than FR209602). The absence of a doublet methyl signal at 1.05 ppm indicated the lack of methyl of the OHMePro residue. This finding was consistent with the molecular formula difference between FR209602 and FR209603. A careful ^{13}C NMR comparison with FR209602 revealed the replacement of methine carbon (39.0 ppm) in FR209602 with methylene (34.6 ppm) in FR209603. This modification was corroborated by the up-field shift of the C-5 methylene (46.9 ppm) in FR209603 from C-5 of OHMePro (52.8 ppm) in FR209602. The ^{13}C NMR data are presented in Table 3.

Structure Elucidation of FR209604

HR-ESIMS defined the molecular formula $C_{50}H_{80}N_8O_{19}S$ (one oxygen atom less than FR209602). The pattern of the hemiaminal proton signal (δ_H 5.38/δ_C 71.9) was changed from doublet (J=3 Hz) in FR209602 to a doublet of doublets (J=10.5 and 3.5 Hz), indicating the presence of methylene at C-4 in the 4,5-dihydroxyornithine (DiOHOrn) residue. With the aid of a combined analysis of ^{1}H-^{1}H COSY and HSQC, a ^{13}C NMR data comparison with FR209602 revealed that the methine (70.7 ppm) was substituted with a methylene (30.9 ppm). With the structure, [5-hydroxyornitine]FR209602, in hand, full ^{1}H and ^{13}C NMR assignments were accomplished by extensive 2D NMR analyses (^{1}H-^{1}H COSY, HSQC and HMBC). The results were in complete agreement with the structure. The ^{13}C NMR data are presented in Table 3.

Discussion

In this paper, we have presented three novel antifungal lipopeptides, FR209602, FR209603 and FR209604, isolated from the fermentation broth of *C. crateriformis* No. 738. These compounds belong to the echinocandin family, a class of antifungal agents that act on the fungal cell wall by inhibiting glucan synthesis. The members of the echinocandin family of fungal origin are FR901379 [14, 15], echinocandin B [16], aclueacins [17], mulundocandin [18] and pneumocandins [19], which are produced by *C. empetri* No. 11899, *Aspergillus nidulans*, *A. aculeatus*, *A. sydowi* and *Glarea lozoyensis*, respectively. Among these, FR901379 has good water-solubility because it has a sulfate moiety. Though FR209602, FR209603 and FR209604 also have a sulfate moiety, the amino acid constitutions of these compounds differ from those of FR901379. The acyl side chain is readily replaced by semi-synthesis as we [2, 20] and others [21] acheived. However, modification of the cyclic peptide portion by chemical synthesis is not easy. The attainment of diversity in the cyclic peptide nucleus by screening for new natural products is important for further research on antifungals of the echinocandin family. Studies of the *in vitro* and *in vivo* antifungal activities of FR209602, FR209603 and FR209604 will be reported in the following paper [22].

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