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Pyrrolidine-containing peptides, JBIR-126, -148, and -149, from *Streptomyces* sp. NBRC 111228

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ABSTRACT

New pyrrolidine containing peptides JBIR-126 (**1**), JBIR-148 (**2**), and JBIR-149 (**3**) were isolated from a culture of *Streptomyces* sp. NBRC 111228 obtained from soil collected in the Okinawa Prefecture, Japan. The planar structures of **1**, **2,** and **3** were established based on 1D and 2D NMR, ESI-TOF-MS, IR, and UV spectra. The absolute configurations of amino acid residues were determined by Marfey's method. Compounds **1**, **2,** and **3** are structurally related to JBIR-34 and -35, in which an alanine residue is replaced by a unique pyrrolidine moiety.

Members of the class *Actinobacteria* have been extensively studied for their tendency to produce pharmaceutically useful compounds. However, the discovery rate of novel compounds from these strains has decreased significantly. Our intention was to show that strains isolated by a variety of microbial-isolation protocols, such as the moist incubation and desiccation method,[1] are producing unique metabolites. For this purpose, we attempted to isolate unique actinomycetes, including new species, from various samples such as soils, plants, lichens, and marine organisms, and then searched for secondary metabolites in the cultures of those strains while testing them for bioactive substances. As a result, we have discovered novel compounds such as a new angucycline JBIR-88,[2] the new butenolide JBIR-89,[2] a phenylacetylated peptide JBIR-96,[3] tetraene macrolides JBIR-100[4] and JBIR-120[5] from the new species of actinomycetes. Further screening resulted in the isolation of novel pyrrolidine-containing peptide compounds, designated as JBIR-126 (**1**), JBIR-148 (**2**), and JBIR-149 (**3**) from a culture of *Streptomyces* sp. NBRC 111228 obtained from a soil sample collected on Iriomote Island, Okinawa Prefecture, Japan. This Letter describes the fermentation process as well as the isolation and structural elucidation of **1**, **2**, and **3**.

The strain *Streptomyces* sp. NBRC 111228 was isolated from the soil of a pineapple culture collected on Iriomote Island, Okinawa Prefecture, Japan according to the SDS/yeast extract method.[6] The 16S rRNA gene sequence of the strain was used for their taxonomical identification. The strain was cultivated in 50 mL test-tubes, each containing 15 mL of a seed medium consisting of 1.0% starch, 1.0% polypeptone, 1.0% molasses, and 1.0% meat extract at pH 7.2 (adjusted before sterilization). The test tubes were shaken on a reciprocal shaker (320 rpm) at 27 °C for 2 days. Aliquots (2.5 mL) of the culture were transferred into 500 mL baffled Erlenmeyer flasks filled with 100 mL of a production medium consisting of 4.0% β-cyclodextrin, 0.5% glycerol, 2.0% Pharmamedia, $CuSO_4$ 5 mg, $MnCl_2$ 5 mg, and $ZnSO_4$ 5 mg, and cultured on a rotary shaker (180 rpm) at 27 °C for 5 days.

The fermentation broth (10 L) was centrifuged to obtain a mycelial cake, which was extracted with acetone (2500 mL). The extract was concentrated in vacuo and the residual aqueous concentrate was successively extracted with EtOAc followed by *n*-BuOH extraction. The *n*-BuOH layer was then concentrated in vacuo. The dried residue (12.5 g) was applied on a reversed-phase medium-pressure liquid column (Purif-Pack ODS-100; Shoko Scientific, Yokohama, Japan), and the column was successively eluted using a H_2O–MeOH solvent system (0%, 10%, 20%, 40%, 60%, 80%, and 100% MeOH). The target eluate (60% MeOH, 2.7 g) was applied on a normal-phase medium-pressure liquid column

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(Purif-Pack SI-30; Shoko Scientific, Yokohama, Japan), and was developed with a stepwise $CHCl_3$–MeOH solvent system (0%, 2%, 5%, 10%, 20%, and 50% MeOH). The target eluate (20% MeOH, 838 mg) was further fractionated by reversed-phase medium-pressure liquid chromatography eluted using a H_2O–MeOH solvent system (20%, 40%, 60%, and 80% MeOH). The 60% MeOH eluate (271 mg) was subjected to preparative reversed-phase HPLC using a CAPCELL PAK C_{18} MGII column (5.0 µm, 20 i.d. × 150 mm; Shiseido, Tokyo, Japan) with a 2996 photodiode array detector (Waters, Milford, USA) and a 3100 mass detector (Waters) developed with 51% aqueous MeOH containing 0.1% formic acid (flow rate: 10 mL/min) to yield fraction A (94.2 mg, R_t = 11.5 min) and B (39.5 mg, R_t = 20.8 min). Final purification was carried out by preparative reverse-phase HPLC using a CAPCELL PAK C_{18} MGII column (5.0 µm, 20 i.d. × 150 mm) with a solvent system of 27% aqueous CH_3CN containing 0.1% formic acid (flow rate: 10 mL/min) to afford **1** (48.4 mg, R_t = 14.3 min) and 2 (9.4 mg, R_t = 17.3 min) from fraction A, and with a solvent system of 30% aqueous CH_3CN containing 0.1% formic acid (flow rate: 10 mL/min) to afford **2** (13.3 mg, R_t = 13.0 min) and **3** (4.1 mg, R_t = 17.7 min) from fraction B.

The structures of these compounds were mainly determined by HRESIMS and NMR (1D, DQF-COSY, HSQC, and CT-HMBC[7]) spectroscopy. The ^{13}C and ^1H NMR assignments are listed in Table 1. JBIR-126 (**1**) was obtained as a colorless amorphous powder: $[\alpha]_D^{24}$ −121 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.98) nm; IR (ATR) ν_{max} 3460, 1640 cm^{-1}. The molecular formula of **1** was established as $C_{24}H_{30}ClN_5O_7$ by high-resolution electrospray ionization mass spectrometry (m/z 536.1910 $[M+H]^+$, calcd for $C_{24}H_{31}{}^{35}ClN_5O_7$, 536.1912). Absorptions at 3460 and 1640 cm^{-1} in the IR spectrum of **1** revealed the presence of hydroxy and amide carbonyl groups, respectively. ^1H and ^{13}C NMR data, recorded in DMSO-d_6, for **1** strongly suggested a peptidic structure, which was confirmed by the analysis of DQF-COSY, HSQC, and CT-HMBC NMR spectra. The ^{13}C NMR spectrum shows eight aromatic carbon signals representing five quaternary sp^2 carbons, C-3 (δ_C 101.4), C-3a (δ_C 113.5), C-4 (δ_C 152.3), C-6 (δ_C 129.4), and C-7a (δ_C 139.3) and three protonated sp^2 carbons, C-2 (δ_C 133.6), C-5 (δ_C 106.9), and C-7 (δ_C 101.8). The ^1H–^{13}C long-range couplings from the singlet methyl protons N-CH_3 (δ_H 3.77) to C-2 and C-7a; from a singlet aromatic proton H-2 (δ_H 7.93) to C-3, C-3a, and C-7a; from a phenolic hydroxy proton 4-OH (δ_H 12.75) to C-3a, C-4, and C-5; from an aromatic proton H-5 (δ_H 6.48), which in turn *meta*-coupled with an aromatic proton H-7 (δ_H 7.07), to C-3a and C-7; and from the aromatic proton H-7 to C-3a and C-5, together with the UV absorption at a particular wavelength (λ_{max} 300 nm) revealed the presence of a tetrasubstituted indole nucleus. Additional weak ^1H–^{13}C long-range couplings from H-5 and H-7 to a quaternary aromatic carbon C-6 established all assignments of the indole moiety.

Table 1
^{13}C (150 MHz) and ^1H (600 MHz) NMR Spectroscopic Data for JBIR-126 (**1**) and JBIR-148 (**2**) in DMSO-d_6, and JBIR-149 (**3**) in $CDCl_3$–CD_3OD (1:1)

Position	JBIR-126 (**1**)		JBIR-148 (**2**)		JBIR-149 (**3**)	
	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)
Indole						
2	133.6	7.93, s	133.7	7.93, s	133.6	7.61, s
3	101.4		101.1		102.7	
3a	113.5		113.4		114.3	
4	152.3		152.3		152.7	
5	106.9	6.48, d (1.2)	107.0	6.48, br s	108.4	6.54, br s
6	129.4		129.4		131.4	
7	101.8	7.07, d (1.2)	101.9	7.08, br s	102.2	6.87, br s
7a	139.3		139.4		140.3	
N-Me	33.6	3.77, s	33.6	3.77, s	33.9	3.75, s
4-OH		12.75, s		12.69, s		
Oxazoline						
1′	163.9		164.1		165.9	
2′	77.0	4.78, d (8.4)	77.5	4.62, d (8.5)	77.9	4.79[a]
		4.38, d (8.4)		4.38, d (8.5)		4.33, m
3′	72.8		72.6		73.9	
4′	24.9	1.58, s	25.0	1.50, s	25.4	1.63, s
5′	173.2		172.6		177.0	
Pyrrolidine						
1″	167.6		169.7		169.9	
2″	52.8	4.81, dd (8.4, 6.6)	51.8	4.06, dd (8.3, 6.4)	54.2	4.80[a]
3″	60.2	3.85, m	57.8	3.96, m	60.5	4.10, m
4″	27.0	1.99, m	25.4	1.77, m	28.2	2.19, m
		1.63, m		1.31, m		1.79, m
5″	23.5	1.84, m	23.8	1.75, m	26.4	1.59, m
		1.73, m				
6″	45.5	3.12, m	45.0	3.15, m	46.4	3.21[a]
				3.05, m		
3″-NH		8.87, br s				
NH		8.18, d (8.4)		7.60, d (8.3)		
O-Me					53.6	3.78, s
methylSer						
1‴	173.9					
2‴	60.8					
3‴	64.2	3.67, d (10.8)				
		3.63, d (10.8)				
4‴	19.6	1.36, s				
NH		8.15, s				

[a] Overlapped with the solvent peak.