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NOTE

Novel aziridine-containing peptides MBJ-0034 and MBJ-0035 from *Streptosporangium* sp. 32552

This article has been corrected since Advance Online Publication, and a corrigendum is also printed in this issue.

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Recently, we have constructed a library of isolated natural products (CB library) primarily of microbial origin, in order to perform efficient screenings.[1] During the exploration of rare microbial products with promising biological and pharmacological properties, we have developed an advanced system for compound identification based on accumulated HPLC-MS profiling data and strain information designated as 'MBJ's special selection'. Using this system, we have already succeeded in discovering novel eremophilane derivatives MBJ-0009 and MBJ-0010 from *Nectria* sp. f26111,[2] MBJ-0011, MBJ-0012 and MBJ-0013 from *Apiognomonia* sp. f24023,[3] cytotoxic chaetoglobosin derivatives MBJ-0038, MBJ-0039 and MBJ-0040 from *Chaetomium* sp. f24230,[4] and a cytotoxic hydroxamate MBJ-0003 from *Micromonospora* sp. 29867.[5] During the course of further screening, we discovered two aziridine-containing linear peptides MBJ-0034 (**1**) and MBJ-0035 (**2**) from the culture of *Streptosporangium* sp. 32552 (Figure 1a). Herein, we report the fermentation, isolation, structure elucidation and in brief, the biological activities of **1** and **2**.

The producing microorganism *Streptosporangium* sp. 32552 was isolated from a plant sample collected in Iwata, Shizuoka Prefecture, Japan. The strain was cultivated in 250-ml Erlenmeyer flasks, each containing 25 ml of a seed medium consisting of 2% potato starch (Tobu Tokachi Nosan Kako Agricultural Cooperative Assoc., Hokkaido, Japan), 2% glucose (Junsei Chemical, Tokyo, Japan), 2% soy bean powder (SoyPro, J-Oil Mills, Tokyo, Japan), 0.5% yeast extract powder (Oriental Yeast, Tokyo, Japan), 0.25% NaCl (Junsei Chemical), 0.32% $CaCO_3$ (Wako Pure Chemical Industries, Osaka, Japan), 0.0005% $CuSO_4 \cdot 5H_2O$ (Wako Pure Chemical), 0.0005% $ZnSO_4 \cdot 7H_2O$ (Wako Pure Chemical Industries) and 0.0005% $MnCl_2 \cdot 4H_2O$ (Junsei Chemical) (pH 7.4). The flasks were shaken on a rotary shaker (220 r.p.m.) at 28 °C for 3 days. Then, the aliquots (0.5 ml) of the broth were transferred to 500-ml Erlenmeyer flasks containing 50 ml of the production medium of the same composition and were cultured on a rotary shaker (220 r.p.m.) at 28 °C for 4 days.

The whole culture broth (2 l) was extracted with an equal volume of *n*-BuOH. After the *n*-BuOH layer was evaporated *in vacuo*, the resulting residue was suspended in brine (350 ml) and then extracted with EtOAc (350 ml × 3) and *n*-BuOH (300 ml × 2), successively. The *n*-BuOH extract (3.1 g) was subjected to reversed-phase medium-pressure liquid chromatography (Purif-Pack ODS-30, size: 60 (39 g), Shoko Scientific Co., Ltd., Yokohama, Japan) with the UV detection wavelength set at 254 nm. A H_2O–CH_3CN stepwise gradient system (100 ml each of 10, 20, 30, 40 and 50% CH_3CN) was used to eluate **1** (63.2 mg, 40% CH_3CN). The EtOAc extract (2.4 g) was fractionated by medium-pressure silica gel column chromatography (Purif-Pack SI-30, size: 60 (27 g), Shoko Scientific) using an *n*-hexane–EtOAc linear gradient system (0–25% EtOAc over 12 min and was kept at 25% for 3 min, flow rate: 20 ml min^{-1}) followed by a $CHCl_3$–MeOH stepwise gradient system (100 ml each of 0, 2, 5, 10, 20, 30 and 100% MeOH). The 20% MeOH fraction (288.1 mg) was subjected to gel filtration chromatography (Sephadex LH-20, GE Healthcare BioSciences AB, Uppsala, Sweden; 2.5 × 45 cm) and eluted using $CHCl_3$–MeOH (1:1) to afford crude **2** (63.5 mg). The final purification of **2** (11.9 mg) was carried out by reversed-phase HPLC using a CAPCELL PAK C18 MGII column (5.0 μm, 20 × 150 mm; Shiseido, Tokyo, Japan) with 35% aqueous CH_3CN containing 0.1% formic acid (flow rate: 10 ml min^{-1}, retention time: 12.5 min).

MBJ-0034 (**1**) was isolated as a colorless amorphous powder: $[\alpha]^{25}_D$ +0.6 (*c* 0.1, MeOH); UV λ_{max} nm (log ε): 241 (4.2) and 305 (3.8) in MeOH; IR (ATR) ν_{max} 3300 and 1650 cm^{-1} (hydroxy and carbonyl). The molecular formula of **1** was established as $C_{26}H_{37}N_7O_9$ by HR-ESIMS (*m/z* 590.2560 $[M–H]^-$, calcd for $C_{26}H_{36}N_7O_9$: 590.2575). The peptide-like nature of **1** was evident from the NMR resonances corresponding to amide NH protons (δ_H 8.49–7.91) and the resonances corresponding to the carbonyl carbons (δ_C 165.1–

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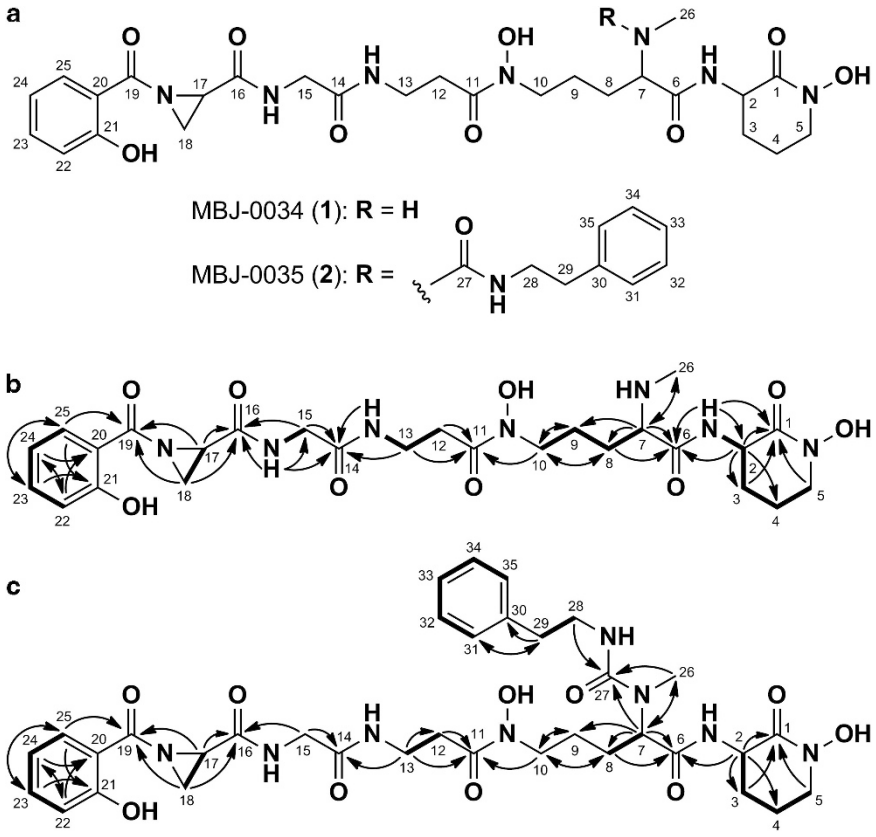

Figure 1 (**a**) Structures of **1** and **2**. (**b**) Structure determination of **1**. COSY and HMBC (^{1}H to ^{13}C) correlations are shown as bold lines and arrows, respectively. (**c**) Structure determination of **2**. COSY and HMBC (^{1}H to ^{13}C) correlations are shown as bold lines and arrows, respectively.

173.7) in the ^{1}H and ^{13}C NMR spectra, respectively. The planar structure of **1** was determined by a series of 2D NMR analyses including double quantum filtered COSY (DQF-COSY), heteronuclear single quantum coherence and constant-time heteronuclear multiple-bond correlation[6] (CT-HMBC). The ^{13}C and ^{1}H NMR data of **1** is listed in Table 1.

The CT-HMBC spectrum showed a ^{1}H–^{13}C long-range coupling from a nitrogen-bearing methyl proton H_3-26 (δ_H 2.20, δ_C 34.4) to a methine carbon C-7 (δ_C 63.9), in which proton H-7 (δ_H 2.86) was long-range coupled to an amide carbonyl carbon C-6 (δ_C 173.7). Together with these HMBC correlations, the ^{1}H sequence from the H-7 through aliphatic methylene protons H_2-8 (δ_H 1.47, 1.40) and H_2-9 (δ_H 1.58) to nitrogen-bearing methylene protons H_2-10 (δ_H 3.46, δ_C 47.2) were observed in the DQF-COSY spectrum, indicating the presence of an *N*-methylornithine moiety (Figure 1b). On the other hand, observation of COSY correlations from a doublet amide NH proton 2-NH (δ_H 8.09) through an α-methine proton H-2 (δ_H 4.31), aliphatic methylene protons H_2-3 (δ_H 1.93, 1.68) and H_2-4 (δ_H 1.88) to nitrogen-bearing methylene protons H_2-5 (δ_H 3.45, δ_C 51.4) and HMBC correlations from H-2, H_2-3 and H_2-5 to an amide carbonyl carbon C-1 (δ_C 165.1) revealed the presence of a δ-lactam of an ornithine moiety.

The presence of a β-alanine moiety was ascertained by ^{1}H–^{1}H spin systems from α-methylene protons H_2-12 (δ_H 2.51) through β-methylene protons H_2-13 (δ_H 3.25, 3.24) to a triplet amide NH proton 13-NH (δ_H 7.91) and ^{1}H–^{13}C long-range couplings from H_2-12 and H_2-13 to an amide carbonyl carbon C-11 (δ_C 171.1). The structure of a glycine moiety was determined by ^{1}H–^{1}H couplings between a triplet amide NH proton 15-NH (δ_H 8.49) and α-methylene protons H_2-15 (δ_H 3.74, 3.65) together with ^{1}H–^{13}C long-range couplings from all the protons to an amide carbonyl carbon C-14 (δ_C 168.5).

The presence of a salicylic acid moiety was established based on a ^{1}H sequence from an aromatic proton H-22 (δ_H 6.99) to an aromatic proton H-25 (δ_H 7.63) through aromatic protons H-23 (δ_H 7.46) and H-24 (δ_H 6.94), in addition to HMBC correlations from H-23 and H-25 to an oxygen-bearing aromatic quaternary carbon C-21 (δ_C 159.2), from H-22 and H-24 to an aromatic quaternary carbon C-20 (δ_C 110.1), and from H-25 to an amide carbonyl carbon C-19 (δ_C 166.0). Furthermore, HMBC correlations from an α-methine proton H-17 (δ_H 5.00) and methylene protons H_2-18 (δ_H 4.64, 4.51), which were mutually ^{1}H spin coupled, to amide carbonyl carbons C-16 (δ_C 170.3) and C-19, revealed the presence of an aziridine moiety, which was acylated with the salicylic acid at the *N*-terminus.

The connectivity among the amino-acid units was determined by ^{1}H–^{13}C long-range couplings from H-2, H_2-10, H_2-13 and H_2-15 to C-6, C-11, C-14 and C-16, respectively. The molecular formula indicated the presence of two hydroxamic acid groups in **1**. The chemical shift value of C-26 (δ_C 34.4) suggested that the nitrogen atom connected to C-7 is protonated.[7] Accordingly, the positions of two hydroxamic acid groups were determined to be C-1 (C-5) and C-10 (C-11). Thus, the gross structure of **1** was elucidated, as shown in Figure 1a.

MBJ-0035 (**2**) was obtained as a colorless amorphous powder: $[\alpha]^{25}_D$ –52 (*c* 0.1, MeOH); UV λ_{max} nm (log ε): 240 (4.1) and 305 (3.7) in MeOH; IR (ATR) ν_{max} 3300 and 1650 cm^{-1} (hydroxy and carbonyl). The molecular formula of **2** was established as

Table 1 ^{13}C and ^{1}H NMR spectroscopic data for MBJ-0034 (1) in DMSO-d_6 and MBJ-0035 (2) in CD_3OD

	1[a]		***2***[b]	
Position	δ_C	δ_H, *mult (J in Hz)*	δ_C	δ_H, *mult (J in Hz)*
1	165.1		167.3	
2	49.6	4.31, m	51.5	4.40, dd (5.4, 10.8)
3	27.9	1.93,m; 1.68, m	28.6	2.03, ovl[c]; 1.79, m
4	20.5	1.88, m	21.8	1.99, m; 1.95, m
5	51.4	3.45, ovl[c]	52.5	3.61, ddd (4.2, 10.8, 10.8); 3.53, ovl[c]
6	173.7		173.94[d]	
7	63.9	2.86, dd (6.5, 6.5)	58.5	4.85, ovl[c]
8	30.4	1.47, m; 1.40, m	26.7	1.86, m; 1.69, m
9	23.0	1.58, m	24.2	1.58, m; 1.52, m
10	47.2	3.46, ovl[c]	48.3	3.75, ddd (7.2, 14.4, 14.4); 3.53, ovl[c]
11	171.1		173.94[d]	
12	32.1	2.51, ovl[c]	32.9	2.73, m; 2.69, m
13	34.8	3.25, t (6.0); 3.24, t (6.0)	36.4	3.47, m
14	168.5		171.3	
15	42.3	3.74, dd (6.0, 16.5); 3.65, dd (6.0, 16.5)	43.6[d]	3.93, d (16.5); 3.85, d (16.5)
16	170.3		173.86	
17	67.6	5.00, dd (8.0, 10.5)	69.5	5.05, dd (8.0, 10.2)
18	69.6	4.64, dd (8.0, 10.5); 4.51, dd (8.0, 8.0)	70.6	4.68, dd (8.0, 10.2); 4.61, dd (8.0, 8.0)
19	166.0		168.7	
20	110.1		111.5	
21	159.2		160.9	
22	116.8	6.99, d (8.0)	117.7	6.97, d (7.6)
23	134.3	7.46, ddd (1.5, 8.0, 8.0)	135.1	7.42, ddd (1.2, 7.6, 7.6)
24	119.3	6.94, dd (8.0, 8.0)	120.0	6.90, dd (7.6, 7.6)
25	128.2	7.63, dd (1.5, 8.0)	129.6	7.69, dd (1.2, 7.6)
26	34.4	2.20, s	30.4	2.77, s
27			161.1	
28			43.6[d]	3.38, ovl[c]
29			37.5	2.80, t (7.2)
30			140.9	
31/35			129.9	7.21, d (7.8)
32/34			129.4	7.27, dd (7.8, 7.8)
33			127.2	7.17, t (7.8, 7.8)
NH		8.49, t (6.0)		
NH		8.09, d (8.5)		
NH		7.91, t (6.0)		
OH		11.70, br s		
OH		9.56, br s		

NMR spectra were taken on a Varian NMR System 600 NB CL (**1**) or 500 NB CL (**2**) in DMSO-d_6 (**1**) or CD_3OD (**2**) with the residual solvent peak as an internal standard (DMSO-d_6: δ_C 39.7, δ_H 2.49 p.p.m.; CD_3OD: δ_C 49.0, δ_H 3.31 p.p.m.).
[a]500 MHz.
[b]600 MHz.
[c]Overlapped with other signals.
[d]Exchangeable.

C_{35} $H_{46}N_8O_{10}$ from the HR-ESIMS data (*m/z* 739.3450 $[M+H]^+$, calcd for $C_{35}H_{47}N_8O_{10}$: 739.3415).

Based on the analyses of 1D and 2D NMR spectra, the partial structure of **2** was found to be the same as that of **1**, as shown in Figure 1c. The structural difference between **1** and **2** is an additional phenethylcarbamic acid moiety, whose presence was confirmed by ^{1}H sequences from aromatic protons H-31/35 (δ_H 7.21) through aromatic protons H-32/34 (δ_H 7.27) to an aromatic proton H-33 (δ_H 7.17) and from nitrogen-bearing methylene protons H_2-28 (δ_H 3.38, δ_C 43.6) to allylic methylene protons H_2-29 (δ_H 2.80), together with HMBC correlations from H_2-28 to carbonyl ketone carbon C-27 (δ_C 161.1) and from H_2-29 to an aromatic quaternary carbon C-30 (δ_C 140.9) and an aromatic methine carbon C-31/35 (δ_C 129.9). Finally, HMBC correlations from an *N*-methyl proton H_3-26 (δ_H 2.77) and a low-field shifted α-methine proton H-7 (δ_H 4.85) to C-27 indicated that the phenethylcarbamic acid moiety is connected to C-7 by an amide bond. Therefore, the structure of **2** was determined as shown in Figure 1a.

The cytotoxic activities of **1** and **2** against human ovarian adenocarcinoma SKOV-3 cells were examined by using the WST-8 [5-(2,4-disulfophenyl)-3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl) -2*H*-tetrazolium, monosodium salt] colorimetric assay (Cell Counting Kit; Dojindo, Kumamoto, Japan). After 72 h of treatment, **2** exhibited weak cytotoxic activity against SKOV-3 cells with the IC_{50} of 37 μM, whereas **1** exhibited no cytotoxicity ($IC_{50} > 50$ μM). Since the derivatives of **1** and **2**, madurastatin A1 and its congeners

were reported to show antibacterial activities against *Micrococcus luteus*,[7] we evaluated the antimicrobial activities of **1** and **2**. As the result, contrary to this report, **1** and **2** did not show antimicrobial activities against *M. luteus* nor *Bacillus subtilis* even at the concentrations of 100 μM. Compounds **1** and **2** did not show the antimicrobial activities against *Escherichia coli*. To the best of our knowledge, peptides with aziridine moiety is extremely rare in nature and have not been reported except for madurastatin A1. The planar structure of **1** appears to be same as madurastatin C1.[8]

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