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NOTE

Acremopeptin, a new peptaibol from *Acremonium* sp. PF1450

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The inhibitor of apoptosis protein (IAP) plays important roles in cancer cells, apoptosis control, tumorgenesis and chemotherapy resistance.[1] The expression and function of IAP are deregulated in many human cancers because of genetic aberrations, an increase in their protein expression or the loss of endogenous inhibitors such as second mitochondria-derived activator of caspase (SMAC).[2] In cell cytoplasm, survivin, a member of IAP family, plays a role as a suppressor of apoptosis, together with X-chromosome-linked IAP (XIAP). Furthermore, survivin controls cell division by its association with Aurora B and inner centromere protein (INCENP). Because apoptosis induced by suppression of IAP can lead to antitumor effects, an IAP such as survivin is considered as a promising molecular target for cancer chemotherapy. YM155, a survivin suppressant, suppresses survivin expression through direct binding to its promoter,[3] and induces regression of established human hormone-refractory prostate tumor xenografts.[4] An SMAC-mimicking IAP antagonist, AT-406 binds to XIAP, c-IAP1 and c-IAP2, and prevents the association of these proteins with caspases and SMAC.[5] An XIAP antisense oligonucleotide, AEG35156, efficiently decreases XIAP mRNA and XIAP protein amounts.[6] In addition, their combination with other cytotoxic agents, small molecule signal transduction inhibitors, proteasome inhibitors and death receptor ligands potentiated their effects.[2]

We screened for the microbial products to identify new candidate compounds that enhance doxorubicin-induced apoptosis in murine monocyte/macrophage RAW264.7 cells. Furthermore, we selected a culture broth that could suppress protein levels of survivin and/or XIAP. As a result of the purification of active compounds, we discovered the new peptide acremopeptin (**1**), which suppressed survivin and XIAP, and inhibited the growth of hormone-refractory prostate cancer PC-3 cells and colorectal adenocarcinoma HT-29 cells *in vitro*. Here we describe the isolation, structural elucidation and biological properties of **1** (Figure 1a).

Acremonium sp. PF1450 was isolated from a soil sample collected from Ishigaki Island in Okinawa prefecture, Japan. The strain was inoculated to 500-ml Erlenmeyer flasks each containing 100 ml of a medium comprising of 2% soluble starch, 1% glucose, 0.5% polypeptone (Nihon Seiyaku), 0.6% wheat germ, 0.3% yeast extract (Nihon Seiyaku), 0.2% soybean meal (Ajinomoto), 0.2% $CaCO_3$, with a pH of 7.0 before sterilization for seed culture. A loop of the slant culture of *Acremonium* sp. PF1450 was inoculated into 20 ml of the seed medium and was cultured on a rotary shaker at 25 °C for 3 days. Two ml of the seed cultured broth was transferred to 100 ml of the same medium and was cultured on a rotary shaker at 25 °C for 4 days.

The culture filtrate (1 l) obtained from the fermented broth (1.2 l) was adjusted to pH 8.0 with 4% $NaHCO_3$, and was applied to a Diaion HP-20 column (200 ml). After washing with 80% aqueous MeOH, the fraction containing the active compounds was eluted with methanol. The active fractions were concentrated *in vacuo*, and further purification was carried out by HPLC (Shiseido, Tokyo, Japan, CapcellPak C_{18} UG120, ϕ 20 × 250 mm, flow rate: 5 ml min^{-1}) using a solvent system of 40% aqueous CH_3CN containing 0.1% acetic acid. The new compound, acremopeptin (**1**, 16 mg), was eluted at 24–25 min and a known compound, adenopeptin[7] (**2**, 80 mg), was eluted at 22–23 min (Figures 1a and b).

Acremopeptin (**1**) was obtained as white powder. The specific rotation of **1** was $[\alpha]_D^{20}$–3.0° (*c* 0.10, MeOH). The melting point exhibited 127 °C–132 °C. The molecular formula of **1** was determined to be $C_{70}H_{121}N_{16}O_{14}$ by high-resolution ESI mass spectrometer; (found, *m/z* 1409.9242 $[M]^+$, calcd for $C_{70}H_{121}N_{16}O_{14}$, 1409.9256). The UV spectrum of **1** exhibited an absorption maximum at 203.5 nm (ε 28 900) in MeOH. The IR spectrum of **1** showed strong absorptions at 3330, 1664 and 1536 cm^{-1}, suggesting the existence of an amide group. The analysis of the partial structures via ^1H- and ^{13}C-NMR spectroscopy suggested that **1** belongs to the peptaibol family.[8] The fragment ion pattern of **1** by LC/MS/MS experiments was similar to that of **2**. By the LC/MS/MS experiment, the fragment ions of *b*-series (*m/z* 140, 239, 338, 409, 466, 551, 636 and 735) from **1** were in accordance with those of **2**. Similarly, the fragment ions of *y*-series (*m/z* 224 and 323) from **1** were also in accordance with those of **2** (Figures 1c and d and Supplementary Figure S1). However, the fragment ion (*m/z* 420) of **1** was not observed in **2**. Furthermore, the

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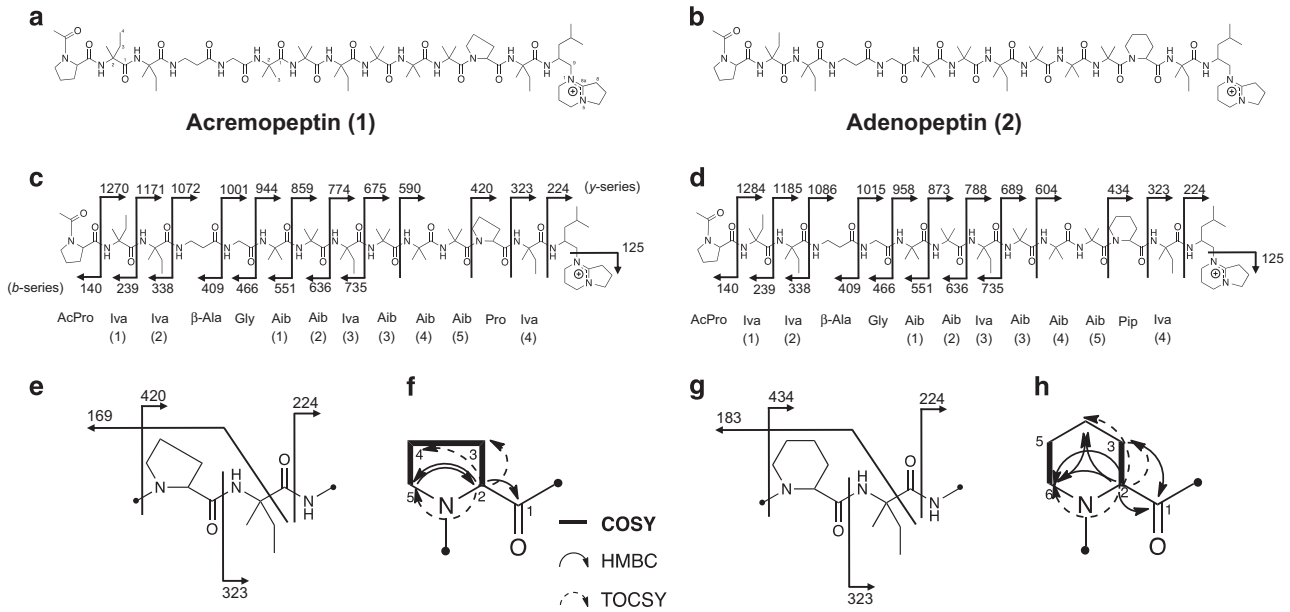

Figure 1 Structures of acremopeptin (**1**) and adenopeptin (**2**). (**a**) Structure of **1** (**b**) Structure of **2** (**c**), (**d**) fragmentation of **1** and **2** by LC/MS/MS. (**e**) Partial structures of 1 by LC/MS/MS/MS. (**f**) Correlation of partial structures of **1** by ^{1}H-^{1}H COSY, HMBC spectroscopy and TOCSY. (**g**) Partial structures of **2** by LC/MS/MS/MS. (**h**) Correlation of partial structures of **2** by ^{1}H-^{1}H COSY, HMBC spectroscopy and TOCSY.

LC/MS/MS/MS experiment of the fragment ions, *m/z* 420 of **1** and *m/z* 434 of **2** was performed. The fragment ion of *m/z* 169 from fragment ion (*m/z* 420) of **1** was observed, while a fragment ion of a *b*-series with *m/z* 183 from the fragment ion (*m/z* 434) of **2** was observed. The results suggested that the structure of **1** contained a proline, replacing the pipecolic acid (Pip) in **2** (Figures 1e and g). The ^{1}H- and ^{13}C-NMR data for **1** in CD_3OH are shown Table 1. The results were in accordance with those of **2** except for the difference caused by the replacement of Pip with proline. Spin-spin networks from the methine proton at H-2 (δ_H 4.07) to the methylene proton at H-5 (δ_H 3.80) in proline of **1** were shown by the ^{1}H-^{1}H COSY (Figures 1f and h). In the HMBC spectrum, the methine proton at H-2 of proline correlated with carbonyl carbon at C-1 (δ_C 174.7) and the methylene carbon at C-5 (δ_C 50.0) and the methylene proton at H-5 of proline correlated with the methine carbon at C-2 (δ_C 65.6). Furthermore, based on the results of TOCSY experiments of **1**, a spin network was observed from the methine proton at H-2 to those at H-3 (δ_H 1.73 and 2.29), H-4 (δ_H 1.91 and 2.00) and H-5 (δ_H 3.80). In contrast, a spin network in **2** was observed from the methine proton at H-2 (δ_H 4.54) to those at H-3 (δ_H 1.74 and 2.14), H-4 (δ_H 1.45 and 1.63) and H-6 (δ_H 3.37 and 4.22) (Figures 1f and h). Taken together, the structure of **1** was determined to be a new peptaibol as shown in Figure 1a.

To determine the absolute configuration of amino acids in **1**, the partial hydrolysis of **1** was performed by the method of Fukushima *et al.*[8] Compound **1** (10 mg) was partially hydrolyzed with a mixture of 6 N HCl and formic acid (1:1) at 37 °C for 16 h, resulting in three fragments. Each fragment was isolated by HPLC to give **1a** (2.4 mg), **1b** (0.7 mg) and **1c** (0.9 mg), and the chemical structures of these fragments were determined by LC/MS/MS analysis (Supplementary Figure S4). The absolute configurations of Pro and Iva in the fragments were determined by the advanced Marfey's method using an authentic sample (L-FDLA derivatives of both Pro and Iva stereoisomers).[9,10] The LC/MS analysis was performed under the following conditions: column; Shiseido Capcellpak C_{18} UG120 ϕ 2.0 × 50 mm, flow rate; 0.2 ml min^{-1}, solvent system; linear gradient elution with aqueous acetonitrile containing 0.1% formic acid (20–60% CH_3CN for Pro or 20–95% CH_3CN for Iva, 10 min), mass spectrometer; positive ion mode, detection; *m/z* 410.1659 ± 0.0021 for Pro and *m/z* 412.1826 ± 0.0021 for Iva. The retention times of the L and D isomers of the Pro derivative were 7.75 and 9.16 min, respectively, while those of the L and D isomers of the Iva derivatives were 7.36 and 8.07 min, respectively. The results indicated that the two Pro of **1** were in the L-configuration. The two Iva of **1a** were in the D-configuration, the one Iva of **1b** was in the L-configuration, and the one Iva of **1c** was in the L-configuration (Supplementary Figure S4).

Apoptosis induction activity of **1** and **2** was estimated by measuring the caspase-3 activity of doxorubicin-treated RAW264.7 cells. The caspase-3 activity was measured with a Caspase 3 Assay Kit, Fluorometric (Sigma-Aldrich, St Louis, MO, USA). Treatment of **1** and **2** enhanced the caspase-3 activity in doxorubicin-treated RAW264.7 cells (Supplementary Figure S2). Compounds **1** and **2** showed growth inhibition against PC-3 cells (GI_{50} values of 0.78 and 0.20 μM, respectively) and HT-29 cells (GI_{50} values of 0.66 and 0.30 μM, respectively) Protein levels of survivin and XIAP in PC-3 cells were estimated by western blot method. Compounds **1** and **2** suppressed protein levels of survivin and XIAP (Supplementary Figure S3). However, mRNA expression of survivin and XIAP was not suppressed by treatment of **1** and **2**.

Survivin associates with the molecular chaperone Hsp90.[11] The disruption of the survivin-Hsp90 interaction results in the proteasomal degradation of survivin. Furthermore, F_1F_0-ATPase functions as a co-chaperone of Hsp90, and the inhibition of F_1F_0-ATPase results in the disruption of the Hsp90-client protein complex.[12,13] Efrapeptin, a member of the peptaibols family, inhibits the mitochondrial F_1F_0-ATPase.[14,15] Because the structure of acremopeptin is similar to that of efrapeptin, acremopeptin might inhibit the mitochondrial F_1F_0-ATPase. Therefore, we deduce that the decrease in survivin protein level by acremopeptin results from the disruption of the survivin–Hsp90 interaction through the inhibition of F_1F_0-ATPase.

Table 1 ^{13}C and ^{1}H NMR assignments for acremopeptin (1) and adenopeptin (2) in CD_3OH

*Acremopeptin (**1**)*			*Adenopeptin (**2**)*			*Acremopeptin (**1**)*			*Adenopeptin (**2**)*		
	δ_C	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C	δ_H
AcPro			AcPro			Aib (3)[a]			Aib (3)[a]		
1	175.0		1	175.0		1	176.9		1	176.9	
2	61.5	4.33	2	61.5	4.33	2	57.8		2	57.9	
3	30.6	2.22, 1.92	3	30.6	2.22, 1.92	3	26.2	1.45–1.46	3	26.2	1.45–1.46
4	25.9	2.09, 1.97	4	25.9	2.09, 1.97	4	24.9	1.45–1.46	4	25–26	1.45–1.46
5	49.5	3.63	5	49.5	3.63	NH		7.92	NH		7.92
MeCO	172.0		MeCO	172.0		Aib (4)[a]			Aib (4)[a]		
MeCO	22.3	2.07	MeCO	22.3	2.07	1	177.6		1	177.5	
Iva (1)			Iva (1)			2	57.9		2	58.0	
1	175.1		1	175.1		3	27.2	1.46	3	26.6	1.47
2	61.3		2	61.3		4	24.8	1.55	4	25.2	1.53
3	32.5	1.7–1.9	3	32.6	1.7–1.9	NH		7.68	NH		7.70
4	8.2–8.4	0.92	4	8.2–8.4	0.91	Aib (5)[a]			Aib (5)[a]		
5	21.4	1.37	5	21.4	1.37	1	175.8		1	176.7	
NH		8.46	NH		8.45	2	58.2		2	57.9	
Iva (2)			Iva (2)			3	23.8	1.57	3	25.0	1.59
1	176.8		1	176.8		4	26.4	1.45	4	27.6	1.46
2	61.5		2	61.5		NH		7.95	NH		8.03
3	32.6	1.7–1.9	3	32.7	1.7–1.9	Pro			Pip		
4	8.2–8.4	0.86	4	8.2–8.4	0.86	1	174.7		1	173.0	
5	20.8	1.43	5	20.8	1.43	2	65.6	4.07	2	58.5	4.54
NH		7.33	NH		7.31	3	30.1	2.29, 1.73	3	26.3	2.14, 1.74
β-Ala			β-Ala						4	20.2	1.63, 1.45
1	175.1		1	175.1		4	26.9	2.00, 1.91	5	24.9	1.72, 1.61
2	37.2	2.49, 2.40	2	37.2	2.49, 2.40	5	50.0	3.80	6	44.0	4.22, 3.37
3	37.4	3.59, 3.31	3	37.4	3.59, 3.31	Iva (4)			Iva (4)		
NH		7.55	NH		7.54	1	177.7		1	177.0	
Gly			Gly			2	61.6		2	61.9	
1	171.8		1	171.8		3	33.7	2.06, 1.78	3	33.2	2.07, 1.82
2	45.0	3.76	2	45.1	3.76	4	8.4–8.6	0.95	4	8.2–8.4	0.91
NH		8.24	NH		8.23	5	20.0	1.51	5	20.7	1.48
Aib (1)			Aib (1)			NH		7.46	NH		7.53
1	176.7		1	176.7		C-terminus			C-terminus		
2	57.7		2	57.7		2	45.1	3.75, 3.35	2	45.1	3.72, 3.35
3	25–26	1.45–1.46	3	25–26	1.45–1.46	3	20.0	2.15, 2.03	3	19.8	2.15, 2.03
4	25–26	1.45–1.46	4	25–26	1.45–1.46	4	43.4	3.39	4	43.4	3.41
NH		8.36	NH		8.35	6	55.5	3.80, 3.72	6	55.5	3.80, 3.72
Aib (2)			Aib (2)			7	18.8	2.22, 2.15	7	18.8	2.22, 2.15
1	177.1		1	177.2		8	31.7	3.48, 2.88	8	31.7	3.25, 2.95
2	57.7		2	57.7		8a	166.9		8a	166.4	
3	25–26	1.42	3	25–26	1.42	9	57.5	3.45	9	57.6	3.40
4	25–26	1.45–1.46	4	25–26	1.45–1.46	10	45.6	4.40	10	45.7	4.41
NH		7.76	NH		7.76	11	41.9	1.58, 1.21	11	42.1	1.54, 1.22
Iva (3)			Iva (3)			12	25.5	1.71	12	25.5	1.71
1	177.2		1	177.3		13	23.8	0.90	13	23.8	0.92
2	61.0		2	60.9		14	21.3	0.87	14	21.5	0.89
3	30.6	1.9–2.0	3	30.6	1.9–2.0	NH		7.27	NH		7.32
4	8.2–8.4	0.89	4	8.2–8.4	0.91						
5	21.6	1.41	5	21.7	1.41						
NH		7.72	NH		7.72						

Abbreviations: Aib, 1-Amino isobutyric acid; β-Ala, 3-amino-propionic acid; Iva, Isovaline; Pip, pipecolic acid.
Chemical shifts of ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were adjusted with solvent signal.
[a]Interchangeable.

We are currently investigating the mechanism of action of acremopeptin in detail.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)