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NOTES

UCS1025A, a Novel Antibiotic Produced by *Acremonium* sp.

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In the course of our screening for antitumor antibiotics, two novel compounds, UCS1025A and B, were isolated from *Acremonium* sp. KY4917. UCS1025A exhibited antimicrobial activity, and antiproliferative activity against human tumor cell lines. In this paper, we describe the taxonomy of the producing organism, fermentation, isolation, physico-chemical properties and biological activities of UCS1025A and B.

The cultural and taxonomical characteristics of the producing fungal strain KY4917 were as follows. Colonies on 2% malt extract agar medium are 28 to 31 mm in diameter after culturing at 25°C for 2 weeks. The surface of the colony is grayish brown at the center and beige or grayish white at the margin. The color of the reverse of the colony is reddish brown at the center and grayish brown at the margin. Soluble orange pigment is produced in the medium. Colonies on Potato-glucose agar medium are about 25 mm in diameter after culturing at 25°C for 2 weeks. The color of the colony surface is cream or pale brown at the center and gray at the margin. The color of the reverse of the colony is pale brown to brown at the center and grayish white at the margin.

Smooth and colorless hyphae are developed on Potato-glucose agar medium. The hyphae are septate and well-branched. Conidia are produced at the tip of phialides developing from the hyphae. Typically forming single orthotropic phialides. Phialides are hyaline, smooth, 10 to 35 μm long and 1.5 to 2.5 μm wide at the base, and gradually tapering towards the tip to 0.5 to 1.5 μm wide. The conidial ontogeny is enteroblastic. The phialo-conidia adhering in slimy heads are single-celled, subspherical to ellipsoidal, hyaline, smooth-walled, and 2.5 to 4.5×1.7 to 2.5 μm. Chlamydospores spherical or subspherical and 2.5 to 5.5 μm in diam. are produced. No teleomorph was observed in this strain. From the characteristics mentioned above, the fungal strain KY4917 was identified as *Acremonium* sp.[1]. The fungus has been deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, as FERM BP-5673.

A loopful of the cells from a mature slant of strain KY4917 was inoculated into each of two 300-ml Erlenmeyer flasks containing 50 ml of the seed medium composed of glucose 10%, dried mashed potato (Yukijirushi Co. Ltd.) 3%, yeast extract (Nihon-Seiyaku Co. Ltd.) 0.5% in deionized water (pH adjusted to 6.5 with NaOH before sterilization). The inoculated flasks were incubated on a rotary shaker at 25°C for 6 days. Fifteen ml of the seed culture were added to a 2-liter Erlenmeyer flask containing 300 ml of the same medium. After incubation on a rotary shaker at 25°C for 2 days, nine hundred ml of seed culture was transferred into each of two 30-liter fermenters containing 18 liters of a fermentation medium composed of glucose 2%, dried mashed potato 2%, peptone (Kyokuto Co. Ltd.) 0.5%, KH_2PO_4 0.5%, $Mg_3(PO_4)_2 \cdot 7H_2O$ in deionized water (pH adjusted to 6.0 with NaOH before sterilization). The fermentation was carried out at 25°C for 7 days with agitation of 300 rpm and aeration of 18 liters per minute.

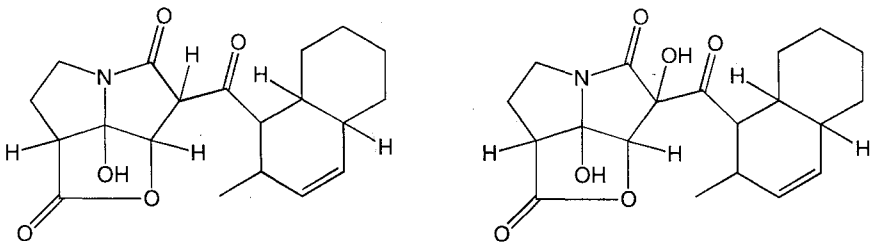
The 30 liters of culture broth was filtered using a centrifugal filter and the filtrate was applied to a column of Diaion HP-20 (2 liters, Mitsubishi Chemical Industries). The column was washed with 40% MeOH and then the active substance was eluted with MeOH (6 liters). The eluate was concentrated and extracted with AcOEt. The extract was concentrated *in vacuo* to yield a brown oil. The oil was applied to a column of silica gel Wakogel C-200 (Wako Pure Chemical Industries). The column was washed with hexane-AcOEt (7:3) and the active substance was eluted with hexane-AcOEt (6:4). The eluate was concentrated *in vacuo* and then applied to a column of silica gel LiChroprep Si 60 (Merck). After the column was washed with hexane-acetone (86:14), UCS1025A and B

Table 1. Physico-chemical properties of UCS1025A and UCS1025B.

	UCS1025A	UCS1025B
Appearance	Colorless needle crystal	Colorless needle crystal
Melting point	135-137°C	221-223°C
$[\alpha]_D^{28}$ (c 0.1, MeOH)	+30.1°	-31.8°
Molecular formula	$C_{20}H_{25}NO_5$	$C_{20}H_{25}NO_6$
HRFAB-MS		
Found	360.1799 $[M+H]^+$	376.1757 $[M+H]^+$
Calcd.	360.1811 (for $C_{20}H_{26}NO_5$)	376.1760 (for $C_{20}H_{26}NO_6$)
UV λ_{max} nm (ε) (MeOH)	260 (7100)	End absorption
IR ν_{max} (KBr) cm^{-1}	3440, 2920, 2850, 1790, 1670, 1445, 1415, 1385, 1320, 1235, 1160, 1110, 1020, 970	3205, 2925, 2875, 1805, 1720, 1670, 1410, 1325, 1300, 1175, 1130, 1040
TLC (Rf value[a])	0.39	0.28
Solubility		
Soluble	MeOH, AcOEt, $CHCl_3$, DMSO	MeOH, AcOEt, $CHCl_3$, DMSO
Insoluble	hexane	hexane

[a] Silica gel TLC (Kieselgel 60 F_{254}, Merck), solvent: hexane - acetone (2 : 1).

Fig. 1. Structures of UCS1025A and UCS1025B.



UCS1025A UCS1025B

were eluted with hexane - AcOEt (84 : 16~82 : 18) and hexane - AcOEt (70 : 30~75 : 25), respectively. Both of the fractions of UCS1025A and B were allowed to stand for 3 days at 4°C for crystallization. By recovering and drying precipitated crystals, 131 mg of UCS1025A and 4.8 mg of UCS1025B were obtained.

The physico-chemical properties of UCS1025A and B are summarized in Table 1. The structures of UCS1025A and B were elucidated by the spectroscopic analysis and the X-ray crystallography (Fig. 1). UCS1025A turned out to be a novel natural product which was constituted from two segments. One of them was an unique tricyclic skeleton including a pyrrolizidine fused with a γ-lactone and another was a decaline moiety. On the other hand, UCS1025B contained a mono-oxidized tricyclic skeleton and the decaline moiety. Antitumor compounds clazamycin A and B from *Streptomyces puniceus*[2] and antimicrobial compounds pyrrolam A~D from *Streptomyces olivaceus*[3] have been isolated as the antibiotics containing a pyrrolizidine moiety. However UCS1025A belongs to a distinct class from these reported antibiotics because UCS1025A possesses the unique pyrrolizidine skeleton. The details in the structure determination of UCS1025A and B will be reported elsewhere.

The antimicrobial activities of UCS1025A and B are shown in Table 2. UCS1025A exhibited antimicrobial activity against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus hirae*, and

Table 2. Antimicrobial activities of UCS1025A and UCS1025B.

Test microorganisms	MIC (μg/ml)	
	UCS1025A	UCS1025B
Staphylococcus aureus subsp. *aureus* ATCC 6538 P	1.3	42
Bacillus subtilis No.10707	1.3	83
Enterococcus hirae ATCC 10541	1.3	42
Proteus vulgaris ATCC 6897	5.2	83
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 10031	>83	>83
Escherichia coli ATCC 26	>83	>83
Pseudomonas aeruginosa BMH No.1	>83	>83
Shigella sonnei ATCC 9290	>83	>83
Candida albicans ATCC 10231	>83	>83

Table 3. Antiproliferative activities of UCS1025A and UCS1025B.

Cell lines	IC_{50} (μM)		
	UCS1025A	UCS1025B	VP-16
ACHN	58	>100	0.31
A431	55	>100	0.27
MCF-7	21	>100	0.57
T24	51	>100	1.3

Gram-negative bacterium, *Proteus vulgaris* with the MIC values ranged from 1.3 μg/ml to 5.2 μg/ml. In contrast, UCS1025B showed a weak antimicrobial activity with the MIC values ranged from 42 μg/ml to 83 μg/ml.

The antiproliferative activities of UCS1025A and B are shown in Table 3. UCS1025A exhibited an antiproliferative activity against human tumor cell lines with the IC_{50} values ranged from 21 μM to 58 μM. In comparison with other antitumor agent like etoposide (VP-16), antiproliferative activity of UCS1025A is weak as an antitumor compound. As in the case of antimicrobial activities, UCS1025B showed no antiproliferative activity against these cell lines at the concentration up to 100 μM, suggesting the structural difference in the tricyclic skeleton between UCS1025A and UCS1025B affect their biological activities. Detailed studies on the mechanism of action and antitumor activity of UCS1025A and B are in progress.

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