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The Synergistic Antibacterial Activity of 1-Acetyl-β-Carboline and β-Lactams Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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1-Acetyl-β-carboline was isolated as an anti-MRSA agent from the fermentation broth of a marine actinomycete isolated from marine sediment. The producing strain was identified to be *Streptomyces* sp. by phylogenetic analysis of the 16S rRNA gene sequence. The anti-MRSA agent was isolated by bioactivity-guided fractionation of the culture extract by solvent partitioning, ODS open flash chromatography, and purification with a reversed-phase HPLC. Its structure was elucidated by extensive 2D NMR and mass spectral analyses. Combination of 1-acetyl-β-carboline with ampicillin exhibited synergistic antibacterial activity against MRSA.

Keywords: *Streptomyces* sp. 04DH52, anti-MRSA agent, 1-acetyl-β-carboline

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a worldwide concern because it is highly prevalent, capable of developing new clones, resistant to almost all currently available antibiotics except vancomycin and teicoplanin, and can potentially cause death [3]. Moreover, glycopeptide-resistant strains have been emerging owing to the increasing use of glycopeptides [6]. Recently, linezolid and daptomycin have also been available for MRSA infections. However, new resistant strains against many drugs including these have already been reported [14]. Furthermore, a decrease in the susceptibility of MRSA to vancomycin and teicoplanin has been reported in several hospitals worldwide [13]. Therefore, new and potent anti-MRSA agents are urgently required.

As a part of our ongoing screening program for bioactive secondary metabolites, we isolated marine actinomycete strains from marine sediments and marine organisms collected at various sites of South Korea and the western Pacific Ocean. Strain 04DH52 was isolated from shallow water sediment taken at −2 m depth of Ayajin Bay, on the East Sea of Korea. This strain was identified as *Streptomyces* sp. by 16S rRNA gene sequence analysis. The crude extracts of strain 04DH52 exhibited anti-MRSA activity. Bioassay-guided fractionation by solvent partitioning, ODS vacuum flash chromatography, and purification with a reversed-phase HPLC gave a pure anti-MRSA compound, 1-acetyl-β-carboline.

Indole alkaloids are pharmacologically active natural products that have been shown to possess a wide range of biological activities, including cytotoxic, antiviral, antimicrobial, antiinflammatory, antiserotonin, and enzyme inhibitiory activities [5]. One important subclass of indole alkaloids is β-carbolines, which possess a common tricyclic pyrido[3, 4] indole ring structure [15]. The β-carbolines skeleton is an important structure in drug discovery [12], and drugs on the market such as tadalafil possess this indole nucleus. β-Carboline alkaloids are widespread in plants, but very rare in microorganisms [1]. In this report, we describe the isolation, physicochemical properties, structure elucidation, and synergistic anti-MRSA activity of 1-acetyl-β-carboline in combination with β-lactams. To the best of our knowledge, this is the first report that describes the anti-MRSA activity of 1-acetyl-β-carboline from a marine-derived actinomycete.

Microorganism and Identification

The bacterial strain 04DH52 was isolated from marine sediments taken at −2 m depth of Ayajin Bay, on the East Sea of Korea in August 2004. The sediment (1 g) was incubated for 50 min at 60°C and resuspended in 9 ml of autoclaved seawater. After filtration and serial dilution with autoclaved seawater, 0.1-ml aliquots were spread onto International *Streptomyces* Project (ISP) medium 2, inorganic salt–starch agar (ISP medium 4) [11], and modified Bennett's agar [2]. The plates were incubated for 14–20 days at 30°C, and the resulting colonies were transferred and maintained on the modified Bennett's agar. Among the

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actinomycete strains isolated, the strain showing significant anti-MRSA activity was designated *Streptomyces* sp. 04DH52. For taxonomic identification, the strain 04DH52 was analyzed following the 16S rDNA sequence method described previously [9]. The BLAST search of 16S rRNA gene sequences available in the DDBJ/EMBL/GenBank databases showed the highest similarity of 99.9% with *Streptomyces albus* subsp. *albus* NBRC 3711 (AB184782).

Culture Conditions, Isolation, and Purification

A 100-ml flask containing 30 ml of the seed medium (modified Bennett's medium) was inoculated with a stock culture of the producing strain 04DH52 maintained on a modified Bennett's agar. After incubation at 30°C for 3 days on a rotary shaker set at 150 rpm, the seed culture was transferred to each of twenty-two 2-l Fernbach flasks and a 20-l fermentor containing 600 ml and 17 l of the production (modified Bennett's) medium, respectively. The fermentation was carried out at 30°C for 7 days with shaking at 200 rpm. Purification and isolation of 1-acetyl-β-carboline was guided by the anti-MRSA activity. After 7 days, the culture broth was centrifuged (2,000 ×*g* for 15 min at 4°C) and then filtrated (0.2-μm pore-size membrane filter) to obtain a cell-free supernatant. Amberlite XAD-7 resin (20 g/l) was added to the supernatant to adsorb excreted organic substances. The resin was collected by centrifugation and extracted three times with acetone. The acetone extract was concentrated *in vacuo* and the residual suspension (4.57 g) was subjected to ODS open flash chromatography with a stepwise gradient mixture of MeOH/H_2O as eluant. The fraction eluted with 80% MeOH in water was purified by a reversed-phase HPLC (YMC ODS-A column, 10×250 mm; 65–85% MeOH; flow rate, 2.0 ml/min; UV detection at 210 nm) to yield an anti-MRSA agent, 1-acetyl-β-carboline (3.8 mg; Fig. 1).

Structure Determination

The molecular formula of 1-acetyl-β-carboline was established as $C_{13}H_{10}N_2O$ by MS analysis [*m/z* 209 $(M-H)^-$, 211 $(M+H)^+$] and ^{13}C NMR spectral data (Table 1). ^1H NMR data (Table 1) of 1-acetyl-β-carboline revealed resonances for 6 aromatic protons (δ 8.45, δ 8.30, δ 8.21, δ 7.70, δ 7.59, δ 7.31). Among these olefinic protons, two *ortho*-coupled doublets at δ 8.21 and δ 7.70 (both J=7.8 Hz) and two triplets at δ 7.59 and δ 7.31 (both J=7.8 Hz) indicated the presence of a 1,2-disubstituted benzene ring. Its ^{13}C NMR data (Table 1) also exhibited resonances for 11 olefinic carbons, of which 6 (δ 138.8, 130.5, 122.8, 121.8, 120.4, 113.6) were sp^2 methine and the remaining 5 (δ 143.6, 137.4, 136.3, 133.4, 121.8) were sp^2 quaternary carbons. ^1H and ^{13}C correlations were indicated by the gHSQC spectrum. Two mutually coupled doublet signals at δ 8.45 (H-3) and 8.30 (H-4) could be assigned to α and β pyridine protons, respectively, according to their chemical shifts, coupling constant (J=4.8 Hz), and the chemical shifts of carbons (C-3, δ 138.8; C-4, δ 120.4) to which they are attached. These spectroscopic characteristics suggested the presence of a C-1-substituted β-carboline, which was also supported by the HMBC correlations. In the HMBC spectrum, all possible $^3J_{CH}$ and $^2J_{CH}$ were displayed between the six protons and the 11 carbons in the β-carboline units. The remaining two carbons (δ 203.4, δ 26.2) were assigned as an acetyl group that was attached to C-1 by the HMBC correlation between H-11 (δ 2.82) and C-1 (δ 203.4). Thus, the gross structure of β-carboline was elucidated to be 1-acetyl-β-carboline (Fig. 1).

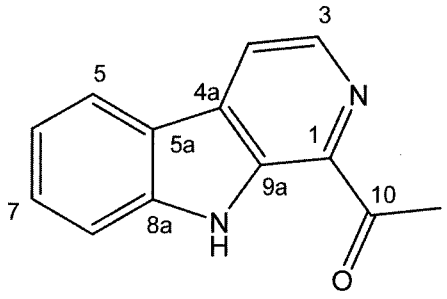

Fig. 1. Structure of 1-acetyl-β-carboline.

Antibacterial Activity

In order to evaluate the antibacterial activity of 1-acetyl-β-carboline against MRSA strains and other bacteria, the minimum inhibitory concentrations (MICs) were determined by the 2-fold serial dilution method as described by the National Committee for Clinical Laboratory Standards [7]. The MICs of 1-acetyl-β-carboline against MSSA (methicillin-

Table 1. ^1H and ^{13}C NMR data of 1-acetyl-β-carboline in CD_3OD-d_4.

No.	δ_H	Mult (J in Hz)	δc	COSY	HMBC
1			137.4		
2					
3	8.45	d (5.4)	138.8	H-4	C-1, -4, -4a
4	8.30	d (5.4)	120.4	H-3	C-3, -9a, -5a
4a			133.4		
5a			121.8		
5	8.21	d (7.8)	122.8	H-6	C-7, -8a
6	7.31	td (7.8, 1.0)	121.8	H-5, -7	C-5a, -8
7	7.59	td (7.8, 1.0)	130.5	H-8	C-5, -8a
8	7.70	d (7.8)	113.6		C-5a, 6
8a			143.6		
9					
9a			136.3		
10			203.4		
11	2.82	s	26.2		C-1, -10

Table 2. Minimum inhibitory concentration (MIC) of 1-acetyl-β-carboline and β-lactams against methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

Strain	Source or reference	MIC (μg/ml)			
		1-Acetyl-β-carboline	Ampicillin	Penicillin	Oxacillin
MSSA (KCTC 1927)	Standard strain	64	< 1	< 1	< 1
MRSA (KCCM40510)	Standard strain	256	512	512	128
MRSA (KCCM40511)	Standard strain	256	512	256	128
MRSA D-3	Clinical isolate[a]	256	128	128	512
MRSA D-4	Clinical isolate	256	128	256	128
MRSA D-5	Clinical isolate	128	256	256	128
MRSA D-6	Clinical isolate	256	256	128	256
MRSA D-8	Clinical isolate	256	256	128	512
MRSA D-10	Clinical isolate	256	128	128	128
MRSA D-12	Clinical isolate	256	128	128	64
MRSA D-13	Clinical isolate	256	128	256	256
MRSA D-14	Clinical isolate	256	128	64	128
MRSA D-17	Clinical isolate	256	128	128	128
MRSA D-18	Clinical isolate	256	128	128	512
MRSA D-19	Clinical isolate	256	128	128	256

[a]MRSA strains were isolated in Dong-A University Medical Hospital.

susceptible *Staphylococcus aureus*) and MRSA strains are shown in Table 2. The MRSA strains tested in the present study were highly resistant to the β-lactams. 1-Acetyl-β-carboline showed antibacterial activity against MRSA strains tested with MICs ranging from 128 to 256 μg/ml. The MICs of 1-acetyl-β-carboline was similar to those of β-lactams against MRSA strains. 1-Acetyl-β-carboline also showed antibacterial activity against MSSA, suggesting the anti-MRSA activity of 1-acetyl-β-carboline may not be related to penicillin-binding protein 2A (PBP2A), which decreases the binding affinity to β-lactams, because the antibacterial activity of 1-acetyl-β-carboline is not specific to MRSA. As is shown in Table 3, 1-acetyl-β-carboline also exhibited antibacterial activity against Gram-negative bacteria with MICs ranging from 64 to 256 μg/ml, even though the compound was less effective against Gram-negative bacteria than against other Gram-positive bacteria. These results suggest that 1-acetyl-β-carboline shows broad-spectrum antibacterial activities.

Combination Effect of 1-Acetyl-β-Carboline and β-Lactams Against MRSA

It has been known that one of the effective approaches to overcome bacterial resistance is restoration of antibiotic activity through the synergistic action of antibacterial materials from natural and old agents [10, 16]. Therefore, we compared MICs of 1-acetyl-β-carboline used alone or in combination with β-lactams against MRSA strains. The synergistic effects between 1-acetyl-β-carboline and β-lactams, including ampicillin, penicillin, and oxacillin, were investigated by the checkerboard method and were evaluated as the fractional inhibitory concentration (FIC) index [8]. As is shown in Table 4, the FIC indices of 1-acetyl-β-carboline and ampicillin were from 0.156 to

Table 3. Antibacterial activity of 1-acetyl-β-carboline against pathogenic bacteria.

	Strain	MIC[c] (μg/ml)	
		1-Acetyl-β-carboline	Vancomycin
Gram-positive	MSSA (KCTC 1927)[a]	64	0.5
	MRSA (KCCM40510)[b]	256	2
	Enterococcus faecalis (KCTC 2011)	64	0.5
	Bacillus subtilis (KCTC 1028)	64	0.5
Gram-negative	*Escherichia coli* (KCTC 1682)	128	512
	Klebsiella pneumonia (KCTC 2242)	128	512
	Legionella birminghamensis (KCTC 2057)	128	256
	Pseudomonas aeruginosa (KCTC 1637)	128	256
	Salmonella typhimurium (KCTC 1925)	256	512

[a]Methicillin-susceptible *Staphylococcus aureus*; [b]methicillin-resistant *S. aureus*; [c]Minimum inhibitory concentration.

Table 4. MICs and FIC[a] indices of 1-acetyl-β-carboline in combination with β-lactams against MRSA.

Strain	Ampicillin					Penicillin					Oxacillin				
	MIC (μg/ml)			FIC index		MIC (μg/ml)			FIC index		MIC (μg/ml)			FIC index	
	A	B	C	b	c	A	B	C	b	c	A	B	C	b	c
MRSA40510	512	16	8	0.156	0.266	512	32	16	0.188	0.281	128	128	64	1.125	0.750
MRSA40511	512	16	8	0.156	0.266	256	32	16	0.250	0.313	128	128	128	1.125	1.250
MRSA D-3	128	16	8	0.250	0.313	128	32	16	0.375	0.375	512	256	256	0.625	0.750
MRSA D-4	128	16	8	0.250	0.313	256	32	16	0.250	0.313	128	128	128	1.125	1.250
MRSA D-5	256	8	4	0.281	0.516	256	32	8	0.375	0.281	128	128	128	1.250	1.500
MRSA D-6	256	16	4	0.188	0.266	128	32	16	0.375	0.375	256	64	64	0.375	0.500
MRSA D-8	256	16	4	0.188	0.266	128	32	16	0.375	0.375	512	256	128	0.625	0.500
MRSA D-10	128	8	4	0.188	0.281	128	16	8	0.250	0.313	128	128	64	1.125	0.750
MRSA D-12	128	16	4	0.250	0.281	128	32	16	0.375	0.375	64	64	64	1.125	1.250
MRSA D-13	128	16	8	0.250	0.313	256	32	16	0.250	0.313	256	256	128	1.125	0.750
MRSA D-14	128	8	4	0.188	0.281	64	16	8	0.375	0.375	128	128	32	1.125	0.500
MRSA D-17	128	8	4	0.188	0.281	128	32	16	0.375	0.375	128	64	64	0.625	0.750
MRSA D-18	128	16	8	0.250	0.313	128	16	8	0.250	0.313	512	512	256	1.125	0.750
MRSA D-19	128	16	8	0.250	0.313	128	32	16	0.375	0.375	64	256	256	1.125	1.250

A, without 1-acetyl-β-carboline; B to C and b to c, 1-acetyl-β-carboline at 32 and 64 μg/ml, respectively.

[a]The FIC was calculated as the MIC of 1-acetyl-β-carboline or each antibiotic in combination divided by the MIC of 1-acetyl-β-carboline or each antibiotic alone. The FIC index was obtained by the sum of FICs. The FIC index indicated synergy: 0.5, synergic; > 0.5 to 1, additive; > 1 to 2, independent; > 2, antagonistic.

0.313 in combination with 32 and 64 μg/ml of 1-acetyl-β-carboline against all tested MRSA strains and a synergistic effect was observed. The FIC indices of penicillin ranged from 0.188 to 0.375 in combination with 1-acetyl-β-carboline (32 and 64 μg/ml) and a synergistic effect was also observed. However, no synergy was observed between 1-acetyl-β-carboline and oxacillin against most of the MRSA. These results are similar to the difference of synergistic effect in dieckol–β-lactams combination. Dieckol showed synergy against MRSA in combination with ampicillin and penicillin, but there was no synergy effect in a dieckol–oxacillin combination [4]. The difference of synergistic effect in an anti-MRSA substance–β-lactams combination was also reported in epigallocatechin gallate (EGCg). EGCg displayed a synergistic effect in combination with penicillin and oxacillin, but no synergy was observed with an EGCg–ampicillin combination [16]. Some significant infections should not be treated with single antibiotic, because the bacteria can rapidly develop resistance when such a single antibiotic is used. Combinations of two or more compounds are generally superior to the use of a single compound, especially for the treatment of serious infections caused by antibiotics-resistant bacteria. The ability of 1-acetyl-β-carboline to enhance the antibacterial activity of ampicillin for MRSA is a useful property and these combinations could decrease the emergence of MRSA. The mechanism of the synergistic effect of 1-acetyl-β-carboline and β-lactams is unknown at present, but the authors speculate that a possible mechanism for the synergistic effect may be attributed to their different structure and they attack the same target site of the cell wall. Studies on the synergistic anti-MRSA mechanism and *in vivo* efficacy of 1-acetyl-β-carboline, alone and in combination with ampicillin, deserve further investigation.

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