NOTES

New Naphthyridinomycin-type Antibiotics, Aclidinomycins A and B, from *Streptomyces halstedi*

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(Received for publication October 12, 2000)

In a search for new antitumor substances as a rec-active antibiotic using a modified rec assay,[1,2] we found that *Streptomyces halstedi* KB012 isolated from a sea sand sample collected from Yasuura seashore (Hiroshima) produced a rec-active antibiotic that exhibited more growth inhibition on a recombination-deficient (*rec*$^-$) strain than a proficient (*rec*$^+$) strain of *Bacillus subtilis*. TLC of the crude-solvent extract from the culture broth revealed that the active entity consisted of at least six yellow spots. However, four of these components were quite labile, so that our efforts to isolate each pure component were unsuccessful. The remaining two components were fortunately stable. In this paper, we describe the isolation and identification of these stable components A and B as new analogs of the bioxalomycins which have been more recently isolated as novel naphthyridinomycin-type antibiotics.[3,4]

S. halstedi KB012 was cultured in a 30-liter jar fermenter containing 15 liters of the medium: glucose 1.5%, sucrose 1.5%, soybean meal 1.5%, dried yeast 0.75%, mineral solution ($CuSO_4 \cdot 5H_2O$ 1.0 g, $ZnSO_4 \cdot 7H_2O$ 0.4 g, $CoSO_4 \cdot 7H_2O$ 0.4 g, $FeSO_4 \cdot 7H_2O$ 1.0 g and $MnSO_4 \cdot 4H_2O$ 1.0 g in 200 ml of deionized water, pH 2.3) 0.15% and halogen salt solution (KBr 1 g, KI 1 g and NaF 1 g in 100 ml of deionized water) 0.1% in sea water, pH 7.0. Seed culture was prepared by 48-hour shake-cultivation at 30°C in 500-ml Erlenmeyer flasks each containing 50 ml of the medium: soluble starch 0.5%, glucose 0.5%, soybean meal 1.0%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1% and NaCl 0.2% in deionized water, pH 7.0. Inoculation was 1%. The jar fermentation was carried out at 30°C for 4 days under aeration of 7.5 liter/hour. The agitation was controlled at 150 rpm for first 2 days and then at 225 rpm. The culture broth from two jar fermenters was centrifuged and the supernatant (about 22 liters) thus obtained was subjected to adsorption chromatography on a column of HP-20 resin. The column was washed with 1 liter of water and eluted with 1 liter of 70% acetone. The eluate was pooled and evaporated to about 500 ml, and was extracted with 500 ml of $CHCl_3$ after pH adjustment to 8.0 with 1 N NaOH. The $CHCl_3$ extract was evaporated to dryness to give a crude yellow material (1.5 g). TLC of the crude preparation on silica gel plate $70F_{254}$ with a solvent of $CHCl_3$-MeOH (5 : 1) afforded six yellow spots with Rf values of 0.69, 0.58, 0.48, 0.32, 0.19 and 0.1. A bioautogram showed that all the spots were active against *rec*$^-$ *B. subtilis*. Of these spots, two yellow spots (components A and B) with a respective Rf value of 0.48 and 0.32 were stable, and these components A and B were partially purified by a column chromatography on silica gel (40×250 mm, Wako gel C-200) with solvents of $CHCl_3$-MeOH (50 : 1~5 : 1). Fractions having component A or B were pooled and evaporated to dryness (A, 112 mg; B, 150 mg). Further purification was done by a semipreparative HPLC with a Luna 5 μ C18(2) column (10×250 mm) (Phenomenex). The mobile phase was 30% CH_3CN and the flow rate 2 ml/minute, The elution was monitored at 210 nm. The components A and B were eluted at a Rt of 15.6 and 10.1 minutes, respectively. Fractions containing A or B were pooled and evaporated to dryness after extraction with $CHCl_3$. The same HPLC was once repeated for complete purification. Thus, pure A (62 mg) and B (22 mg) were obtained and named aclidinomycin A (**1**) and B (**2**), respectively.

Physico-chemical properties of **1** were: $[\alpha]_D$ ±0° (*c* 0.02, MeOH); UV λ_{max}^{MeOH} nm (ε) 282 (19,000), 403 (3,500);

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Table 1. ^{1}H and ^{13}C NMR spectral data for aclidinomycin A (**1**) and B (**2**) in $CDCl_3$.

No	**1**		**2**	
	δ_C ppm (125 MHz)	δ_H ppm (J = Hz) (500 MHz)	δ_C ppm (125 MHz)	δ_H ppm (J = Hz) (500 MHz)
1	48.5	2.24 dt (9.2 d, 8.6 t)	48.5	2.24 dt (9.2 d, 8.3 t)
		2.53 ddd (8.3, 7.3, 1.8)		2.48 m
2	65.9	3.71 ddd (9.5 d, 7.2 t)	65.9	3.68 dt (9.2 d, 7.3 t)
		3.79 ddd (8.4, 7.6, 1.8)		3.77 ddd (8.3, 7.3, 1.8)
3a	91.3	3.94 d (3.7)	91.1	3.92 d (3.8)
4	37.6	2.86 m	37.6	2.85 m
4'	19.2	1.6 9 ddd (13.8, 7.3, 18)	19.1	1.56 ddd (14.0, 8.0, 1.5)
		1.76 ddd (13.8, 11.0, 7.3)		1.77 ddd (14.0, 11.0, 7.6)
5'	41.1	2.52 s	40.9	2.51 s
6	63.4	3.31br d (7.3)	62.9	3.36 br d (7.6)
7	91.6	5.33 br q (2.8)	90.7	5.59 br s (7.6)
9	159.0		157.4	
9'	72.6	4.98 ddd (15.6, 2.6, 1.2)	97.7	6.49 d (1.5)
		5.42 dd (15.6, 2.8)		
9a	96.0		96.8	
10	175.4		175.8	
11	162.8		162.4	
11'	61.2	4.19 s	61.3	4.12 s
12	124.9		126.5	
12'	9.2	1.92 s	9.5	1.94 s
13	196.6		195.8	
13a	71.4		71.1	
13b	56.2	3.07 d (1.8)	55.2	3.14 d (1.9)
13c	52.5	4.08 br s	52.5	4.13 m

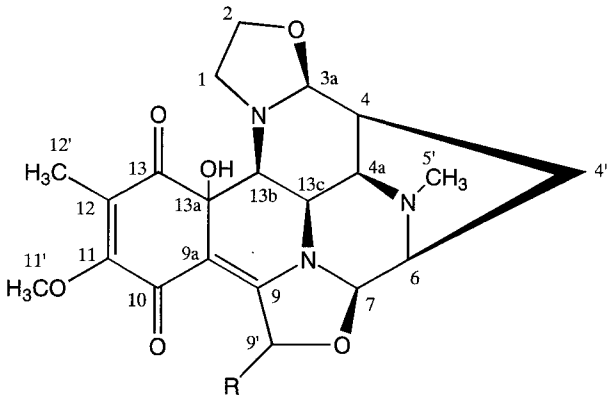
IR ν_{max} (film) cm^{-1} 3370, 1657, 1628, 1604, 1547. The molecular formula was determined to be $C_{21}H_{25}N_3O_6$ from the high resolution FABMS (*m/z* 416.1803 $[M+H]^+$, $\Delta -1.9$ mmu) in conjunction with the ^{1}H and ^{13}C NMR data (Table 1), which were similar to those of bioxalomycin $\beta 2$.[3,4]. The molecular weight is 16 amu larger than that of bioxalomycin $\beta 2$. The IR spectrum indicated the presence of a hydroxyl group (3370 cm^{-1}) in addition to conjugated carbonyl functionalities (1657, 1628, 1604 cm^{-1}). All the protonated carbons of **1** were assigned by the analysis of the HMQC spectrum. In the HMBC spectrum, the H-13b signal at δ_H 3.07 showed long-range correlations to C-9a (δ_C 96.0), C-13 (δ_C 196.6) and C-13a (δ_C 71.4). The H-9′ signal at δ_H 4.98 showed long-range correlations to C-9 (δ_C 159.0) and C-9a. These findings indicate the presence of a hydroxyl group at C-13a and the presence of C-C double bond between C-9 and C-9a. On addition of deuterium oxide, 0.05, 0.12 and 0.03 ppm-upfield shifts of C-9a, C-13a and C-13b signals, respectively, and a 0.03 ppm-downfield shift of C-13 signal were observed in the ^{13}C NMR spectrum, indicating the presence of the hydroxyl group at C-13a. The stereochemistry of **1** except for C-13a was revealed by NOE experiments. Thus, the structure of **1** was determined as shown in Fig. 1. The stereochemistry of C-13a remains to be determined.

Physico-chemical properties of **2** were: $[\alpha]_D$ +188° (*c* 0.16, MeOH); UV λ_{max}^{MeOH} nm (ε) 286 (21,500), 398 (5,000); IR ν_{max} (film) cm^{-1} 3350, 1655, 1628, 1609, 1551. **2** had a molecular formula of $C_{21}H_{25}N_3O_7$, as established by the high resolution FABMS data (*m/z* 432.1796 $[M+H]^+$, $\Delta +2.5$ mmu). The UV and IR spectra of **2** were nearly identical to those of **1**. The ^{13}C NMR spectrum (Table 1)

was almost the same as that of **1** except that it lacked an oxygenated methylene signal and contained an oxygenated methine signal at δ_C 97.7, which is appropriate for a hemiacetal carbon. The proton signal at δ_H 6.49 in the ^1H NMR spectrum of **2** (Table 1) was assigned to the hemiacetal proton (H-9′) by the analysis of the HMQC spectrum. The stereochemistry of **2** except for C-9′ and C-13a was revealed by NOE experiments. Thus, the structure of **2** was determined to be 9′-hydroxy compound of **1** (Fig. 1). The stereochemistry of C-9′ and C-13a remains to be determined.

1 and **2** resemble to naphthyridinomycin[5] but differed from it by the oxazoline ring forming between C-9′ and C-7 as in the bioxalomycins.[3,4] They were also clearly different from bioxalomycins by a hydrogenated benzoquinone structure and a hydroxyl at C-13a (**1**) or an additional hydroxyl at C-9′(**2**). Aclidinomycins producer was a different *Streptomyces* species strain from producers of naphthyridinomycin[5~8] and bioxalomycins,[3] and also from dnacin (2-aminosubstituted naphthylriddinomycin analog) producer, *Actinosynnema pretiosum*.[8] Thus, **1** and **2** have never been obtained from the above producers.

Fig. 1. Structures of aclidinomycin A (**1**) and B (**2**).



1 R = H
2 R = OH

The antimicrobial activity of **1** and **2** is shown in Table 2. They exhibited antimicrobial activity against the Gram-positive bacteria, *B. subtilis*, *B. cereus* and *Micrococcus luteus* at a concentration of 6.25 to 25 μg/ml, but not against Gram-negative bacteria, *Candida albicans* and *Aspergillus fumigatas* even at the concentration of 100 μg/ml. The antimicrobial activity of **1** and **2** were about 50 fold lower than that of naphthyridinomycin.[3] In the rec assay, the concentrations of **1** and **2** to give a 15-mm inhibitory zone on the cup-assay plates with *rec*$^-$ and *rec*$^+$ *B. subtilis* were 16.8 μg/ml and 28.8 μg/ml and 13.6 μg/ml and 23.5 μg/ml, resepectively. Thus, both the rec potencies, expressed as a ratio of the inhibitory concentrations (*rec*$^+$/*rec*$^-$), of **1** and **2** were about 1.7. Criteria on the rec potency[1,2] indicate that both **1** and **2** are DNA-targeting drug. Doxorubicin, used as a reference, gave approximately the same rec potency, 1.7, in the rec assay and its 15-mm inhibitory concentration on *rec*$^-$ *B. subtilis* plate was as low as 10 μg/ml, indicating that antimicrobial activities of **1** and **2** are near to that of doxorubicin when assayed on the agar plate. However, both the antiproliferative activities of **1** and **2** on human leukemic K562 cell culture were as low as 10 μg/ml at the IC_{50} value and were about 500 fold weaker than that of doxorubicin.

Table 2. Antimicrobial activity of aclidinomycin A (**1**) and B (**2**).

Microorganisms	MIC (μg/ml)	
	1	**2**
Bacillus subtilis ATCC 6633	25.0	6.25
B. cereus ATCC 9634	12.5	6.25
Escherichia coli K12 B-1536	>100	>100
Staphylococcus aureus FAD 209P	50	50
Micrococcus luteus ATCC 9341	25.0	12.5
Mycobacerium segmatis ATCC 607	>100	>100
Candida albicans 3147	>100	>100
Aspergillus fumigumates 0063	>100	>100

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