Communication to the Editor

AZICEMICIN A, A NEW ANTIMICROBIAL ANTIBIOTIC FROM *Amycolatopsis*

Sir:

In the course of a screening program for new antibiotics, we isolated a new type antibiotic, azicemicin A (**1**) from a culture broth of the strain MJ126-NF4, which was closely related to *Amycolatopsis sulphurea*. In this paper we report the production, isolation, physico-chemical properties and biological properties of **1**.

A slant culture of the azicemicin-producing organism was inoculated into a 500 ml Erlenmeyer flask containing 110 ml of a seed medium containing galactose 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, corn steep liquor 0.5%, glycerol 1.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). The inoculated medium was incubated at 30°C for 8 days on a rotary shaker. Two ml of the seed culture was then transferred to each of 500 ml Erlenmeyer flasks containing 110 ml of a production medium. The production medium was composed of glycerol 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, yeast extracts 0.3%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$

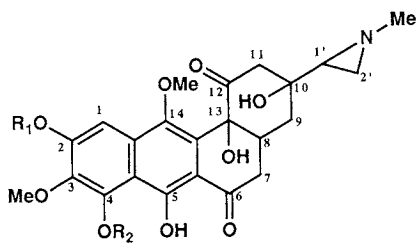
Table 1. Physico-chemical properties of azicemicin A.

Appearance	Yellow solid
Nature	Amphoteric
Molecular formula	$C_{23}H_{25}NO_9$
FAB-MS (*m/z*)	$(M+H)^+$ 460
	$(M-H)^-$ 458
HRFAB-MS (*m/z*)	
Calcd:	459.1529 (as $C_{23}H_{25}NO_9$)
Found:	459.1524 (M^-)
UV λ_{max} (ε)	
in MeOH	234 (16,850), 277 (28,530), 322 (4,490), 335 (sh, 3,550), 410 (8,860)
in 0.01 N NaOH-MeOH	241 (10,490), 290 (27,790), 414 (14,190)
in 0.01 N HCl-MeOH	234 (16,460), 278 (26,950), 322 (4,130), 335 (sh, 3,220), 409 (9,090)
IR ν_{max} (KBr; cm^{-1})	3430, 2955, 1720, 1630, 1615, 1525, 1395
$[\alpha]_D^{24}$	−190° (*c* 0.1, MeOH)
Rf[a]	0.26
Rm[b] (L-alanine: **1**)	0.51

[a] Silica gel TLC (Merck Art. No. 5715) $CHCl_3$-MeOH (10:1).

[b] 3,500 V, 15 minutes (Formic Acid-Acetic acid-Water 25:75:900).

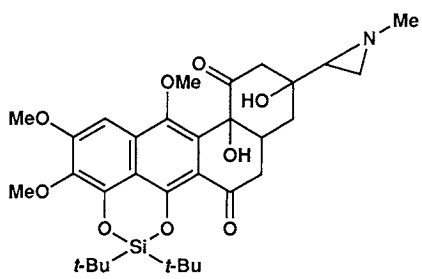
Fig. 1. The structure of azicemicin A and its derivatives.



Azicemicin A (**1**) $R_1=R_2=H$

2 $R_1=R_2=Me$

3 $R_1=Me$ $R_2=H$



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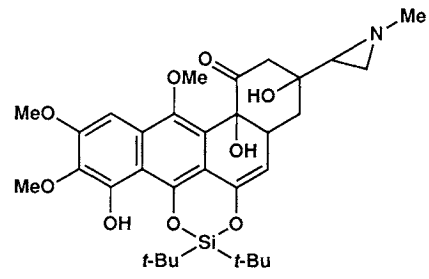

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Table 2. ^{13}C NMR data (100 MHz) and ^{1}H NMR data (400 MHz) of azicemicin A (**1**) in $CDCl_3$[a].

Position	^{13}C (**1**)	^{1}H (**1**)
1	98.3	6.93 (s)
2	154.2	
3	133.5	
4	150.7	
4a	110.2	
5	164.6	
5a	105.7	
6	200.7	
7	37.5	2.53 (dd, 2.0, 17.6)[b], 3.49 (dd, 5.4, 17.6)
8	41.1	2.21 (m)
9	41.5	1.9 (m)[c]
10	70.8	
11	47.2	2.57 (dd, 2.0, 12.7), 2.95 (d, 12.7)
12	206.5	
13	75.5	
13a	123.9	
14	143.9	
14a	131.5	
1′	44.0	1.53 (dd, 3.4, 6.3)
2′	31.7	1.35 (d, 6.3), 1.9[c]
N–Me	46.9	2.38 (s)
3-OMe	60.9	4.07 (s)
14-OMe	62.3	3.64 (s)
2-OH		
4-OH		10.2 (s)
5-OH		
10-OH		
13-OH		4.93 (s)

[a] Chemical shifts in ppm from TMS as an internal standard.
[b] Coupling constants in J=Hz.
[c] These signals were obscured by overlapping signals.

0.2% (adjusted to pH 7.4 before sterilization). The fermentation was carried out at 27°C for 5 days on a rotary shaker.

The culture broth was filtrated and the filtrate (4.4 liters) was adsorbed on a column of Diaion HP-20 (350 ml). The column was washed with water (2.0 liters) and then with 30% aqueous MeOH (1.5 liters). The azicemicin A and minor components were eluted with 80% aqueous MeOH (1.5 liters). The active fractions were collected and concentrated to dryness under reduced pressure. The dried residue (1.4 g) was chromatographed on a silica gel (70 g) using mixtures of $CHCl_3$-MeOH (20:1, 10:1 and 7:1). The fractions which gave Rf 0.26 on silica gel TLC ($CHCl_3$-MeOH, 10:1) were collected and concentrated under reduced pressure to give a dark yellow solid (0.202 g). A small amount of this sample (13 mg) was purified by silica gel TLC ($CHCl_3$-90% aqueous MeOH, 10:1) providing 9.5 mg of azicemicin A (**1**) as a yellow solid.

Table 3. The antimicrobial activities of azicemicin A.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA209P	>100
S. aureus Smith	>100
Micrococcus luteus FDA16	50
M. luteus IFO3333	12.5
Bacillus subtilis PCI 219	>100
Corynebacterium bovis 1810	25
Escherichia coli NIHJ	50
E. coli K-12	>100
Shigella dysenteriae JS11910	50
Mycobacterium smegmatis ATCC 607	50
M. 60R Rifamycin fast	50
M. vaccae ATCC 15483	50
Erwinia aroideae	25

Physico-chemical properties of azicemicin A (**1**) are summarized in Table 1. The antibiotic is soluble in methanol, chloroform, ethyl acetate, acetone, acidic water, alkaline water, slightly soluble in neutral water and toluene but insoluble in hexane. The molecular formula $C_{23}H_{25}NO_9$ was determined by HRFAB-MS. The IR spectrum of **1** showed the nonchelated carbonyl and chelated carbonyl at 1720 and 1630 cm^{-1}, respectively. The UV spectra showed absorption maxima at 234, 277, 322, 335 and 410 nm. These absorption appeared similar to those of the aureolic acid group such as chromomycins and indicative of the presence of an anthracenone moiety[1,2] contained in the molecule of (**1**) shown in Fig. 1. The azicemicin A was reacted with diazomethane to give methyl derivatives **2** and **3**. The methyl derivative (**3**) was treated with di-*tert*-butylsilyl bis(trifluoromethanesulfonate) and 2,6-lutidine affording sililated compounds **4** and **5**. The ^{1}H and ^{13}C NMR data for **1** in $CDCl_3$ are shown in Table 2. The structure of azicemicin A (**1**) was determined by NMR spectral analysis of **1** and its derivatives. Azicemicin A is new type antibiotic that contains an anthracenone and an aziridine moieties in its structure. Details of the structure determination of **1** and its derivatives, and of the analogs of **1** will be reported later.

Biological properties of azicemicin A (**1**) are shown in Table 3. The antimicrobial activities of **1** are moderated against *Mycobacteria*. Azicemicin A did not show any toxic sign in mice at a dose of 150 mg/kg when administered intraperitoneally.

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