EM5400, A FAMILY OF MONOBACTAM ANTIBIOTICS PRODUCED BY *AGROBACTERIUM RADIOBACTER*

I. TAXONOMY, FERMENTATION AND BIOLOGICAL PROPERTIES

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A series of novel monocyclic β-lactam antibiotics were isolated from a strain of *Agrobacterium radiobacter*. The compounds show weak antibacterial activity but are highly stable to hydrolysis by β-lactamases.

Monocyclic β-lactams have been recently reported to be produced by bacteria[1~4]. These compounds based on the nucleus, 3-aminomonobactamic acid (Fig. 1) have been given the family name "monobactams"[2]. During a screening program developed to detect β-lactams produced by bacteria[2,4], we isolated strains of *Agrobacterium radiobacter* that produced a mixture of novel monobactams referred to as EM5400. The structure of five of the compounds produced by *A. radiobacter* strain No. SC 11,742 are shown in Fig. 2.

This paper describes *A. radiobacter* SC 11,742, its fermentation, and the biological properties of the monobactams it produces. Isolation and structure determination are described in the accompanying paper[5].

Taxonomy

A. radiobacter SC 11,742 was isolated from a *Rumex* sp. plant collected in Germany. Although nearly one million bacteria were isolated and screened, including a large number of agrobacteria isolated on specialized media[3~8], monobactam-producing strains of agrobacteria were isolated on only two additional occasions from 1) *Lythrum saliaria* leaves collected in Germany and 2) mud and leaf litter from stagnant water in New Jersey. Furthermore, three strains of *A.*

Fig. 1. Structure of 3-aminomonobactamic acid.

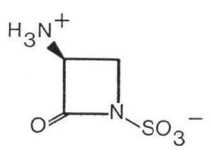
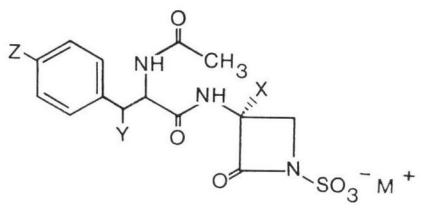


Fig. 2. Structures of monobactams produced by *A. radiobacter* SC 11,742.



	X	Y	Z	M
SQ 26,823	OCH_3	H	H	Na
SQ 26,875	OCH_3	H	OH	K
SQ 26,700	H	H	OH	K
SQ 26,970	OCH_3	OH	$OSO_3^-Na^+$	Na
SQ 26,812	OCH_3	$OSO_3^-Na^+$	$OSO_3^-Na^+$	Na

tumefaciens and one strain of *A. radiobacter* obtained from the American Type Culture Collection were non-producers. The monobactam-producing strains of *A. radiobacter* had the following characteristics.

Morphology

A. radiobacter SC 11,742 is a Gram-negative rod, motile by means of sub-polar to peritrichous flagella.

Physiological and Biochemical Characteristics

The organism is aerobic and grew optimally between 25 and 30°C with no growth at 41°C. Glucose was metabolized oxidatively with the production of acid on synthetic medium low in organic nitrogen. The test for cytochrome oxidase[9] was positive.

Copious extracellular polysaccharide slime was produced on nutrient agar with 5% glucose or sucrose. 3-Ketolactose was produced from lactose[10] and congo red was taken up by the cells when grown on congo red - mannitol agar[11].

Pathogenicity

No galls were produced on abraded stems of sunflower and tomato seedlings inoculated with a heavy suspension of *A. radiobacter* SC 11,742. Galls were produced on tomato seedlings infected with *A. tumefaciens* ATCC 15955.

The production of 3-ketolactose separated this organism from *A. rhizogenes* and *A. rubi*. Failure to produce galls on test seedlings separated it from *A. tumefaciens*. Therefore, we have considered it to be a strain of *A. radiobacter*, a saprophytic species.

Fermentation

Seed culture was prepared by transferring a loopful of surface growth from an agar slant of *A. radiobacter* SC 11,742 into 500-ml Erlenmeyer flasks containing 100 ml of the following medium: oatmeal 2.0% and tomato paste 2.0% in tap water. The pH was adjusted to 7.0 (NaOH) before sterilization. The flasks were incubated at 25°C on a rotary shaker (300 rpm; 5 cm stroke) for approximately 24 hours. A 1% (v/v) transfer of this seed culture was used to prepare a second stage seed culture in a 4-liter Erlenmeyer flask containing 1.5 liters of the medium described above. The flasks were then incubated for approximately 24 hours under the conditions described above. A 1% (v/v) transfer of the second seed culture was then used to inoculate a 380-liter stainless steel fermentation tank containing 250 liters of production medium consisting of; yeast extract 0.5% and glucose 1.0% in distilled water. The fermentation was continued for approximately 40~45 hours at 25°C using an agitation rate of 155 rpm and an air flow of 283 liters/minute. Antibiotic production was determined by a paper disc agar diffusion assay using *B. licheniformis* SC 9262 as the test organism.

Biological Properties

Antibacterial Activity

Antibacterial activities of the five monobactams produced by *A. radiobacter* against a range of bacteria are shown in Table 1. Activity of the five compounds was weak and primarily directed against Gram-positive bacteria.

Inhibition of *Streptomyces* R61 DD-Carboxypeptidase

The monobactams were also tested for their ability to inhibit DD-carboxypeptidase from *Streptomyces* R61 (Table 2). Good inhibitory activity against the enzyme was seen with all compounds. Methoxylation increased inhibitory activity by an order of magnitude (SQ 26,700 *vs.* SQ 26,875) and anionic

Table 1. Antibacterial activity of monobactams produced by *A. radiobacter* SC 11,742.

Organism	SC #	SQ 26,823	SQ 26,875	SQ 26,700	SQ 26,970	SQ 26,812
Staphylococcus aureus	1,276	12.5	25	25	>100	>100
S. aureus	2,399	12.5	50	25	>100	>100
S. aureus	2,400	25	100	25	>100	>100
S. aureus	10,165	>50	>100	>100	>100	>100
Streptococcus faecalis	9,011	>50	>100	100	>100	>100
S. agalactiae	9,287	25	50	12.5	>100	>100
Micrococcus luteus	2,495	12.5	100	12.5	>100	>100
Escherichia coli	8,294	>50	>100	>100	>100	>100
E. coli	10,857	>50	100	>100	>100	>100
E. coli	10,896	25	6.3	>100	25	>100
E. coli	10,909	>50	100	>100	>100	>100
Klebsiella aerogenes	10,440	>50	>100	>100	>100	>100
K. pneumoniae	9,527	>50	>100	>100	>100	>100
Proteus mirabilis	3,855	>50	>100	>100	>100	>100
P. rettgeri	8,479	>50	>100	>100	>100	>100
P. vulgaris	9,416	>50	>100	>100	>100	>100
Salmonella typhosa	1,195	>50	>100	>100	>100	>100
Shigella sonnei	8,449	>50	>100	>100	>100	>100
Enterobacter cloacae	8,236	>50	>100	>100	>100	>100
E. aerogenes	10,078	>50	>100	>100	>100	>100
Citrobacter freundii	9,518	>50	>100	>100	>100	>100
Serratia marcescens	9,783	>50	>100	>100	>100	>100
Pseudomonas aeruginosa	9,545	50	50	>100	>100	>100
P. aeruginosa	8,329	>50	>100	>100	>100	>100
Acinetobacter calcoaceticus	8,333	>50	>100	>100	>100	>100

Minimum inhibitory concentrations were determined by a two fold agar dilution method on DST agar (Oxoid). Final inoculum level was 10^4 colony-forming units.

Table 2. The inhibition of DD-carboxypeptidase by monobactams produced by *A. radiobacter* SC 11,742*.

Compound	I_{50}[a] (M)	
	0 minute[b]	30 minutes
SQ 26,823	3.5×10^{-6}	1.0×10^{-6}
SQ 26,875	2.4×10^{-6}	4.8×10^{-7}
SQ 26,700	3.7×10^{-5}	2.2×10^{-6}
SQ 26,970	3.7×10^{-5}	2.2×10^{-5}
SQ 26,812	2.0×10^{-4}	3.3×10^{-5}

* Partially purified *Streptomyces* R61 DD-carboxypeptidase was incubated at 30°C with the appropriate monobactam for 0 and 30 minutes in a total volume of 20 μl. Two nmoles of substrate, [^{14}C]diacetyl-L-Lys-D-Ala-D-Ala was added and the incubation continued for 30 minutes. Reaction was stopped by the addition of 5 μl of 0.25 N HCl. The hydrolysis product was separated from the substrate by high voltage electrophoresis (pH 3.5). Percentage hydrolysis was determined from liquid scintillation counting of the radioactive spots.

a) Concentration to cause 50% inhibition.

b) Preincubation time.

charges in the side chain decreased activity (SQ 26,875 *vs.* SQ 26,970 and SQ 26,812).

β-Lactamase Interactions

The five compounds were tested for their interactions with various β-lactamases. Experimental details for the β-lactamase studies have been described previously[2].

As shown in Table 3, β-lactamases in general did not hydrolyze monobactams containing a 3-α-methoxy group. An exception to this was the

Table 3. Interactions of β-lactamases with compounds isolated from *A. radiobacter* SC 11,742*.

β-Lactamase	Compound	Relative V_{max}	*Km* (mM)	I_{50}[a] (mM)
S. aureus	Penicillin G	100	0.03	—
	SQ 26,823	2	0.47	>0.5
	SQ 26,700	12	0.98	—
	SQ 26,875	<0.02	—	>0.4
	SQ 26,970	<0.05	—	>0.4
	SQ 26,812	N.D.	N.D.	N.D.
TEM-2	Penicillin G	100	0.08	—
	SQ 26,823	<0.01	—	>0.05
	SQ 26,700	7.1	0.28	—
	SQ 26,875	<0.01	—	>0.4
	SQ 26,970	<0.01	—	>0.4
	SQ 26,812	<0.02	—	>0.8
K1	Penicillin G	100	0.17	—
	SQ 26,823	<0.02	—	>0.5
	SQ 26,700	57	1.28	—
	SQ 26,875	<0.01	—	>0.04
	SQ 26,970	<0.01	—	>0.04
	SQ 26,812	<0.03	—	>0.08
P-99	Cephaloridine	100	0.58	—
	SQ 26,823	<0.01	—	0.0008
	SQ 26,700	0.15	0.19	—
	SQ 26,875	<0.01	—	0.002
	SQ 26,970	<0.01	—	0.0007
	SQ 26,812	<0.02	—	0.002

* All studies were performed using spectrophotometric assays at 25°C in 0.1 M phosphate buffer, pH 7.0. Hydrolysis studies of SQ 26,823, SQ 26,970 and SQ 26,812 were performed at 205 nm. Hydrolysis studies of SQ 26,875 and SQ 26,700 were performed at 206 and 235 nm, respectively.

[a] I_{50} values were determined using 0.5 mM penicillin G as substrate for *S. aureus*, TEM-2 and K-1 β-lactamases and 1.0 mM cephaloridine as the substrate for P-99 β-lactamase.

observed hydrolysis of SQ 26,823 by the *S. aureus* penicillinase. SQ 26,823 contains no polar substituents on the phenylalanyl side chains and may represent a characteristic of substrates which can be recognized by β-lactamases from Gram-positive organisms but not Gram-negative strains. SQ 26,700, the non-methoxylated monobactam corresponding to SQ 26,875, was hydrolyzed by all the β-lactamases studied.

For this series of compounds significant binding was observed only with the P-99 β-lactamase, a Class I cephalosporinase. Strong inhibition of this enzyme was observed by the methoxylated monobactams which had I_{50} values less than 2 μM. Inhibition was readily reversed following dialysis of the enzyme-inhibitor complex, indicating that irreversible, or suicide, inhibition did not occur.

Conclusion

The monocyclic β-lactams produced by *A. radiobacter* SC 11,742 are all related to the compounds (monobactams) previously reported to be produced by strains of *Gluconobacter* or *Acetobacter*[2,12],

Pseudomonas acidophila[1] and *Chromobacterium violaceum*[4]. Although strains of *A. radiobacter* producing monobactams were isolated only rarely, the ability to produce these monocyclic β-lactams is possessed by a number of bacterial genera and is in fact rather widespread in nature.

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