A NEW AMINOGLYCOSIDE ANTIBIOTIC G-367 S_1, 2′-*N*-FORMYLSISOMICIN

FERMENTATION, ISOLATION AND CHARACTERIZATION

SHUZO SATOI, MASASHI AWATA, NAOKI MUTO, MITSUO HAYASHI,
HITOSHI SAGAI and MASARU OTANI

Research Laboratories, Toyo Jozo Co., Ltd.
Ohito, Shizuoka, 410-23, Japan

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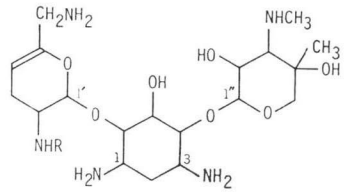
A new aminoglycoside antibiotic, G-367 S_1 (2′-*N*-formylsisomicin, $C_{20}H_{37}N_5O_8$) produced by a rare actinomycetes, *Dactylosporangium thailandense* G-367 (FERM-P 4840) has been isolated by column chromatography on a cation-exchange resin. G-367 S_1 is active against Gram-positive and Gram-negative bacteria.

In the course of our screening program for new antibiotics, actinomycete strain G-367, identified as *Dactylosporangium thailandense*, was found to produce an antimicrobial agent. The organism was isolated from a soil sample collected at Fuji City, Shizuoka Prefecture, Japan. A new water-soluble basic antibiotic named G-367 S_1 was purified from the broth filtrate by a cation-exchange resin process and column chromatography. In this paper, the fermentation, isolation and characterization of G-367 S_1 is reported.

Structures of G-367 S_1 and sisomicin.



G-367 S_1 R = –CHO
Sisomicin R = –H

Fermentation

Dactylosporangium thailandense G-367 was cultured in an Erlenmeyer flask which contained 100 ml of a medium composed of 1% dextrin, 1% glucose, 0.5% NZ-Amine type A, 0.5% yeast extract and 0.1% $CaCO_3$ (pH was adjusted to 7.0 before sterilization) on a rotary shaker at 30°C for 120 hours.

One thousand ml of the culture broth was inoculated into 20 liters of the above-mentioned medium in a 30-liter fermentor. The fermentation was conducted at 30°C under aeration of 20 liters/minute and agitation of 300 rpm.

Ten liters of the culture broth was inoculated into 200 liters of a medium composed of 5% dextrin, 0.5% glucose, 3% soybean meal, 0.7% $CaCO_3$ and 1.3 ppm $CoCl_3$ (pH was adjusted to 7.0 before sterilization) in a 250-liter fermentor. The fermentation was conducted at 30°C under aeration of 100 liters/minute and agitation of 250 rpm. The potency of the cultured broth was estimated by a disc plate method against *Bacillus subtilis* PCI 219. After 120 hours incubation, a maximum concentration (30 μg/ml as G-367 S_1) was obtained.

Isolation

The 120 hours cultured broth (200 liters) containing the G-367 S_1 was adjusted to pH 2.0. After 30 minutes, the broth was adjusted to pH 7.0 and filtered. G-367 S_1 in the filtrate was absorbed on a column of Amberlite IRC-50 (NH_4^+) resin (10 liters). The column was washed with water (20 liters)

and the antibiotic was eluted with 2 M NH_4OH (20 liters). Active fractions were combined and concentrated to 100 ml *in vacuo*. The aqueous solution was adjusted to pH 7.0 and charged on a column of CM-Sephadex C-25 (NH_4^+) (4×30 cm). After washing with distilled water, the column was eluted with aqueous ammonia with a concentration gradient from water (2.5 liters) to 0.35 M (2.5 liters). The eluate was collected in 20 ml fractions and each fraction was monitored by disc plate assay. Fractions containing G-367 S_1 (fractions Nos. 175~185) were concentrated and lyophilized to give a colorless solid (750 mg) of pure G-367 S_1.

Physico-chemical Properties

G-367 S_1 is a water-soluble, basic, white powder. It is stable at pH 2.0, 7.0 and 9.0 at 60°C for 30 minutes. G-367 S_1 gives positive reactions to ninhydrin and $KMnO_4$ tests. The physico-chemical properties of G-367 S_1 are listed in Tables 1 and 2. G-367 S_1 was clearly differentiated from known aminoglycoside antibiotics by these properties.

Table 1. Physico-chemical properties of G-367 S_1.

Nature	Basic, white powder	
Mp	130~133°C	
$[\alpha]_D^{24}$	+188.9°C (*c* 1.0, H_2O)	
UV $\lambda_{max}^{H_2O}$ (nm)	End absorption	
Elementary analysis	$C_{20}H_{37}N_5O_8$	
(%)	Found	Calcd.
C	50.14	50.51
H	7.60	7.84
N	14.42	14.73
CI-MS (*i*-C_4H_{10}) *m/z*	476 (MH^+)	
IR ν_{max}^{KBr} (cm^{-1})	3350, 2920, 1660, 1390, 1380, 1140, 1100, 1050, 1000, 950	

Table 2. Rf values of aminoglycoside antibiotics.

Antibiotics	A	B
G-367 S_1	0.36	0.56
Sisomicin	0.44	0.50
Verdamicin	0.53	0.59
Gentamicin C_{1a}	0.36	0.42
Gentamicin C_2	0.48	0.52
Gentamicin C_1	0.54	0.57

Solvent system, A: $CHCl_3$ - MeOH - 28% NH_4OH (1:1:1, lower layer).
B: $CHCl_3$ - MeOH - 14% NH_4OH (1:2:1).
Silica gel TLC: TLC plastic sheets silica gel 60 F_{254} pre-coated (Merck Art. 5735).

The protonated molecular ion at *m/z* 476 of the chemical ionization mass spectrum using *iso*-butane as the reagent gas and the elemental analysis for G-367 S_1 agreed with the molecular formula of $C_{20}H_{37}N_5O_8$. The IR and ^1H NMR spectra of G-367 S_1 are shown in Figs. 1 and 2, respectively. The ^1H NMR spectrum (100 MHz, D_2O) of G-367 S_1 revealed the presence of one *N*-formyl proton[1] (8.08 ppm, 1H, s) and two methyl groups assigned to N–CH_3 (2.53 ppm, 3H, s) and C–CH_3 (1.22 ppm, 3H, s). The ^{13}C NMR carbon chemical shifts of G-367 S_1 are shown in Table 3 together with those of sisomicin[2], and these data are very similar. The structural distinction between G-367 S_1 and sisomicin[3,4] was the lack of an *N*-formyl group in sisomicin.

Therefore, these data indicated that G-367 S_1 is 2′-*N*-formylsisomicin.

Fig. 1. IR spectrum of G-367 S_1 in KBr disc.

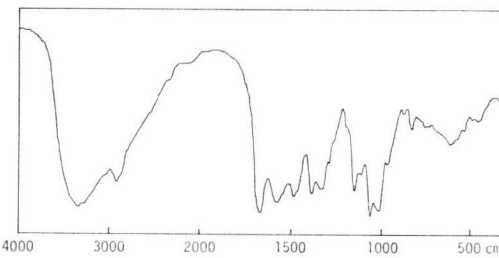


Fig. 2. ^1H NMR spectrum of G-367 S_1 in D_2O.

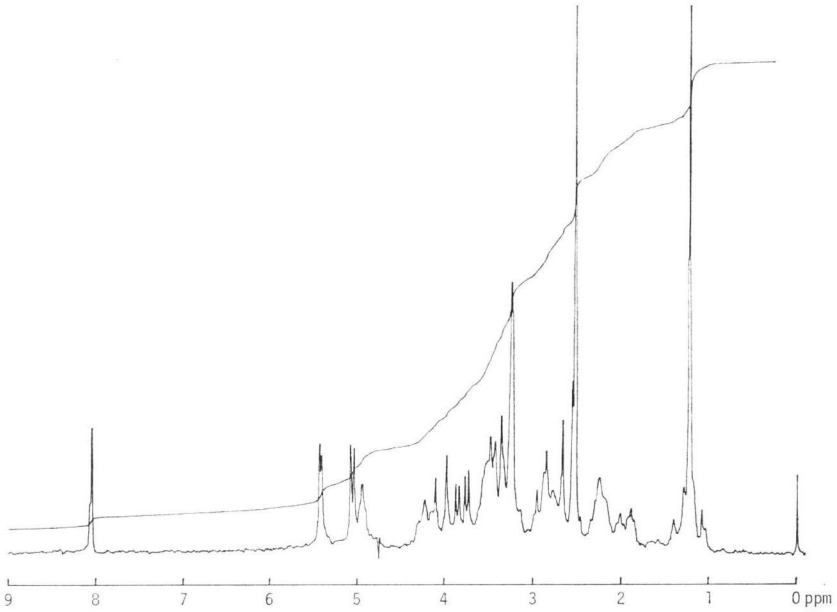


Table 3. ^{13}C NMR chemical shifts of G-367 S_1 and sisomicin.

Carbon	Chemical shifts δ (ppm)*		Carbon	Chemical shifts δ (ppm)*	
	G-367 S_1	Sisomicin		G-367 S_1	Sisomicin
1	51.7	51.8	5′	150.8	150.4
2	36.3	36.4	6′	43.3	43.5
3	50.2	50.4	1″	101.4	101.5
4	85.3	85.3	2″	70.1	70.0
5	75.3	75.4	3″	64.2	64.3
6	87.8	87.8	4″	73.2	73.0
1′	98.1	100.6	5″	68.6	68.5
2′	45.5	47.6	3″-CH_3	37.8	37.9
3′	23.4	25.6	4″-CH_3	22.6	22.9
4′	96.2	96.5	N-CHO	164.7	—

* Measured in D_2O (pD 11), dioxane (67.4 ppm) as an internal standard.

Biological Properties

The antimicrobial activity of G-367 S_1 was determined in a nutrient agar. It has an activity against many of the microorganisms shown in Table 4.

Acknowledgements

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Table 4. Antibacterial activity of G-367 S_1 and sisomicin.

Standard strains			G-367 S_1 (μg/ml)	Sisomicin (μg/ml)
Escherichia coli	MLI629	APH(3′)	3.1	0.8
Pseudomonas aeruginosa	GN4496		12.5	0.8
	GN237		>100	12.5
	GN4490		12.5	0.8
	GN4492		>100	100
Alkaligenes	GN573	+APH(6)	>100	50
Escherichia coli	ML4849	+AAD(2″)	>100	6.3
	GN3684	APH(3″)	3.1	0.4
	GN3644		3.1	0.8
Staphylococcus aureus	MS11446	APH(2″)+AAC(3)	3.1	≤0.2
	ML4844	APH(2″)	3.1	≤0.2
Pseudomonas aeruginosa	GN1567	AAC(6′)-I	25	1.6
	GN4925	AAC(6′)-III	>100	50
	GN315	AAC(6′)-IV	>100	12.5
	GN362		>100	12.5
	GN208		>100	100
	GN3052		>100	>100
Proteus inconstans	GN1554	AAC(2′)	6.3	12.5
	GN626		100	100
Proteus sp.	GN7777		50	50
Escherichia coli	ML4846	AAC(3)-I	3.1	25
Pseudomonas aeruginosa	ML4847	AAC(3)-III	>100	>100
	GN3054	AAC(3)	100	100
	GN3055		>100	>100
	GN4471		100	100
	GN4495		25	50
	GN4470		50	100
	GN4493		25	25
	GN4489		50	50
	GN7884		>100	100
	GN4487		50	100
Enterobacter cloacae	GN8282		1.6	0.4
Escherichia coli	GN3451	AAD(3″)	6.3	0.8
	GN4861		3.1	0.8
	GN4469		6.3	1.6
Staphylococcus aureus	MS27	AAD(6)	6.3	0.4
Klebsiella pneumoniae	GM3057	AAD(2″)	>100	50
	GN3058		>100	50
Serratia marcescens	GN7979		>100	25
Klebsiella pneumoniae	GN3057		>100	12.5
Serratia marcescens	GN6944		>100	25
Staphylococcus epidermidis	ML4843	AAD(4′)	1.6	≤0.2
Staphylococcus aureus	ML4845		0.8	≤0.2

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