Communications to the editor

2-HYDROXYSAGAMICIN: A NEW ANTIBIOTIC PRODUCED BY MUTATIONAL BIOSYNTHESIS OF *MICROMONOSPORA SAGAMIENSIS*

Sir:

Mutational biosynthesis has been shown to be a valuable technique for preparing new antibiotics. Recently, several groups[1~9] have reported an isolation of 2-deoxystreptamine (DOS) idiotrophic mutants from various aminoglycoside-producing organisms and a preparation of new aminoglycoside antibiotics using this technique.

Micromonospora sagamiensis was isolated by NARA *et al.*[10] as a producer of sagamicin, a novel broad spectrum aminoglycoside antibiotic. In the present investigation, the authors attempted to utilize the DOS idiotrophic mutants for preparation of new mutasynthetic antibiotics. When streptamine, a structural analog of DOS, was fed to the mutants, a new antibiotic, 2-hydroxysagamicin, was produced. In this communication, isolation, identification and biological properties of the 2-hydroxysagamicin are described.

M. sagamiensis KY 11505, a sagamicin producing strain, and its mutants were maintained on agar slants of a seed medium. After two stages of cultivation in a seed medium (3 days and 1 day, respectively), the seed medium was transferred to a fermentation medium. Fermentation was conducted at 30°C for 7 days. The composition of the seed medium was (per liter): Stabilose K (soluble starch) 20 g, glucose 5 g, peptone 5 g, yeast extract 5 g, meat extract 3 g and calcium carbonate 2 g. The fermentation medium consisted of (per liter): Stabilose K 40 g, soybean meal 10 g, Pharmamedia (cottonseed flour) 20 g, corn oil 1 g, casein 5 g, phytate (1 Ca, 2 Mg) 2 g, $FeSO_4 \cdot 7H_2O$ 150 mg, $MgSO_4 \cdot 7H_2O$ 500 mg and KH_2PO_4 250 mg.

M. sagamiensis KY 11505 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) at a concentration of 1 mg/ml for 20 minutes. The NTG treated cells were plated on agar plates of the fermentation medium, and incubated at 30°C for 7 days. The plates were overlaid with soft agar seeded with *Escherichia coli* KY 8301. After incubation at 37°C for overnight, mutants showing no inhibition zone were selected and transferred to the agar plates of the seed medium supplemented with 25 μg/ml of sagamicin to eliminate the test organism. The mutants were cultured in 10 ml of the production medium, supplemented with or without 50 μg/ml of DOS. After incubation for 7 days sagamicin production was examined.

Antibiotics were detected by bioautography against *Bacillus subtilis* KY 4273 after paper chromatography (PPC) or silica gel thin-layer chromatography (TLC). PPC was developed on Toyo No. 51 paper using the lower phase of chloroform - methanol - 17% ammonium hydroxide (2: 1: 1 v/v). TLC was developed on Merck silica gel 60 TLC plate in the lower phase of chloroform - methanol - conc. ammonium hydroxide (1: 1: 1 v/v). The amount of antibiotics were determined by fluorometric measurements of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD chloride) according to the procedure of KABASAKALIAN *et al.*[11]

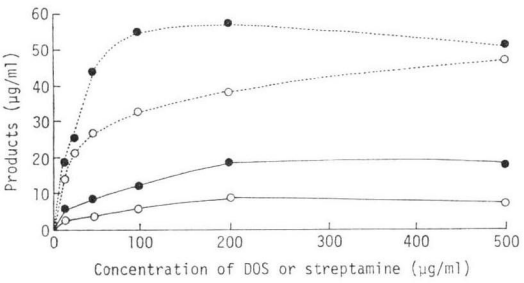
A number of non-sagamicin-producing mutants were derived from the sagamicin producing strain KY 11505. Four mutants, which were able to produce sagamicin when DOS was supplemented in the production medium, were selected as DOS idiotrophs. An unknown antibacterial compound was detected when three of the DOS idiotrophs were cultivated in the fermentation medium supplemented with streptamine. One mutant, KY 11525, was found to be the best strain to convert streptamine to the unknown antibiotic (named HSGM).

In order to obtain mutants possessing higher activity of the biotransformation of streptamine to HSGM, mutant KY 11525 was mutated further with NTG. Colonies, showing larger inhibition zones against *E. coli* when grown on the production agar plates containing 25 μg/ml of streptamine, were selected as desired mutants. Biotransformation activity of a selected mutant, designated KY 11530, was approximately three times higher than that of KY 11525 (Fig. 1).

The antibiotic HSGM was isolated from the

Fig. 1. Bioconversion activities of DOS and streptamine in KY 11530 and KY 11525.

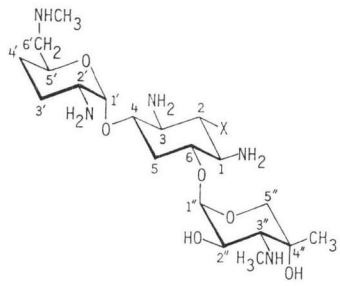
DOS idiotrophs KY 11530 (●) and KY 11525 (○) were cultivated in 10 ml of the production medium supplemented with various concentrations of DOS or streptamine in large test tubes. Production of 2-hydroxysagamicin (—) or sagamicin (···) in 7 days-culture broths were determined by fluorometric assay with NBD chloride.



fermentation broth of KY 11530. The mutant was grown in 150 liters of the fermentation medium supplemented with 50 μg/ml of streptamine. The fermentation broth was harvested at 116 hours, and pH was adjusted to 2.0 with conc. sulfuric acid. After filtration, the filtrate was neutralized and applied to ion-exchange chromatography (Amberlite IRC-50, NH_4^+ form). The column was eluted with 2 N ammonium hydroxide. Crude antibiotic was obtained from the eluates. Further purification was achieved *via* Amberlite CG-50 (NH_4^+ form) column chromatography. The column was eluted with gradients of ammonium hydroxide, ranging from 0.1 to 1.0 normal. Fractions containing HSGM were combined, concentrated and lyophilized to obtain a crude of HSGM. The crude was further purified by silica gel column chromatography (Mallinkrodt silica gel G) using solvent system of chloroform - methanol - conc. ammonium hydroxide (5: 1: 1 v/v).

The compound thus obtained showed the following physicochemical properties: m.p. 102~112°C (dec.); $[\alpha]_D^{25}$ +158° (*c* 0.5, H_2O); IR (KBr) 3350, 2940, 1580, 1480, 1380, 1345, 1050 and 1020 cm^{-1}; *Anal.* Calcd. for $C_{20}H_{41}N_5O_8 \cdot H_2O$; C, 48.28, H, 8.71, N, 14.72; Found.; C, 48.07, H, 8.78, N, 14.65; mass spectrum (*m/z*) 479 (M^+), 436, 366, 362, 349, 338, 321, 320, 207, 179, 161, 160 and 143, PMR (D_2O, pD 10.6) 5.23 (1H, d, *J*=3.9), 5.04 (1H, d, *J*=3.9), 2.52~4.20 (14H, m), 2.51 (3H, s), 2.40 (3H, s), 1.20~1.90 (4H, m) and 1.20 (3H, s). Its ^{13}C NMR chemical shifts

Table 1. Structure and ^{13}C NMR chemical shifts of 2-hydroxysagamicin and its related compounds.



	X=OH		X=H	
	2-Hydroxy-sagamicin (pD=11.0)	Strept-amine[9]	Saga-micin (pD=10.9)	DOS[9]
1	57.4	57.7	51.4	51.6
2	73.9	73.2	36.7	51.6
3	56.2	57.7	50.4	51.6
4	83.9	73.9	87.6	78.5
5	75.0	76.3	75.1	76.6
6	83.1	73.9	87.9	78.5
1'	101.4		101.8	
2'	50.5		50.7	
3'	26.7		27.0	
4'	28.1		28.9	
5'	68.7		69.0	
6'	55.3		55.7	
6'-N-Me	35.5		36.0	
1''	101.3		101.1	
2''	70.3		70.1	
3''	64.2		64.4	
4''	73.2		73.2	
5''	67.4		68.5	
4''-Me	22.5		22.9	
3''-N-Me	37.7		38.1	

are shown in Table 1 together with those of sagamicin and streptamine. These data indicate that the structure of HSGM is 2-hydroxysagamicin.

2-Hydroxysagamicin was tested against a number of Gram-positive and Gram-negative organisms (Table 2). Compared with sagamicin, 2-hydroxysagamicin was slightly less potent against sagamicin-sensitive organisms but more potent against some sagamicin-resistant strains such as *E. coli* KY 8536, which is known to possess gentamicin 2''-adenylating enzyme.

Table 2. *In vitro* activities of 2-hydroxysagamicin and sagamicin.

Test organisms	Marker	Minimum inhibitory concentration (μg/ml)	
		2-Hydroxysagamicin	Sagamicin
S. aureus Smith	Gram (+)	0.4	0.1
S. epidermidis	〃	0.2	<0.05
S. faecalis ATCC 10541	〃	6.25	3.12
B. subtilis ATCC 6633	〃	0.4	0.1
E. coli NIHJ JC-2	Gram (−)	0.78	0.4
K. pneumoniae 8045	〃	0.78	0.2
Ps. aeruginosa #1	〃	3.12	0.78
Ser. marcescens T-55	〃	0.78	0.4
Pr. mirabilis 1287	〃	1.56	0.78
E. coli KY 8349	APH (3′)-I	1.56	0.78
Ps. aeruginosa KY 2445	AAC (6′)-3	>100	100
Ps. aeruginosa KY 8510	AAC (6′)-4	3.12	1.56
Providencia KY 8464	AAC (2′)	50	>100
E. coli KY 8348	AAC (3)-I	25	12.5
Ps. aeruginosa KY 8565	AAC (3)-III	>100	>100
E. coli KY 8536	AAD (2″)	12.5	100
S. aureus KY 8970	AAD (4′)	0.4	0.1

Assayed by agar dilution method at pH 8.0.

Table 3. Acute toxicity (i.v.) of 2-hydroxysagamicin compared with sagamicin and gentamicin C complex in the mouse.

Compound	LD_{50} (mg/kg)	Relative toxicity
2-Hydroxysagamicin	>250	<0.22
Sagamicin	68.3	0.82
Gentamicin C complex	55.9	1.00

The acute (intravenous) toxicity (LD_{50}) of 2-hydroxysagamicin was compared with sagamicin and gentamicin C complex, and it was determined that the toxicity of 2-hydroxysagamicin was one-fourth to one-fifth as compared to those of sagamicin or gentamicin C complex (Table 3).

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