ASPICULAMYCIN, A NEW CYTOSINE NUCLEOSIDE ANTIBIOTIC

III. BIOLOGICAL ACTIVITIES, *IN VITRO* AND *IN VIVO*

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Aspiculamycin was weakly active against gram-positive and gram-negative bacteria but strongly against *Mycoplasma*. However, versatility of the antibiotic in biological activity was also recognized by its activities, such as anthelmintic and acaricidal activities *in vivo*.

As described in the preceeding papers[1,2], aspiculamycin was obtained from the cultured broth of *Streptomyces toyocaensis* var. *aspiculamyceticus*. Based on the chemical structure, it was classified as one of the members of cytosine nucleoside antibiotics. Bacteria, especially mycobacteria, were reported to be susceptible to many of the antibiotics of this family, such as gougerotin[3], blasticidin S[4], amicetin B[5,6], bamicetin[6], plicacetin[6], anthelmycin[7], hikizimycin[8], and oxamicetin[6,10] except ezomycin[11,12] and moroyamycin[13]. Antifungal activity was observed for blasticidin S, ezomycin and hikizimycin. Anti-tumor activity was reported for blasticidin S and amicetin. Although the structure has not yet been presented for anthelmycin, an anthelmintic antibiotic, its physical and chemical properties suggest the presence of cytosine chromophore in its molecule. Asteromycin, anti-mycoplasma antibiotic, reported by ISHIDA *et al.*[14] was recently found to be identical with gougerotin. Acaricidal activity was reported for moroyamycin, in which the presence of cytosine chromophore has also been assigned by its physical and chemical characteristics. The present paper describes the existence of these versatile biological activities also in aspiculamycin.

Antimicrobial Activity of Aspiculamycin

The minimal inhibitory concentrations (MIC) of aspiculamycin against bacteria, fungi and yeasts were determined by serial two-fold dilution method as shown in Table 1. Aspiculamycin was inactive against yeasts and fungi and only weakly active against some strains of *Escherichia coli* and *Mycobacterium smegmatis* ATCC 607 with the lowest MIC value of 50 mcg/ml. However, it was strongly active against many species of *Mycoplasma* with the MIC values ranging between 0.78 and 25 mcg/ml. The details of the anti-mycoplasma activity *in vivo* as well as *in vitro* will be reported elsewhere.

Anthelmintic Activity

One of the characteristic activities of aspiculamycin was anthelmintic activity against com-

Table 1. Minimal inhibitory concentrations of aspiculamycin

Test organism	Medium*	MIC (mcg/ml)	Test organism	Medium*	MIC (mcg/ml)
Staphylococcus aureus 209PJC-2	B	400	*E. coli* SMf	B	100
S. aureus 56	〃	>400	*E. coli* KMf	〃	50
S. aureus 1557	〃	>400	*E. coli* CM, TCf	〃	50
S. aureus 52-34	〃	400	*E. coli* 97	〃	>400
S. aureus 193	〃	400	*Mycobacterium smegmatis* ATCC 607	〃	50
S. aureus STf	〃	>400	*Candida albicans* YU1200	S	>400
Bacillus subtilis PCI 219	〃	200	*Saccharomyces cerevisiae*	〃	>400
Sarcina lutea PCI 1001	〃	200	*Trichophyton interdigitale*	〃	>400
Corynebacterium xerosis	〃	400	*Cryptococcus neoformans*	〃	>400
Aeromonas liquefaciens	〃	>400	*Penicillium chrysogenum*	P	>400
Pseudomonas aeruginosa	〃	400	*Aspergillus niger*	〃	>400
Proteus vulgaris OX-19	〃	400	*Hormodendrum pedrosoi*	〃	>400
Escherichia coli NIHJJC-2	〃	200	*Botrytis cinerea*	〃	>400
E. coli K-12	〃	200	*Fusarium moniliforme*	〃	>400
E. coli STf	〃	200	*Gloeosporium kaki*	〃	>400

*B: 1% Glycerol-bouillon agar. S: SABOURAUD's glucose agar. P: Potato-glucose agar.

Table 2. Effect of aspiculamycin on mouse pinworm

		Days for treatment											*Syphacia obvelata*	*Aspiculris tetraptera*
		0	5	7	12	14	18	25	29	32	35	39		
Group I	1	###	+	+	+	+	−	##	##	###	+	+	2	4
	2	+	−	−	###	###	+	+	###	+	+	+	4	0
	3	###	###	###	###	###	###	+	##	+	##	+	7	5
	4	##	+	+	+	###	−	###	+	−	###	+	7	3
	5	+	##	+	##	###	−	##	###	###	###	+	4	0
II	1	+	+	+	−	−	−	−	−	−	−	−	0	0
	2	+	###	##	−	−	−	−	−	−	−	−	0	0
	3	###	−	+	−	−	−	−	−	−	−	−	0	0
	4	+	###	###	−	−	−	−	−	−	−	−	0	0
	5	+	+	−	−	−	−	−	−	−	−	−	0	0
III	1	##	###	##	+	−	−	−	−	−	−	−	0	0
	2	+	−	+	−	−	−	−	−	−	−	−	0	0
	3	###	##	+	−	−	−	−	−	−	−	−	0	0
	4	##	###	###	−	−	−	−	−	−	−	−	0	0
	5	###	##	##	+	−	−	−	−	−	−	−	0	0
IV	1	+	−	−	−	−	−	−	−	−	−	−	0	0
	2	+	−	−	−	−	−	−	−	−	−	−	0	0
	3	+	−	−	−	−	−	−	−	−	−	−	0	0
	4	##	−	−	−	−	−	−	−	−	−	−	0	0
	5	+	−	−	−	−	−	−	−	−	−	−	0	0
V	1	+	##	+	+	−	−	−	−	−	−	−	0	0
	2	###	−	−	−	−	−	−	−	−	−	−	0	0
	3	###	+	−	+	−	+	+	+	−	−	−	0	0
	4	##	−	###	##	+	−	−	−	−	−	−	0	0
	5	+	−	−	−	−	−	−	−	−	−	−	0	0

Fecal egg counts by cellophane-tape method, in which a cellophane tape, 1.0×2.0 cm in size, was stuck on the anus of mouse to collect pinworm eggs followed by sticking that tape on the slide glass to count the number of eggs. 0; −; 1~10; +; 11~50; ##; 50; ###.

mon mouse pinworm, such as *Aspiculris tetraptera* and *Syphacia obvelata*. The effectiveness of the anthelmintic activity based on the reduction of fecal egg counts and the number of worms was examined using commercially-available anthelmintic antibiotic, destomycin A. (Meiji Seika Co., Tokyo, Japan) as a reference sample. Twenty-five mice maintained on a basal diet for 20 days were divided into five groups. Group I was fed with basal diet without antibiotic. Groups II and III were administered destomycin A, 100 and 50 mg/kg of basal diet, respectively. Groups IV and V were administered aspiculamycin of the same amount as destomycin A in groups II and III. As shown in Table 2, complete inhibition of ovulation was observed from 5th day of treatment in group IV and from 4th week in group V. After termination of the test, complete elimination of pinworms from intestinal tract was observed in both aspiculamycin-treated groups, while heavy worm population was found in control mice (group I). Comparison of the activity of the two antibiotics showed that the activity of aspiculamycin was very similar to that of destomycin A.

Acaricidal Activity

The effectiveness of acaricidal activity of aspiculamycin was evaluated against *Tetranychus urticae* in a laboratory test and against *Panonychus citri* in a field test.

In the laboratory test, leaves of asparagus bean (*Vigna sesquipedalis*) each inoculated with about 50 adult female mites (*Tetranychus urticae*) were placed in a 9 cm petri dish. The diluted preparation of the antibiotic was sprayed on the mites. Mortality of the mites was deter-

Table 3. Acaricidal activity of aspiculamycin in laboratory test

	Days after treatment	Mortality (%)			
		100 ppm	30 ppm	10 ppm	3 ppm
Aspiculamycin	1	1.9	3.1	1.8	0
	3	94.5	88.8	30.7	14.7
Dicofol*	1	93.7	73.2	0	
	3	97.7	84.2	23.6	
Control	1	2.2			
	3	11.2			

* Dicofol, (an acaricide; 1,1-bis (*p*-chlorophenyl)-2,2,2-trichloroethanol) 40% emulsified solution.

Table 4. Acaricidal effect of aspiculamycin in field test

	Conc. (%) a.i.	Number of adult mite*					
		Before treatment	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks
Aspiculamycin	0.05	133	1	5	30	42	135
Dicofol	0.02	112	1	19	146**	209	992
Control		128	84	201	676	551	1,005

* Average of duplicates.
** The lower effectiveness of Dicofol suggests a possibility that the mites had developed some resistance to the compounds.

mined after 1 and 3 days. Table 3 indicates that the acaricidal activity of aspiculamycin is as high as that of Dicofol, though the former has delayed activity.

In the field test, seven-year-old *Citrus unshu*, on which mites (*Panonychus citri*) inhabited, were sufficiently sprayed with the diluted preparations. After treatment, 30 leaves per tree were selected at random and the number of adult mites on the leaves was examined weekly. Table 4 shows that the effect of aspiculamycin was more persistent than that of Dicofol, and the mite population was supressed by the antibiotic for 4 weeks.

Acute Toxicity in Mice

The acute toxicity (LD_{50}) of aspiculamycin in mice by intravenous or by oral administration was 13 or 125 mg/kg, respectively.

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