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Axenomycins, New Cestocidal Antibiotics

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Axenomycins are a new group of macrolide antibiotics isolated from the fermentation broth of *Streptomyces lisandri* n.sp. They exhibit anthelmintic activity against tapeworms (*Cestoda*). Three different fractions, A, B, and D, have been obtained, the most active fraction being axenomycin D. The activities of the axenomycin complex and axenomycin D against *Hymenolepis nana* in mice, *Taenia pisiformis*, *Dipylidium caninum*, and *Diphyllobothrium* sp. in dogs, and *Moniezia expansa*, *M. benedeni*, and *Avitellina centripunctata* in lambs were studied in experimentally and naturally infected animals. Axenomycins were effective and well tolerated by the oral route. Worm reduction rates after a single oral dose were 90 to 100% with 5 to 10 mg of axenomycin D/kg and 50 to 100% with 20 mg of axenomycin complex/kg.

The chemotherapeutic agents effective against helminthic infections include a range of substances derived from plant material, organic and inorganic metallic compounds, and a number of more recent nonmetallic synthetic compounds (4, 6, 8, 10). Although some anthelmintic antibiotics produced by microorganisms are known (2, 3, 5, 7, 9, 11), so far this field has been scarcely explored. During the past few years, a screening program has been developed in our laboratory for the detection of anthelmintic substances produced by streptomycetes, and a new group of antibiotics, named axenomycins, was isolated from the mycelium of *Streptomyces lisandri* n.sp.

Three different components, A, B, and D, were separated, and the latter two were obtained in the crystalline state (1). Some physicochemical properties of axenomycins B and D have been determined (Table 1); the ultraviolet spectrum (Fig. 1) is characteristic for the 1,4-naphtoquinone chromophore.

The axenomycins represent a new group of antibiotics with high molecular weight which have a complex structure containing a macrocyclic lactone, two sugar residues, and a 1,4-naphtoquinone chromophore. The structure of axenomycin B (Fig. 2) was recently determined (1). The chromophore and the sugar moieties appear to be common to all three components; in the aglycone of axenomycin D, one hydroxyl group is esterified by a molecule of sulfuric acid.

Axenomycin is particularly effective against cestodes.

This paper reports the data obtained with axenomycins, mainly axenomycin D, against experimental and natural infections of tapeworms in animals.

MATERIALS AND METHODS

Axenomycin was always administered by the oral route in a single dose to the infected animals as a powder in water suspension containing 0.5% (vol/vol) Tween 80 for mice and in enteric-coated capsules for dogs.

Hymenolepis nana infections. Groups of 10 male Swiss COBS mice were experimentally infected with eggs of *H. nana* obtained from gravid proglottides (100 eggs/mouse orally). After a 12-day prepatent period, the mice were dosed with axenomycin. At 24 h after the treatment, the animals were sacrificed and examined for the presence of intestinal *H. nana*.

Taenia pisiformis infections. All dogs used were experimentally infected with cysts obtained from artificially infected rabbits (three or four cysts/dog orally). The feces were periodically examined for the appearance of tapeworm segments and, when manifestly infected, dogs were dosed with axenomycin D. Feces were collected 2 to 5 days after dosing and examined for worms, eggs, and segments. The dogs were sacrificed 12 days after treatment (this period of time allowing any scolices not removed to redevelop), and necropsy of the whole intestine was performed.

Diphyllobothrium sp. infections. Dogs were experimentally infected with plerocercoids obtained from lake fish (trout and perch). The fish were sectioned for plerocercoids, which were isolated and administered by the oral route to the dogs (two to four plerocercoids/dog).

The feces of each dog were periodically collected and observed for the presence of eggs. The animals

TABLE 1. *Some physicochemical properties of axenomycins B and D*

Property	Axenomycin B	Axenomycin D
Melting point, C	142–143	174–175
Analysis, %		
C	61.86	57.78
H	8.61	7.37
O	29.9	
S		1.85
Na		1.58
Molecular weight[a]	1,530	
$E^{1\%}_{1\,cm}$ (255 nm)	154	135

[a] Determined by the vapor pressure method.

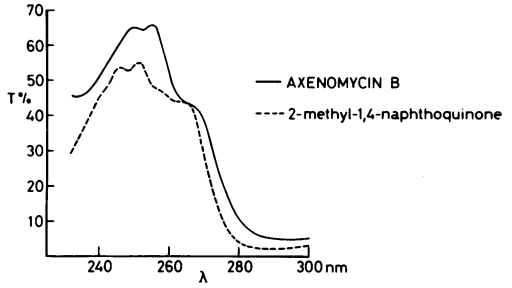

FIG. 1. *Ultraviolet spectrum of axenomycin B (solid line) and of 2-methyl-1,4-naphthoquinone (dashed line) in methanol.*

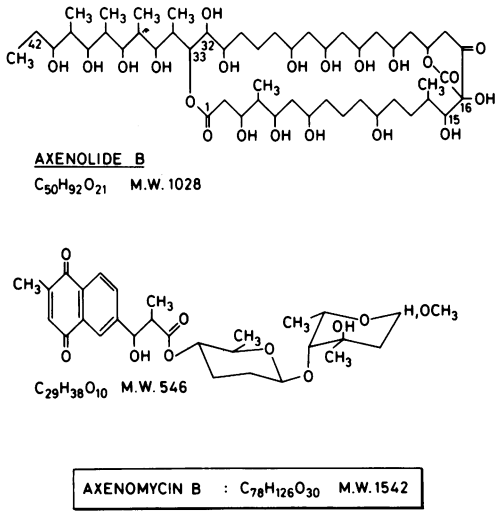

FIG. 2. *Axenomycin B.*

manifested mature infection after 18 days and were dosed with axenomycin D. The feces were again examined at 1 to 5 and 20 days after treatment.

Some dogs were sacrificed for necropsy of the whole intestine 10 days after treatment.

RESULTS

H. nana. The results of several experiments with the three components of axenomycin are summarized in Table 2. Fraction A showed little activity at the dose levels tested, and fraction B was clearly effective only at the highest dose of 20 mg/kg. Fraction D, however, proved to be the most effective in bringing about a cure rate of 65% at 2.5 mg/kg. Niclosamide tested at the same time was active at higher doses.

In other experiments where the dosage and feeding were varied, there was an indication that the antibiotic was more rapidly effective when administered to fasting animals. Figure 3 shows the effectiveness of axenomycin D at 5

TABLE 2. *Activity of axenomycins A, B, and D against Hymenolepis nana in experimentally infected mice*

Compound	Dose (mg/kg)	No. of mice/group	Percentage of mice cleared
Axenomycin A	20	30	17.6
	10	80	25
	5	30	13.3
Axenomycin B	20	80	73.3
	10	80	23.7
	5	30	16.6
Axenomycin D	20	100	97
	10	100	92
	5	100	73
	2.5	100	65
Niclosamide	200	20	100
	100	20	60
Control[a]	—	260	5.5

[a] The average number of *H. nana* in the controls was 4.2 ± 1.6.

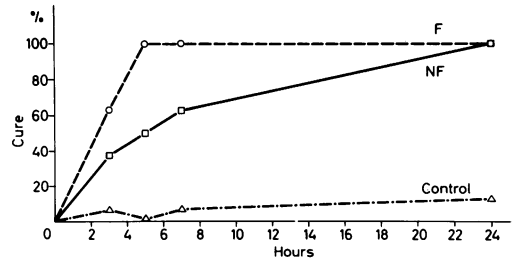

FIG. 3. *Activity of axenomycin D on H. nana in fasted (F) and not fasted (NF) mice. The percentage of animals cleared is plotted against the time of expulsion of the parasites.*

mg/kg against *H. nana* in mice treated after overnight fasting or in normal feeding conditions. The expulsion of *H. nana* began 2 h after treatment, and the parasites appeared to be damaged in the external cuticule.

Axenomycin D showed some activity even when administered in the diet. In experiments where normal feeding was interrupted 15 h before dosing and replaced with food containing 0.05% axenomycin D, a clearing of 50% of the mice was observed.

No activity was demonstrated on the developing parasites when axenomycin D was simultaneously administered with the *H. nana* eggs and subsequently once a day for 11 days at a dose of 10 mg/kg. Normal *H. nana* developed in the intestine of infected mice.

T. pisiformis. Two doses of axenomycin D were tested against *T. pisiformis* in dogs. A complete clearing of the tapeworms from all of 12 dogs was observed after treatment with 10 mg/kg. Clearing also occurred in one of two dogs treated with 5 g/kg.

The worms eliminated appeared to be partially or completely lysed, and sometimes it was impossible to recover the parasites in the feces. The clearance was demonstrated by necropsy.

Diphyllobothrium sp. Axenomycin D proved to be highly effective against *Diphyllobothrium* in dogs. Doses of 20, 10, and 5 mg/kg administered to four, three, and six dogs, respectively, resulted in a 100% cure of the infected dogs.

The worms began to be eliminated 8 to 12 h after dosing and appeared severely damaged on the external tegument.

Preliminary results were obtained in the treatment of natural infections of *Moniezia expansa*, *M. benedeni*, and *Avitellina centripunctata* in lambs and of *D. caninum* in dogs. Crude axenomycin complex was used in these cases at a dose of 20 mg/kg. Nine of 10 lambs so treated and 7 of 9 dogs were cleared of the parasites.

Toxicity. The oral LD_{50} of axenomycin D in mice was 100 mg/kg and the intraperitoneal LD_{50} was 1.0 mg/kg.

Oral administration of 10 and 20 mg/kg to dogs for 30 days was well tolerated. Sporadic vomiting was the only side effect observed.

DISCUSSION

The anthelmintic activity of axenomycin D appears to be of interest based on the high effectiveness demonstrated in several experimental and natural infections of cestodes in animals. From the available data, axenomycin D has been shown to be a broad-spectrum cestocidal antibiotic, well tolerated orally and active after a single dose by the oral route.

The novel structure of the antibiotic, the good experimental results, and the interest in new cestocidal compounds for human chemotherapy, as well as in the veterinary field, recommend that further work be carried out with axenomycin D.

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