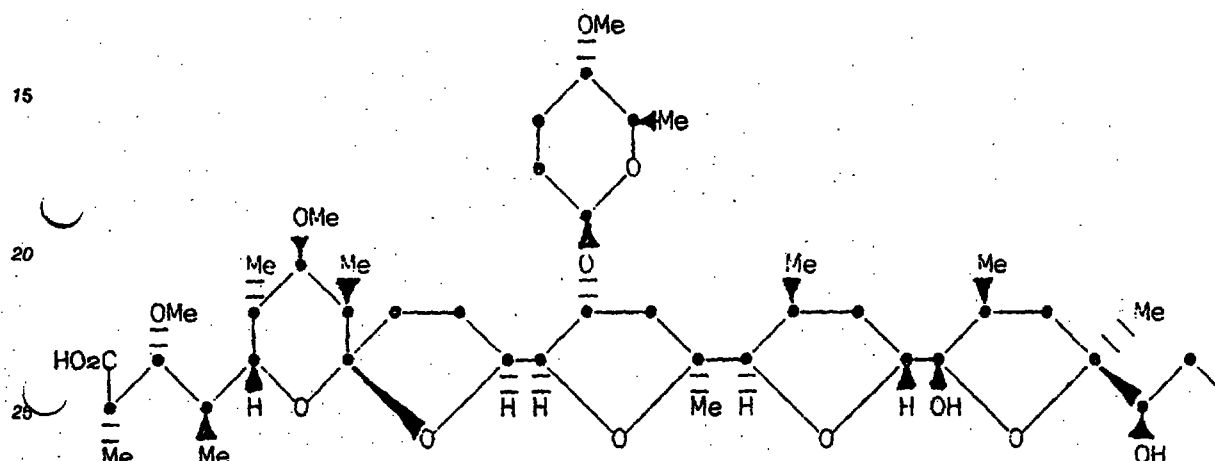


Description

This invention relates to a new polyether antibiotic A80190, to certain derivatives thereof, and to its preparation by cultivation of novel strains of microorganisms.

Westley (John W. Westley, "Polyether Antibiotics: Naturally Occurring Acid Ionophores, Vol. 2, Chemistry," Marcel Dekker: New York, 1983) has separated existing polyethers by class and type. Using Westley's system, A80190 is a new member of the Class 1b, type (1), group of polyethers because it has one spiroketal system. Other members of this group include A-28695 A and B (U.S. Patent No. 3,839,558); A204I and II (U.S. Patent No. 3,705,238) and A-32887 (U.S. Patent No. 4,133,876).

A80190 has the following structure:



Characteristics of A80190

Antibiotic A80190 has been assigned structure 1 based on X-ray crystallographic studies. A80190 (in its free acid form) has the following characteristics:

State: white crystals (from acetone-water)

Mp: 98-100°C or 120-122°C (more frequently); probably varies with the degree of solvation

pKa: = 6.2 (66% aqueous dimethylformamide)

$[\alpha]_D^{25}$: -26° (c 1, CHCl₃)

Molecular weight: 828 (field desorption mass spectrometry)

Empirical formula: C₄₄H₇₆O₁₄

UV: no absorbance

IR: (CHCl₃) Figure 1; shows absorption at the following frequencies (cm⁻¹): 3019, 2970, 2936, 2827, 1721, 1457, 1402, 1376, 1314, 1163, 1105, 1092, 1083, 1056, 1022, 1006, 989, 980, 945, 934, 917, 892 and 859

Elemental Analysis:		
	Found	Calcd
Carbon	63.35	63.77
Hydrogen	9.17	9.18
Oxygen	27.10	27.05

Solubility: Insoluble in water; soluble in lower alcohols such as methanol, ketones such as acetone, esters such as ethyl acetate, halogenated hydrocarbons such as chloroform and hydrocarbons such as diethyl ether, benzene, toluene and warm hexane.

A80190 has an acid function capable of forming salts and ester derivatives and has at least one hydroxyl group which can be esterified or which can form ether derivatives or urethane derivatives. The acyl

and alkyl ester and alkyl ether derivatives of A80190, urethane derivatives, and the pharmaceutically-acceptable salts of A80190 and of these derivatives are also useful as antibiotics, coccidiostats and as agents which increase feed-utilization efficiency.

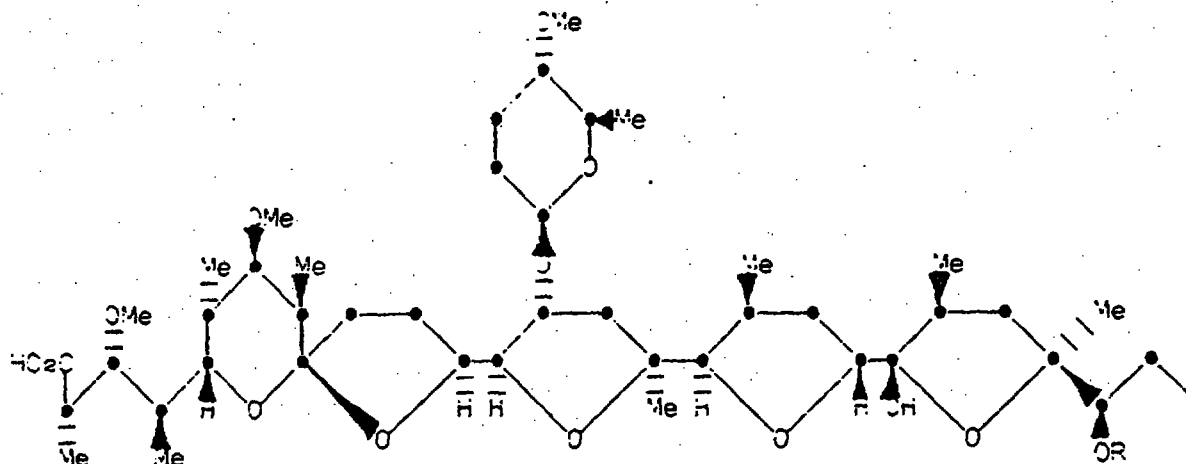
According to one aspect of the invention there is provided a compound of formula (I):

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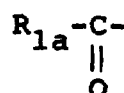


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wherein R is hydrogen or, -CONHR₁ and R₁ is C₁ to C₇ alkyl, phenyl, pyridyl, furyl, (C₁ to C₇ alkyl)-phenyl, phenyl-C₁ to C₇ alkyl, halo = phenyl, nitro = phenyl, halo = phenyl-C₁ to C₇ alkyl, (C₁ to C₇ alkoxy)-phenyl, phenoxyphenyl, phenyl-C₃ to C₇ cycloalkyl optionally substituted by phenyl, acyl = phenyl in which acyl is a radical of the formula

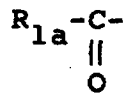
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wherein R_{1a} is C₁ to C₆ alkyl or hydrogen, and C₃ to C₇ cycloalkyl; or an acyl derivative thereof, in which acyl is a radical of the formula wherein R_{1a} is C₁ to C₆

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alkyl or hydrogen, and C₃ to C₇ cycloalkyl, a C₁ to C₇ alkyl ester derivative thereof, or an alkyl ether derivative thereof in which one or more of the hydroxyl groups has been replaced by a YR'' group wherein Y represents O or S; and R'' represents C₁-C₅-alkyl, C₁-C₄-alkoxy-C₂-C₅-alkyl, C₁-C₄-alkoxy-carbonyl-C₂-C₅-alkyl, amino-C₂-C₅-alkyl, mercapto-C₂-C₅-alkyl, hydroxyalkyl,

haloalkyl, or

$(R')_m$ -phenyl $(CH_2)_n$ -,

wherein R' represents C₁-C₄-alkyl, C₁-C₄-alkoxy, or hydroxy;

m represents 0-2; and

n represents 0-3, and

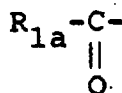
wherein hydroxyalkyl refers either to a monohydroxy-C₂-C₅-alkyl moiety or, when Y is 0, to the 2,3-dihydroxyprop-1-yl moiety and haloalkyl refers to a C₂-C₅-alkyl moiety having from one to three halogen substituents selected from the group consisting of bromine, chlorine and fluorine; or

a salt thereof.

A80190, and its derivatives, are useful as antibacterial and anticoccidial agents. They improve feed-utilization efficiency in ruminants and act as a growth promotant in monogastric animals. In addition, they have insecticidal, herbicidal and antiviral activity. Further, they are useful as ionophores. Methods and compositions pertaining to these uses are also provided.

This invention further relates to synergistic compositions of A80190, its acyl and alkyl ester, alkyl ether and urethane derivatives or a pharmaceutically acceptable salt thereof (an "A80190 compound"), together with a compound selected from nicarbazin, 4,4'-dinitrocarbanilide, metichlorpindol and certain naphthalenamine or benzenamine compounds. These compositions are useful in controlling coccidiosis in animals.

The term "acyl" means a C₁ to C₇, preferably a C₁ to C₄, alkanolic acid moiety, i.e., radicals of the formula



wherein R_{1a} is C₁ to C₆-alkyl or hydrogen, e.g., formyl, acetyl, propionyl, butyryl and the like.

The term "cycloalkyl" means cyclic hydrocarbon groups containing from 3 to 7 carbon atoms, such as, cyclopropyl, cyclobutyl, cyclohexyl and the like, cyclohexyl being preferred. The cycloalkyl group may be substituted by an aryl residue, as defined herein, to form an arylcycloalkyl residue, e.g., 2-(phenyl)-cyclopropyl.

The term "alkoxy" means a C₁ to C₇ lower alkyl group having an oxygen function substituted therein, such as, methoxy, ethoxy, propoxy and the like.

The group R₁ can also be phenyl, pyridyl or furyl, especially phenyl, optionally substituted by various groups. A substituent on a phenyl nucleus is preferably on the 4-position. Examples are 4-alkylaryl, e.g., 4-methylphenyl (4-tolyl); 4-halophenyl, e.g., 4-chlorophenyl; 4-nitrophenyl; 4-aryloxy-aryl, e.g., 4-phenoxyphenyl; 4-alkoxyphenyl, e.g., 4-methoxyphenyl; and 4-(alkyl-carbonyl)phenyl, e.g., 4-(methylcarbonyl)-phenyl or 4-(phenylcarbonyl)phenyl.

The term "alkyl" means a C₁ to C₇ straight or branched chain hydrocarbon, preferably a C₁ to C₄ hydrocarbon, e.g., methyl, ethyl, propyl, isopropyl, n-butyl, etc. The alkyl group may be substituted by an aryl residue, as defined supra, to form an arylalkyl residue, e.g., phenylethyl or 2-phenylethyl or by a haloaryl residue to form a haloarylalkyl residue, e.g., 4-bromophenethyl.

The salts of A80190 and of its derivatives are useful for separating and purifying the antibiotics. The pharmaceutically-acceptable salts are particularly useful. Such salts should have little or no toxicity towards warm-blooded animals. Examples of salts are the alkali-metal, alkaline-earth-metal and amine salts of A80190 and of its derivatives.

Representative and suitable alkali-metal and alkaline-earth metal salts of A80190 include the sodium, potassium, lithium, cesium, rubidium, barium, calcium and magnesium salts. Suitable amine salts of A80190 include the ammonium and the primary, secondary, and tertiary C₁-C₄-alkylammonium and hydroxy-C₂-C₄-alkylammonium salts. Illustrative amine salts include those formed by reaction of A80190 with ammonium hydroxide, methylamine, *sec*-butylamine, isopropylamine, diethylamine, di-isopropylamine, ethanolamine, triethylamine, 3-amino-1-propanol and the like.

It is well known in the veterinary pharmaceutical art that the form of an antibiotic is not ordinarily of great significance when treating an animal with the antibiotic. In most cases, conditions within the animal change the drug to a form other than that in which it was administered. The salt form in which it may be administered is, therefore, not of great significance. The salt form may, however, be chosen for reasons of

economy, convenience, and lack of toxicity.

Antibiotic A80190 can be produced by culturing an A80190-producing strain of *Actinomadura oligospora* under submerged aerobic conditions in a suitable culture medium until substantial antibiotic activity is produced. The antibiotic is recovered using various isolation and purification procedures understood in the art.

The new *Actinomadura oligospora* microorganism of this invention, which produces antibiotic A80190, was isolated from a soil sample from India. Subsequently, a substantially improved variant which produces increased amounts of A80190 was isolated by LaVerne D. Boeck.

Cultures of these two A80190-producing organisms have been deposited and made part of the stock culture collection of the Northern Regional Research Center, Agricultural Research, North Central Region, 1815 North University street, Peoria, Illinois, 61604, from which they are available to the public under the accession numbers NRRL 15877 (parent strain) and NRRL 15878 (the variant strain).

Taxonomic studies of the variant were carried out by Frederick P. Mertz of the Lilly Research Laboratories. Based on these studies, the two new organisms are classified as members of a new species of the genus *Actinomadura* for which the name *Actinomadura oligospora* sp. nov. is proposed. This classification is based on direct laboratory comparisons with similar species and examination of published descriptions [M. Goodfellow and G. Alderson, "Numerical Taxonomy of *Actinomadura* and Related Actinomycetes," *J. Gen. Microbiol.* 112:95-111 (1970); M. Goodfellow and K. P. Schaal, "Identification Methods for *Nocardia*, *Actinomadura* and *Rhodococcus*," p. 261-276 In F. A. Skinner and D. W. Lovelock (ed.), "Identification Methods for Microbiologists," 2nd ed., The Society for Applied Microbiology Technical Series No. 14, Academic Press, New York, 1979; L. H. Huang, "*Actinomadura macra* sp. nov., the Producer of Antibiotics CP-47,433 and CP-47,434," *Int. J. Syst. Bacteriol.* 30:565-568 (1980); and H. A. Lechevalier and M. P. Lechevalier, "A Critical Evaluation of the Genera of Aerobic Actinomycetes," p. 393-405, In H. Prauser (ed.), "The Actinomycetales," Gustav Fischer Verlag, Jena].

Methods Used

The methods recommended by the International Streptomyces Project (ISP) for the characterization of Streptomyces species [E. B. Shirling and D. Gottlieb, "Methods for Characterization of Streptomyces Species," *Int. J. Syst. Bacteriol.* 16:313-340 (1966)] have been followed along with certain supplementary tests (D. J. Blazevic and G. M. Ederer, "Principles of Biochemical Tests in Diagnostic Microbiology," John Wiley and Sons, Inc., New York, 1975).

Carbon utilization was determined with ISP No. 9 basal medium to which filter-sterilized carbon sources were added to equal a final concentration of 1.0 percent. Plates were incubated at 30°C and read after 14 days.

Melanoid pigment production (chromogenicity) was determined with ISP No. 1 (tryptone-yeast extract broth), ISP No. 6 (peptone-yeast extract iron agar), ISP No. 7 (tyrosine agar) and modified ISP No. 7 which has tyrosine removed.

Starch hydrolysis was determined by testing for the presence of starch with iodine on ISP No. 4 (inorganic salts-starch agar) plates (See Blazevic and Ederer, *supra*).

Morphology was studied using an optical light microscope. A scanning electron microscope (SEM) was used to study the spore surface ornamentation.

NaCl tolerance was measured by adding NaCl to ISP No. 2 agar to equal the concentration desired.

ICSS-NBS Centroid Color Charts, standard sample No. 2106 (National Bureau of Standards, 1958, U.S. Department of Commerce, Washington, D.C.) and the Color Harmony Manual (4th ed., Container Corporation of America, Chicago, Illinois, 1958) were used to assign color names.

The isomers of diaminopimelic acid (DAP) and the carbohydrates in hydrolysates of whole cells were established by the chromatographic methods of Becker et al. [B. Becker, M. P. Lechevalier, R. E. Gordon, and H. A. Lechevalier, "Rapid Differentiation between *Nocardia* and *Streptomyces* by Paper Chromatography of Whole-cell Hydrolysates," *Appl. Microbiol.* 12:421-423 (1964)] and of Lechevalier [M. P. Lechevalier, "Identification of Aerobic Actinomycetes of Clinical Importance," *J. Lab. Clin. Med.* 71:934-944 (1968)].

Resistance to lysozyme was measured by methods recommended by Gordon [R. E. Gordon and D. A. Barnett, "Resistance to Rifampin and Lysozyme of Strains of Some Species of *Mycobacterium* and *Nocardia* as a Taxonomic Tool," *Int. J. Syst. Bacteriol.* 27, 176-178 (1977)].

Resistance to antibiotics was measured by padding antibiotic sensitivity discs onto the surface of seeded ISP No. 2 agar plates.

Phosphatase and urease were determined by methods described by Blazevic, *supra*.

Mycolic acids were determined by a method based on techniques described by Minnikin [D. E. Minnikin, L. Alshamaony and M. Goodfellow, "Differentiation of *Mycobacterium*, *Nocardia*, and Related Taxa by Thin-Layer Chromatographic Analysis of Whole-organism Methanolysates," J. Gen. Microbiol. 88:200-204 (1975)].

Phospholipid analysis was performed as described by Lechevalier [M. P. Lechevalier, C. De Bievre and H. Lechevalier, "Chemotaxonomy of Aerobic Actinomycetes: Phospholipid Composition," Biochemical Systematics and Ecology 5, 249-260 (1977)].

Cultural Characteristics

Growth of the organism was generally poor on chemically defined media but better on complex organic media. Aerial mycelia were absent except for trace amounts on ISP No. 4 and sodium butyrate agar. When spores were present, their color was oyster white in the Tresner and Backus System [H. D. Tresner and E. J. Backus, "System of Color Wheels for Streptomycete Taxonomy," Appl. Microbiol. 11:335-338 (1956)].

The color of the reverse side was yellowish gray to brown. No soluble pigments were produced, except for the production of a very light brown soluble pigment in ISP No. 2 and a dark brown soluble pigment in yeast-dextrose agar. Table I presents these cultural characteristics.

Table I: Cultural Characteristics of NRRL 15878 and A. macra on Various Agar Media^a

Agar Media	NRRL 15878	<u>A. macra</u>
ISP No. 2	G: Good (moist surface) R: 61.gy.Br Am: None Sp: Very light brown	Abundant (39.gy.r0) 43.m.rBr→56.deep Br. None Very light brown
ISP No. 3	G: Poor R: 93.y Gray Am: Trace: <u>b</u> White few scattered individual clumps SP: None	Fair 5.m.Pink None None
ISP No. 4	G: Poor R: 93.y Gray Am: Trace: <u>b</u> White few scattered individual clumps SP: None	Fair to poor 5 m. Pink None None
ISP No. 5	G: Poor R: 93.y Gray Am: None Sp: None	Good 48.v.0 None None

Table I (continued)

Agar Media	NRRL 15878	<u>A. macro</u>
ISP No. 7	G: Poor (moist surface) R: 79.1.gy.y.Br Am: None Sp: Light-brown	Fair 48.v.0 None None
Czapek's	G: Fair R: 93.y Gray Am: None Sp: None	Fair 93.y Gray None None
Emerson's	G: Good (edges peeled back) R: 77.m.yBr Am: None Sp: None	Good 267. Black Trace: <u>5fe</u> 1.gy.rBr None
Glucose Aspara- gine	G: Good (moist surface) R: 90.gy.Y Am: None Sp: None	Good 26.s.y Pink Fair: a White→7ca 1.y Pink None
Tomato- paste Oatmeal	G: Abundant (moist surface) R: 79.1.gy.y.Br Am: None Sp: None	Good 39.gy.r0 Trace: 2 White (edges only) None

Table I (continued)

Agar Media	NRRL 15878	<u>A. macra</u>
	G: Abundant (moist surface)	Abundant (moist surface)
Yeast-dextrose	R: 81.d.gy.y Br	59.d.Br
	Am: None	Slight trace: Gray
	Sp: Dark-brown	Dark-brown
Sodium Butyrate	G: Good	Fair
	R: 93.y Gray	78.d.yB
	Am: Fair: b White	None
	Sp: None	None
Calcium Malate	G: Fair	Fair
	R: 93.y Gray	26.s.y Pink
	Am: None	None
	Sp: None	None

^aG = growth R = reverse Am = aerial mycelium Sp = soluble pigment

Morphological Characteristics

Spore chains were sparsely produced on ISP No. 4 and sodium butyrate agar. Sporophores contained approximately 10 spores per chain, and were generally flexuous as in the Rectus-flexibilis (RF) configuration. However, hooked sporophores were also observed. The aerial hyphae had a tendency to clump together. Spore shape was oblong and ranged in size from 0.5-0.7 μm by 0.9-1.3 μm . The average spore size measured 1.1 by 0.6 μm . The spore surface ornamentation was smooth.

Physiological Characteristics

Table II lists the carbohydrate utilization pattern of the strain as compared to that of A. macra. ISP medium No. 9 was used as a basal medium. The addition of vitamin B₁ or the use of Luedemann's medium [G. M. Luedemann and B. Brodsky, "Micromonospora carbonacea sp. n., an Everinomycin-producing Organism," Antimicrob. Agents Chemother. 1964, 47-52] produced slightly better growth, but no change in carbon utilization. Adonitol, cellobiose, glucose, and ribose were utilized. Questionable utilization was noted with fructose and xylose. Arabinose, cellulose, dextran, galactose, i-inositol, inulin, lactose, mannitol, mannose, melizitol, melibiose, raffinose, rhamnose, salicin, sucrose, trehalose, and xylitol were not utilized for growth.

Table II

Carbon Utilization Pattern of NRRL 15878 and <i>A. macra</i> ^a		
Carbon Source	NRRL 15878	<i>A. macra</i>
control	-	-
adonitol	+	-
L-arabinose	-	-
cellobiose	+	-
cellulose	-	ND
dextran	-	ND
D-fructose	±	-
D-galactose	-	±
D-glucose	+	+
D-inositol	-	±
inulin	-	ND
D-lactose	-	-
mannitol	-	-
D-mannose	-	-
D-melezitose	-	-
D-melibiose	-	-
raffinose	-	-
L-rhamnose	-	-
ribose	+	±
salicin	-	-
sucrose	-	+
trehalose	-	+
xylitol	-	ND
D-xylose	±	±

^a + = utilized, - = not utilized,
 ± doubtful if utilized, ND = not done

Table III lists the resistance of the strain to various antibiotics at the concentrations indicated and compares it to that of *A. macra*.

Table III

Resistance to Antibiotics by NRRL 15878 and <i>A. macra</i> ^a			
Antibiotic	Concentration	NRRL 15878	<i>A. macra</i>
Bacitracin	10 units	+	-
Cephalothin	30 µg	+	+
Gentamicin	10 µg	-	-
Lincomycin	2 µg	+	+
Neomycin	30 µg	-	-
Oleandomycin	15 µg	-	-
Pencillin G	10 units	+	+
Rifampin	5 µg	+	+
Streptomycin	10 µg	-	+
Tetracycline	30 µg	-	-
Tobramycin	10 µg	-	-
Vancomycin	30 µg	-	-

^a + = resistant (no zones of inhibition)
 - = sensitive (zones of inhibition)

NRRL 15878 grew at temperatures from 15-42°C, and tolerated up to 2 percent NaCl. It produced catalase, phosphatase and urease.

NRRL 15878 degraded casein, DNA, esculin, and gelatin, but not adenine, calcium malate, chitin, elastin, guanine, hippurate, hypoxanthine, keratin, starch, testosterone, tyrosine or xanthine.

Cell-wall Analysis

Hydrolyzed whole cells contained the meso isomer of diaminopimelic acid. Sugars present in whole cell hydrolysates were as follows: glucose, mannose, madurose, and ribose. The cell-wall type according to Becker, supra, is type III, and the sugar pattern is type B (Lechevalier, 1968). A qualitative analysis of whole-cell methanolysates for mycolic acids yielded questionable results. It is doubtful that the culture contains mycolic acids. A type PI phospholipid pattern was found. Type PI contains no nitrogenous phospholipids and is characteristic of the genus Actinomadura (Lechevalier, 1977).

Identity of the Strain

NRRL 15878 has a Type III cell wall, Type B whole-cell sugar pattern, and a Type PI phospholipid pattern. This chemotaxonomic information plus its general cultural characteristics are consistent with the assignment of the strain to the genus Actinomadura Lechevalier and Lechevalier (Lechevalier, 1970).

Comparison of its characteristics to those in published descriptions of known species of Actinomadura show that the culture is similar to A. pelletieri (Laveran 1906) Lechevalier and Lechevalier, 1970 and to A. macra (Huang, 1980).

The culture resembles Actinomadura pelletieri mainly by the absence, or at least the rare occurrence, of aerial mycelia. Morphology of sporophores, when produced, is similar to that described in the literature (Goodfellow, 1979). The two cultures also possess a number of physiological characteristics in common. However, the cultural and physiological differences are sufficient to separate them as distinct species.

According to Lechevalier (1970), A. pelletieri is represented exclusively by a group of clinical isolates. The original description of A. pelletieri by Gordon [R. E. Gordon, "Some Criteria for the Recognition of Nocardia madurae (Vincent) Blanchard," J. Gen. Microbiol 45:355-364 (1966)] describes it as being a bright red culture. NRRL 15878 does not produce this pigment. A. pelletieri degrades elastin, hypoxanthin, keratin, tyrosine, utilizes trehalose, reduces nitrate, and grows at 45°C; but NRRL 15878 does not have these characteristics. NRRL 15878 utilizes adonitol and cellobiose, degrades esculin and DNA, and is resistant to lysozyme; but A. pelletieri does not have these characteristics. The new culture is, therefore, considered to be a different species than A. pelletieri.

Because of the culture's similarity to A. macra, simultaneous laboratory comparisons were made. NRRL 15878 and A. macra share many properties. Both are unable to degrade adenine, calcium-malate, chitin, elastin, guanine, keratin, starch, testosterone, tyrosine, and xanthine. Neither produces H₂S or melanoid pigments. Both cultured degrade casein, DNA and gelatin, produce catalase and phosphatase, and synthesize a polyether antibiotic. They have the same tolerance to NaCl; both grow on sodium butyrate; and they have the same cell-wall type. A. macra and NRRL 15878 differ in carbon-utilization pattern, esculin and hypoxanthine degradation, resistance to antibiotics, temperature range, urease production, and the reduction of nitrate.

A. macra and NRRL 15878 share many cultural characteristics, notably the absence of aerial mycelia. However, there are significant differences. The reverse side of NRRL 15878 is gray to a yellowish brown; A. macra on many media produces a red color. This distinction is most clearly seen on glucose-asparagine agar. On this medium A. macra produces a pink aerial growth, whereas NRRL 15878 produces none. These cultural comparisons are shown in Table I.

The morphology of NRRL 15878 is similar to that of A. macra. Both have poorly developed aerial mycelia that belongs in the Rectus-flexibilis (RF) section of Pridham [T. G. Pridham, C. W. Hesseltine, and R. C. Benedict, "A Guide for the Classification of Streptomycetes According to Selected Groups," Appl. Microbiol. 6:52-79 (1957)]. Spore surface ornamentation is smooth.

The differences and similarities between NRRL 15878 and A. macra are summarized in Table IV.

Table IV

Summary Comparison of NRRL 15878 and <u>A. macra</u>	
Similarities	Differences
Morphology	Antibiotic resistance
Physiological properties	Carbon-utilization pattern
Polyether synthesis	Cultural characteristics
Scarcity of aerial hyphae	Degradation of esculin
	Degradation of hypoxanthine
	Nitrate reduction
	Spore shape and size
	Temperature range
	Urease production

Table V shows these similarities and differences in greater detail.

Table V: Comparison of NRRL 15878 and A. macra

Test	NRRL 15878	<u>A. macra</u>
Aerial hyphae formation	rare	rare
Carbon-utilization pattern	different (see Table II)	
Catalase degradation of:		
adenine	-	-
calcium malate	-	-
casein	+	+
chitin	-	-
DNA	+	+
elastin	-	-
esculin	+	+
gelatin	+	+
guanine	-	-
hypoxanthine	-	+
keratin	-	-
starch	-	-
testosterone	-	-
tyrosine	-	-
xanthine	-	-

Table V: (Continued)

Test	NRRL 15878	<u>A. macra</u>
Growth in liquid media	colorless	red-orange
Growth on Na butyrate	+	+
H ₂ S production	-	-
Melanoid pigments	-	-
NaCl tolerance	2 percent	2 percent
Nitrate reduction	-	+
Phosphatase	+	+
Polyether synthesis	+	+
Resistance pattern to antibiotics	different (see Table III)	
Resistance to lysozyme	+	ND
Reverse side color	gray + brown	brown → orange
Soluble pigment	brown	brown
Spore chains	RF	RF
Spore surface	smooth	smooth
Temperature range	15-42°	15-37°
Urease production	+	-
Spore shape	oblong	oval
Spore size	0.5-0.7 x 0.9-1.3µm 0.8-1.0 x 1.2-2.0µm	

These comparisons indicate that the NRRL 15877 and NRRL 15878 cultures are significantly different from other species of *Actinomadura* and represent strains of a new species for which the name *Actinomadura oligospora* sp. nov. is proposed. The specific epithet (*O. oligospora*: L. Adj. *oligo* few, L.n. *sporus* spored, *oligospora* few spored) refers to the relative absence of sporophores in the organism. Strain NRRL 15878 is the type strain of *A. oligospora*.

As is the case with other organisms, the characteristics of the A80190-producing culture of this invention, *Actinomadura oligospora* NRRL 15877, are subject to variation. Recombinants, mutants or variants of the strain may be obtained by methods in the art. For example, mutants can be obtained by treatment with various known physical and chemical mutagens such as ultraviolet light, X rays, gamma rays and chemicals such as N-methyl-N'-nitro-N-nitrosoguanidine. Natural and induced variants, mutants and recombinants of *Actinomadura oligospora* NRRL 15877 which retain the characteristic of A80190 production are considered part of this invention.

The culture medium used to grow the *Actinomadura oligospora* cultures can be any one of a number of media. For economy in production, optimal yield, and ease of product isolation, however, certain culture media are preferred. Thus, for example, a preferred carbohydrate source in large-scale fermentation is

glucose, although ribose, xylose, fructose, galactose, mannose, mannitol, potato dextrin and the like can also be used. Glycerol and lipids support little or no growth or antibiotic production when used as the primary carbon source. In combination with glucose, they enhance biomass, but depress antibiotic production.

A preferred nitrogen source is collagen hydrolysate, although enzyme-hydrolyzed casein, meat peptones, fish meal, liver meal, and the like are also useful. Among the nutrient inorganic salts which can be incorporated in the culture media are the customary soluble salts capable of yielding zinc, sodium, magnesium, calcium, ammonium, chloride, carbonate, sulfate, nitrate and like ions.

Essential trace elements necessary for the growth and development of the organism should also be included in the culture medium. Such trace elements commonly occur as impurities in other constituents of the medium in amounts sufficient to meet the growth requirements of the organism. Foaming is not usually a problem, but small amounts (i.e. 0.2 ml/L) of an antifoam agent such as polypropylene glycol may be added to large scale fermentation media if needed.

For production of substantial quantities of antibiotic A80190, submerged aerobic fermentation in tanks is preferred. Small quantities of A80190 may be obtained by shake-flask culture. Because of the time lag in antibiotic production commonly associated with inoculation of large tanks with the spore form of the organism, it is preferable to use a vegetative inoculum. The vegetative inoculum is prepared by inoculating a small volume of culture medium with the spore form or mycelial fragments of the organism to obtain a fresh, actively growing culture of the organism. The vegetative inoculum is then transferred to a larger tank. The vegetative inoculum medium can be the same as that used for larger fermentations, but other media are also suitable.

A80190 is produced by the A80190-producing organism when grown at temperatures between about 25° and about 37° C. An optimum temperature for A80190 production appears to be about 30-32° C.

As is customary in submerged aerobic culture processes, sterile air is blown into the vessel from the bottom while the medium is stirred with conventional turbine impellers. The maximum oxygen uptake of the fermentation under the conditions used thus far has not exceeded about 0.2 mM/L/minute. In a fully baffled 165-liter fermentor containing approximately 115 liters of broth, an aeration rate of 0.125 v/v/m with an agitation rate of 200 rpm is sufficient to maintain the level of dissolved oxygen at or above 30% of saturation.

Production of antibiotic A80190 can be followed during the fermentation by testing samples of the broth for antibiotic activity against organisms known to be sensitive to the antibiotic. One assay organism useful in testing A80190 is Bacillus subtilis ATCC 6633. The bioassay is conveniently performed by the agar-well plate test.

Following its production under submerged aerobic fermentation conditions, A80190 can be recovered from the fermentation medium by methods used in the fermentation air. The antibiotic activity produced during fermentation of the A80190-producing organism occurs both in the filtered broth and in the mycelial mass. Maximum recovery of A80190 is accomplished, therefore, by initially filtering the medium to separate the broth from the mycelial mass. The filtered broth and the mycelial mass can then be purified separately to give their respective portion of A80190. A variety of techniques may be used in this purification. A preferred technique for purification of the filtered broth involves adjusting it to a pH of about 9 and extracting with a suitable solvent such as, for example, ethyl acetate. The extracting solvent can then be evaporated under vacuum to give the broth portion of A80190. A preferred method of purifying the mycelial mass is to extract the separated mycelial filter cake with a suitable solvent such as, for example, methanol or acetone. The extracting solvent is then evaporated under vacuum to give a concentrated aqueous solution. This aqueous solution is then adjusted to a pH of about 9 and is extracted with a suitable solvent such as, for example, ethyl acetate. The extracting solvent is then concentrated under vacuum to give the mycelial portion of A80190. The broth and mycelial portions of the A-80190 complex are further purified by similar procedures. A preferred procedure involves silica gel chromatography.

Alternatively, the culture solids, including medium constituents and mycelium can be used without extraction or separation, but preferably after removal of water, as a source of A80190. For example, after production of A80190, the whole fermentation broth can be dried by lyophilization, by drum-drying, or by azeotropic distillation and drying. The dried broth is then mixed directly into feed premix.

The alkali-metal and alkaline-earth-metal cationic salts of A80190 are prepared according to procedures commonly used for the preparation of cationic salts. For example, the free acid of A80190 is dissolved in a suitable solvent such as acetone; a 1/3 volume of water is added; and this solution is adjusted to a pH of about 9 to 10 with the base of the desired cationic salt (e.g. NaOH, KOH). The salt thus formed can be isolated by routine methods, such as filtration or evaporation of the solvent.

A preferred method of forming salts is to dissolve A80190 (acid form) in a water-immiscible solvent

such as ethyl acetate, add an equal volume of water, and adjust the mixture to pH 10 with the corresponding cationic base (e.g. NaOH, KOH, etc.). The separated organic phase is washed with water and concentrated to dryness. The residue is lyophilized from dioxane. The salt can be crystallized from an appropriate solvent, such as pentane.

The salts formed with organic amines can be prepared similarly. For example, the gaseous or liquid amine can be added to a solution of A80190 in a suitable solvent such as acetone; the solvent and excess amine can be removed by evaporation.

A80190 acyl-ester derivatives are prepared by treating A80190 with a corresponding acid anhydride or acid chloride. Esterification occurs at one of the A80190 hydroxyl groups. Such esters are typically prepared by reacting A80190 with, for example, the corresponding acid anhydride at room temperature.

A80190 alkyl ester derivatives are prepared by esterification of the carboxyl group, using standard procedures. The A80190 alkyl ester derivatives are typically less active when tested *in vitro*. When administered to an animal, however, such esters can act as pro drugs which are converted to A80190 *in vivo*.

The alkyl ether derivatives of A80190 are those compounds wherein one or more of the hydroxyl groups has been replaced by a YR group wherein:

Y represents O or S; and

R represents C₁-C₆-alkyl,

C₁-C₄-alkoxy-C₂-C₅-alkyl,

C₁-C₄-alkoxycarbonyl-C₂-C₅-alkyl,

amino-C₂-C₅-alkyl,

mercapto-C₂-C₅-alkyl,

hydroxyalkyl,

haloalkyl, or

(R')_m-phenyl(CH₂)_n,

wherein R' represents C₁-C₄-alkyl, C₁-C₄-alkoxy, or hydroxy;

m represents 0-2; and

n represents 0-3.

The terms alkyl and alkoxy have the meaning discussed *supra*, but are limited to the number of carbon atoms specified.

The term "hydroxyalkyl" refers either to a monohydroxy-C₂-C₅-alkyl moiety or, when Y is O, to the 2,3-dihydroxyprop-1-yl moiety.

The term "haloalkyl" refers to a C₂-C₅-alkyl moiety having from one to three halogen substituents, selected from the group consisting of bromine, chlorine, and fluorine. When the alkyl moiety is dihalo- or trihalo-substituted, the halo-substituents must be the same halogen moiety.

Preferred A80190 ether derivatives are those compounds wherein Y represents O and R represents C₁ to C₆ alkyl. The ether derivatives are prepared by reacting A80190, or a salt thereof, with a corresponding primary alcohol or thiol.

With some of the starting alcohols or thiols it may be necessary to add an acid catalyst to the reaction. Suitable catalysts include hydrochloric acid, sulfuric acid, methanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, selenium dioxide, and boron trifluoride.

A solvent such as, for example, water, acetone, benzene, ether, tetrahydrofuran, or dioxane may be added to facilitate the reaction. Reactions generally occur at room temperature, although higher temperatures, for example up to 100°C, may be used.

Although ordinary reaction work-up procedures are sometimes sufficient, additional purification may be required to obtain the compounds of this invention. Such purification may be accomplished by well-known methods, such as, for example, column chromatography, thin-layer chromatography, fractional crystallization and the like.

The A80190 urethane derivatives of formula 1 can be prepared by treating A80190 or an A80190 salt with an isocyanate of formula 3



wherein R is as defined *supra*.

Preferably, a salt of A80190, in particular the sodium salt, is used. The isocyanate of formula 3 should be added in slight excess, e.g., about 10% excess, in order to form the mono derivative in optimum quantity. The reaction is preferably carried out in an inert solvent such as a chlorinated hydrocarbon, e.g., carbon tetrachloride, methylene chloride or chloroform, ether, ethyl acetate or in an aromatic hydrocarbon

solvent such as benzene or toluene. The reaction temperature is not critical but can be between above 0°C. and the boiling point of the reaction mixture, but is preferably about room temperature.

The A80190 compounds inhibit the growth of bacteria and fungi which are pathogenic to animal and plant life. The inhibitory activity of A80190 is illustrated in Table VI. Activity was measured by the conventional disc-diffusion method (6-mm pads were dipped in solutions containing 1 mg of compound per ml of solution; pads were placed on agar plates seeded with test organism).

Table VI

Antibacterial Activity of A80190	
Test Organism	Zone of Inhibition (mm diameter)
<u>Staphylococcus aureus</u>	17
<u>Bacillus subtilis</u>	23
<u>Micrococcus luteus</u>	20
<u>Mycobacterium avium</u>	21
<u>Saccharomyces pastorianus</u>	10
<u>Neurospora crassa</u>	14
<u>Candida albicans</u>	14

One important aspect of the antimicrobial activity of A80190 compounds relates to their activity against anaerobic bacteria. The minimal inhibitory concentrations (MIC's) at which A80190 inhibits various anaerobic bacteria, as determined by standard agar-dilution assay, are summarized in Table VII. End points were read after 24-hour incubation.

Table VII

Susceptibility of Anaerobic Bacterial Isolates to A80190	
Anaerobic Bacteria	MIC (µg/ml)
<u>Clostridium difficile</u> 2994	<0.5
<u>Clostridium perfringens</u> 81	<0.5
<u>Clostridium septicum</u> 1128	<0.5
<u>Eubacterium aerofaciens</u> 1235	<0.5
<u>Peptococcus asaccharolyticus</u> 1302	1
<u>Peptococcus prevoti</u> 1281	<0.5
<u>Peptostreptococcus anaerobius</u> 1428	1
<u>Peptostreptococcus intermedius</u> 1624	1
<u>Propionibacterium acnes</u> 79	1
<u>Bacteroides fragilis</u> 111	>128
<u>Bacteroides fragilis</u> 1877	>128
<u>Bacteroides fragilis</u> 1936B	>128
<u>Bacteroides thetaiotaomicron</u> 1438	>128
<u>Bacteroides melaninogenicus</u> 1856/28	>128
<u>Bacteroides melaninogenicus</u> 2736	>128
<u>Bacteroides vulgatus</u> 1211	16
<u>Bacteroides corrodens</u> 1874	>128
<u>Fusobacterium symbiosum</u> 1470	8
<u>Fusobacterium necrophorum</u> 6054A	16

Anticoccidial activity is an important property of the A80190 compounds. Table VIII summarizes the results of an in vitro tissue culture screen of A-80190 compounds vs. Eimeria tenella.

Table VIII

Activity of A80190 Compounds vs. <i>Eimeria tenella</i> In Vitro					
Compound	Concentration (μg/ml)				
	5	1	0.2	0.04	0.008
A80190	C ^a	C	A	A	A
Acetyl A80190	A	A	A	A	N
Propionyl A80190	A	A	A	A	S

^aC = cytotoxic

A = active

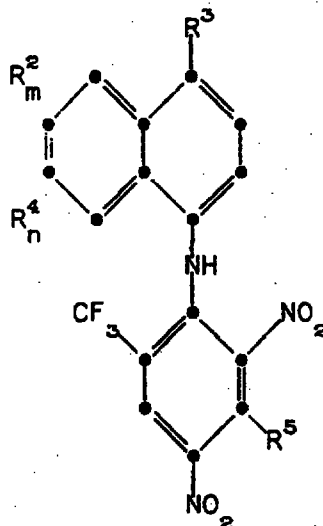
S = slightly active

N = not active

For treating coccidiosis in poultry, a non-toxic anticoccidial amount of an A80190 compound is administered to infected or susceptible birds, preferably orally on a daily basis. The A80190 compound can be supplied in many ways, but it is most conveniently supplied with a pharmaceutically acceptable carrier, preferably the feed ingested by the birds. Although a variety of factors must be considered in determining an appropriate concentration of A80190 compound, the rates of administration are generally in the range of about 2 to about 100 ppm in the feed and are preferably in the range of about 15 to about 50 ppm of feed ration. In another aspect, this invention relates to compositions for treating coccidiosis comprising an effective amount of an A80190 compound for treating coccidiosis, together with a suitable vehicle.

Compositions for controlling coccidiosis in poultry of this invention comprise:

- 1) a compound of formula (I) in combination with
- 2) a compound selected from the group consisting of
 - a) nicarbazin,
 - b) 4,4'-dinitrocarbanilide,
 - c) a naphthalenamine compound of formula 4:

4

wherein:

- R^2 is C_1 - C_4 alkyl;
 R^3 is halogen, C_1 - C_4 fluoroalkyl, C_1 - C_4 fluoroalkoxy or C_1 - C_4 fluoroalkylthio;
 R^4 is halogen;
 R^5 is hydrogen or halogen;

m is 0, 1 or 2; and

n is 0 or 1;

with the proviso that, when an R⁴ substituent exists, it is at other than the 2-position;

d) a benzenamine selected from 2,4-dinitro-N-[4-(trifluoromethoxy)phenyl]-6-(trifluoromethyl)-benzenamine; 2,4-dinitro-N-[4-(1,1,2,2-tetrafluoroethoxy)phenyl]-6-(trifluoromethyl)benzenamine or 2,4-dinitro-N-[4-(pentafluoroethoxy)phenyl]-6-(trifluoromethyl)benzenamine;

e) metichlorpindol; or

f) a pharmaceutically acceptable salt of an (a)-(e) compound.

In formula 4, C₁-C₄ alkyl includes methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, t-butyl, and the like.

The term "halogen" represents fluorine, chlorine, bromine and iodine.

C₁-C₄ Fluoroalkyl is a C₁-C₄ alkyl group bearing one or more fluorine atoms. Such fluoroalkyl groups include trifluoromethyl, 1,1,2,2-tetrafluoroethyl, pentafluoroethyl, 1,2,3,3-tetrafluoropropyl, nonafluorobutyl, and the like.

C₁-C₄ Fluoroalkoxy is a C₁-C₄ alkoxy group bearing one or more fluorine atoms. Such fluoroalkoxy groups include difluoromethoxy, trifluoromethoxy, 1-fluoroethoxy, 1,1,2,2-tetrafluoroethoxy, pentafluoroethoxy, 1,2,2,3,3-pentafluoropropoxy, heptafluoropropoxy, 4,4,4-trifluorobutoxy, and the like.

C₁-C₄ Fluoroalkylthio is a C₁-C₄ alkylthio group bearing one or more fluorine atoms. Such fluoroalkylthio groups include trifluoromethylthio, 1,1,2,2-tetrafluoroethylthio, pentafluoroethylthio, 4,4,4-trifluorobutylthio, and the like.

Preferred formula 4 compounds are those wherein m and n are 0 and R⁵ is hydrogen.

Typical formula 4 compounds are:

4-Fluoro-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

4-Iodo-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

4-Trifluoromethyl-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

4-Pentafluoroethyl-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

6,7-Dimethyl-4-(1,1,2,2-tetrafluoroethoxy)-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

2-Isopropyl-4-chloro-N-[3-chloro-2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

8-n-Butyl-4-(4,4,4-trifluorobutoxy)-N-[3-bromo-2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

3-Methyl-6-propyl-4-heptafluoropropyl-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

3,4-Dichloro-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

4-(1,1-Difluoroethoxy)-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine

4-(1,1,2,2-Tetrafluoroethoxy)-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine and

4-(1,1,2,2-Tetrafluoroethylthio)-N-[2,4-dinitro-6-(trifluoromethyl)phenyl]-1-naphthalenamine.

Nicarbazin and 4,4'-dinitrocarbanilide are taught in U.S. Patent 2,731,382. Nicarbazine is a complex of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine, but the 4,4'-dinitrocarbanilide alone exhibits anticoccidial activity. See Science 122, 244 (1955).

The components of the combinations of an A80190 compound with compounds 2(a)-(e) are used in amounts which, in combination, are synergistic as to at least one coccidiosis-causing organism. In general, the maximum amounts to be used in the combinations are the same as the maximum amounts for anticoccidial treatment by the individual components. The lower limits are generally less than that required for therapy by the individual components. Accordingly, the present invention is generally practiced with compositions containing 1) from about 2 to about 100 ppm of an A80190 compound and 2) a) from about 5 to 125 ppm of nicarbazine, b) from about 25 to about 150 ppm of 4,4'-dinitrocarbanilide, c) from about 1 to about 1000 ppm of the specified naphthalenamine, d) from about 5 to about 125 ppm of a specified benzenamine, or e) from about 20 to about 70 ppm of metichlorpindol. The A-80190 compounds are particularly effective when administered with nicarbazine. Preferred combinations contain from about 2 to about 20 ppm of an A80190 compound with from about 5 to about 50 ppm of nicarbazine.

Another important property of the A80190 compounds is the ability to improve feed-utilization efficiency in animals. For example, the A80190 compounds improve feed-utilization efficiency in ruminants which have a developed rumen function.

The efficiency of feed use can be monitored by observing the production and concentration of propionate compounds in the rumen using the method described by Arthur P. Raun in U.S. Patent 3,794,732 (see especially Example 5). Table IX shows the ratio of volatile-fatty-acid (VFA) concentrations in A80190-treated flasks to concentrations in control flasks in this test.

Table IX:

Effect of A80190 Compounds on Ruminant Feed-Utilization Efficiency^a

Compound ^b	Dosage mcg/ml	Molar % Propionate	Molar % Acetate	Molar % Butyrate	Total VFA/ Control VFA mm/L
A80190	1	1.185*	0.986	0.851	0.916
"	1	1.403*	0.892	0.813	0.887
A80190	0.3	1.220*	0.970	0.819	0.967
AcA80190	0.3	1.091*	0.995	0.912	1.003
PrA80190	0.3	1.049	1.022	0.908	0.948
A80190	1	1.247*	1.016	0.706	0.857
AcA80190	1	1.192*	1.006	0.783	0.909
PrA80190	1	1.158*	1.021	0.792	0.961
A80190	3	1.368*	0.998	0.611	0.963
AcA80190	3	1.213*	1.041*	0.696	1.010
PrA80190	3	1.195*	1.033	0.731	0.930
A80190	1	1.566*	0.926	0.529	0.913
AcA80190	1	1.196*	0.976	0.833	0.777
PrA80190	1	1.244*	0.983	0.764	0.883

*LSD; two-tailed t-test; significant at $P < 0.01$; $C_3 > 95$ percent upper confidence limit

^aThree test runs

^bAcA80190 = acetyl derivative of A80190

PrA80190 = propionyl derivative of A80190

The A80190 compounds are typically effective in increasing propionates and, thereby, the efficiency of feed utilization when administered to ruminants orally at rates of from about 0.02 mg/kg/day to about 1.5 mg/kg/day. Preferable rates of administration are from about 0.05 mg/kg/day to about 0.5 mg/kg/day.

A preferred method of administration is to mix the compound with the animals' feed; however, it can be administered in other ways, for example, tablets, drenches, boluses, or capsules. Formulation of these various dosage forms can be accomplished by methods well known in the veterinary pharmaceutical art. Each individual dosage unit should contain a quantity of a compound of this invention directly related to the proper daily dose for the animal to be treated.

This invention further relates to feed compositions adapted to increase feed utilization comprising feed ration and from 2.5 to 25 grams per ton of an A80190 compound.

As described *supra*, A80190 compounds are active against anaerobic bacteria, particularly *Clostridium perfringens*. A80190 compounds are, therefore, beneficial in the treatment or prevention of enteritis in chickens, swine, cattle and sheep. A80190 compounds are also useful in the treatment of enterotoxemia in ruminants.

The A80190 compounds are also anthelmintic agents. For example, A80190 is active against

Caenorhabditis elegans at levels as low as 20 ppm.

In another aspect the A80190 compounds are useful in the treatment of swine dysentery. A80190 inhibits the growth of *Treponema hyodysenteriae*, a causative agent of swine dysentery, at levels as low as 0.39 mcg/ml. A preferred method of administration to swine is by incorporation of an appropriate amount of an A80190 compound into the feed ration or drinking water. An appropriate amount will relate to whether the treatment is to prevent or to cure infection. Usually, a lower concentration of active compound is needed to prevent infection than is required for to eliminate infection in animals already afflicted. In general, amounts in the range of from about 20 to about 100 grams of A80190 compound per ton of feed are effective to prevent infection. Amounts in the range of from about 100 to about 500 g of A80190 compound per ton of feed are recommended for treating swine suffering from dysentery. These amounts provide from about 1 to about 5 mg/kg of body weight per day (prophylactic treatment) or from about 5 to about 25 mg/kg of body weight per day (treatment of infected animal). When added to the drinking water, amounts of from about 0.04 to about 0.2 g (prophylactic) or from about 0.2 to about 1 g (therapeutic) of A80190 compound per gallon of water are recommended.

This invention further relates to feed compositions for treating swine dysentery comprising swine ration and an effective amount of an A80190 compound for this purpose. As discussed, an effective amount is typically one in the range of from about 20 to about 500 grams of A80190 compound per ton of feed.

The A80190 compounds can be administered to animals orally or parenterally. They can also be administered by insufflation, i.e. by blowing the antibiotic, in the form of a medicated dust, into an enclosed space or room wherein the animals or poultry are held. The animals or poultry breathe the medicated dust present in the air; the medicated dust also is taken into the body through the eyes (a process called intraocular injection).

The most practical way to administer the A80190 compounds is by formulation into the feed supply. A variety of feeds, including the common dry feeds, liquid feeds, and pelleted feeds, may be used. Although the preferred method of administration is by mixing it with the animals' feed, it can also be administered in other ways, for example, tablets, drenches, boluses, or capsules. Each individual dosage unit should contain a quantity of A80190 compound directly related to the proper daily dose for the animal to be treated.

The methods of formulating drugs into animal feeds are well known. A preferred method is to make a concentrated drug premix which in turn is used to prepare medicated feeds. Typical premixes may contain from about 1 to about 200 grams of drug per pound of premix. Premixes may be either liquid or solid preparations.

The final formulation of feeds for animals or poultry will depend upon the amount of drug to be administered. The common methods of formulating, mixing, and pelleting feeds may be used to prepare feeds containing an A80190 compound.

The A80190 compounds may be formulated for parenteral administration by methods recognized in the veterinary pharmaceutical art. Effective injectable compositions containing the A80190 compounds may be in either suspension or solution form. In the solution form, the A80190 compound is dissolved in a physiologically acceptable carrier. Such carriers comprise a suitable solvent, preservatives such as benzyl alcohol, if needed, and buffers. Useful solvents include, for example, alcohols, glycols, or inert oils such as vegetable oils or highly refined mineral oils.

Injectable suspension compositions are prepared using a nonsolvent for the compound with adjuvants, as a carrier. The nonsolvent can be, for example, water or a glycol such as polyethylene glycol.

Suitable physiologically acceptable adjuvants are necessary to keep the compound suspended in suspension compositions. The adjuvants may be chosen from among thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful for suspending the compounds. Lecithin, alkylphenol polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters are useful suspending agents in liquid nonsolvents.

Many substances which affect the hydrophilicity, density, and surface tension of the liquid nonsolvent can assist in making injectable suspensions in individual cases. For example, silicone antifoams, glycols, sorbitol, and sugars can be useful suspending agents.

In the preparation of dusts or powders for administration by insufflation, the compounds are typically mixed with talc, diatomaceous earth, or some other inert substance as an adjuvant.

The A80190 compounds are also useful as insecticides and acaricides. For example, A80190 is active against the following insects at levels as low as the level indicated in parenthesis: mosquito larvae (<50 ppm), blowfly larvae (35 ppm), adult housefly (35 ppm), and Mexican bean beetle and southern armyworm (<100 ppm). A80190 is also active against mites such as two-spotted spider mite when applied at a rate as low as 25 ppm.

In another aspect, the A80190 compounds have herbicidal activity. For example, A80190 is active

against crabgrass, mustard and pigweed at 2 lb/acre (200 gpa) either pre-emergence or post-emergence.

The A80190 compounds are active against polio virus. For example, tissue-culture tests show that A80190 is active against Polio III virus at a level of 2000 $\mu\text{g/ml}$.

Antibiotic A80190 exhibits ion-transport properties and is, therefore, an ionophore (ion-bearer) (see B. C. Pressman, Alkali metal chelators the ionophores, in "Inorganic Biochemistry," Volume 1, G. L. Eichhorn, Elsevier, 1973). At a 0.5 $\mu\text{g/ml}$ concentration A80190 showed ionophorous activity; its activity with Na^+ , K^+ and Rb^+ was better than that with Cs^+ or Li^+ . At a lower concentration (0.2 $\mu\text{g/ml}$), however, A80190 exhibited an even greater ionophorous effect with Cs^+ than with Na^+ as cation.

A80190 can be used, therefore, when the selective removal of particular cations is desired. Examples of such uses include the removal and recovery of silver ions from solutions in photography, the removal of toxic cations from industrial waste streams before such streams are discharged to the environment, and desalinization of sea water. A80190 can be used as one component of an ion-specific electrode (see O. Kedem, et al., U.S. Patent 3,753,887, Aug. 21, 1973).

A80190 alters the cation permeability of both natural and artificial membranes. A80190 can be used, therefore, as a component in a membrane used for the selective transport of cations against a concentration gradient. One potential application of this property is in recovery of heavy and precious metals on a commercial basis [see E. L. Cussler, D. F. Evans, and Sister M. A. Matesick, Science 172, 377 (1971)].

In yet another aspect, the A80190 compounds are active as inhibitors of the enzyme ATPase. ATPase, an alkali-metal-sensitive enzyme found in cell membranes, is involved in the energy necessary for active transport. "Active transport" refers to the energy requiring series of operations whereby intracellular and extracellular fluids maintain their compositions. Inhibitors of ATPase reduce the energy required for active transport. In vitro tests have shown that A80190 inhibits mitochondrial ATPase with a half-effective concentration (IC_{50}) of 0.5 $\mu\text{g/ml}$.

The A80190 compounds are also potential cardiogenic agents.

In order to illustrate more fully the operation of this invention, the following examples are provided:

Example 1

Preparation of A80190

A. Shake-flask Fermentation of A80190

The culture *Actinomadura oligospora* NRRL 15878, either as a lyophilized pellet or as a suspension maintained in liquid nitrogen, is used to inoculate a seed medium having the following composition:

SEED MEDIUM

<u>Ingredient</u>	<u>Amount (%)</u>
Glucose	1.0
Soluble starch	2.0
Yeast extract	0.5
Enzymatic hydrolysate of casein*	0.5
CaCO_3	0.1
Deionized water	q.s. 1 liter

NaOH was added to raise the pH of the medium to about 7.2 before sterilizing.

*NZ Amine A, Sheffield Chemical Co.,
Norwich, N.Y.

Slants or plates are prepared by adding 2.5% agar to the seed medium. The inoculated slant is

incubated at 30°C. for from about 10 to about 14 days. The mature slant culture is scraped with a sterile tool to loosen the spores and remove and mascerate the mycelial mat. About one-fourth of the loosened spores and culture growth thus obtained is used to inoculate 50 ml of a first-stage seed medium.

The inoculated first-stage medium is incubated in a 250-ml Erlenmeyer flask at 30°C. for about 48 hours on a shaker orbiting in a two-inch (5.08 cm) circle at 250 rpm.

This incubated first-stage medium (0.4 ml) is used to inoculate 50 ml of a production medium having the following composition:

<u>Ingredient</u>	<u>Amount (%)</u>
Glucose	3.0
N-Z Amine A	0.4
Collagen hydrolysate*	0.5
MgSO ₄ ·7H ₂ O	0.05
CaCO ₃	0.2
Cold tap water	q.s. 1 liter
(Presterilization pH adjusted to 7.0)	
*IPC 3, Inland Industrial Molasses Co., Dubuque, Iowa	

The inoculated production medium is incubated in a 250-ml wide-mouth Erlenmeyer flask at 30-32°C. for 8 to 10 days on a shaker orbiting in a two-inch circle at 250 rpm.

B. Tank Fermentation of A80190

In order to provide a larger volume of inoculum, 10 ml of incubated first-stage medium, prepared as described in Section A, is used to inoculate 400 ml of a second-stage growth medium having the same composition as that of the first-stage medium. This second-stage vegetative medium is incubated in a two-liter wide-mouth Erlenmeyer flask for about 48 hours at 30°C. on a shaker orbiting in a two-inch circle at 250 rpm.

Incubated second-stage vegetative medium (800 ml) thus prepared is used to inoculate 100 liters of sterile production medium, prepared as described in Section A. The inoculated production medium is allowed to ferment in a 165-liter stirred fermentation tank for 8 to 10 days at a temperature of 30-32°C. Low airflow (0.12-0.25 v/v/m) and low rpm (150-200) in the stirred vessel maintain a dissolved oxygen level above 30% of air saturation.

Example 2

Isolation of A80190

Whole fermentation broths from two 100-L tanks were combined (207 L) and filtered through a filter press with the aid of Hyflo Supercel. The mycelial filter cake was extracted by circulating methanol (40 L) through the filter press. Acetone can also be used as an extractant. The methanol extract, concentrated in vacuo to a volume of about 15 L, was combined with the broth filtrate (182 L). This was adjusted to pH 9 with 1N sodium hydroxide, and the resulting solution was extracted with an equal volume of ethyl acetate. The ethyl acetate extract was concentrated to a volume of about 700 ml. Water (1 L) was added to the concentrated extract; the pH was adjusted to 9.0 with sodium hydroxide; and the mixture was extracted twice with toluene (1 L), maintaining the pH at 9.0. The toluene extracts were combined and concentrated in vacuo to give an oily residue containing A80190.

The residue was dissolved in toluene (100 ml) and applied to a column containing 2 L of silica gel (Woelm, 70-150 mesh) in toluene. The column was eluted first with toluene (10 L) and then with toluene:ethanol mixtures (49:1, 10 L) and (48:2, 10 L), collecting 1-L fractions. Elution was monitored by bioassay and TLC. Fractions containing A80190 were combined and concentrated. The residue was

dissolved in dioxane and freeze-dried to yield 13.6 g of crude A80190.

Example 3

Purification of A80190

Crude A80190 (20.4 g), obtained from four 100-L tanks as described in Examples 1 and 2, was dissolved in acetonitrile (200 ml). The solution was applied to a column containing 2 L of silica gel (Woelm, 70-150 mesh) in acetonitrile. The column was washed with acetonitrile (10 L) and eluted sequentially with acetonitrile:acetone mixtures (95:5, 2 L), (9:1, 10 L), (4:1, 10 L) and (7:3, 10 L), collecting 1-L fractions. Elution was monitored by bioassay, using *Bacillus subtilis*. Fractions containing A80190 were combined and concentrated. The residue was dissolved in dioxane and freeze-dried to yield 15.8 g of purified A80190.

Example 4

Crystallization of A80190

Purified A80190 (28.2 g), was dissolved in acetone (500 ml). Water (500 ml) was added, and the pH was adjusted to 5.0 with dilute hydrochloric acid. The resulting solution was allowed to stand at room temperature for 20 hours for crystallization to occur. The crystals were separated by filtration, washed with water and dried in a vacuum oven to yield 25.9 g of crystalline A80190 (acid form). Figure 1 shows the infrared absorption spectrum of A80190 in chloroform.

Example 5

Preparation of A80190 Sodium Salt

A80190 (acid form, 300 mg) was dissolved in acetone (30 ml); water (10 ml) was added; and the pH was adjusted to 10 with NaOH. The solution was concentrated to an oily residue which was dissolved in dioxane and freeze-dried to yield a white powder (m.p. 145-150°). The molecular weight of the product by field desorption mass spectrometry (FDMS) was 850. IR in CHCl_3 , shown in Figure 2, shows absorption maxima at the following frequencies (cm^{-1}): 2962, 2934, 2885, 2825, 1719, 1569, 1455, 1397, 1377, 1358, 1314, 1285, 1243, 1162, 1104, 1074, 1057, 1025, 987, 979, 936, 922, 895 and 847.

Example 6

Alternate Preparation of A80190 Sodium Salt

A80910 (acid form, 5 g) was dissolved in ethyl acetate (500 mg); water (500 ml) was added. The mixture was adjusted to pH 10 with 5N NaOH, stirring for 15 minutes to maintain the pH. The ethyl acetate phase was separated and washed with water (500 ml) and concentrated under vacuum to dryness. The residue was dissolved in dioxane (100 ml) and freeze-dried to give 4.1 g of the sodium salt of A80190.

The water wash was allowed to stand at room temperature overnight, and the crystals which formed were filtered and dried. The crystals were A80190 in the acid form (mp 115-120° C).

Example 7

Crystallization of A80190 Sodium Salt

A80190 sodium salt (1 g) was dissolved in pentane (10 ml) and allowed to stand at room temperature for three days (solvent evaporates to about 5 ml). The crystals which formed were filtered and dried under vacuum to give 593 mg of crystalline A80190 sodium salt, mp 225-230° C.

Example 8

Preparation of Acetyl A80190

A80190 (acid form, 200 mg) was dissolved in pyridine (4 ml); acetic anhydride (4 ml) was added; and

the mixture was allowed to stand for 72 hours at room temperature. Water (10 ml) was added, and the aqueous solution was extracted with chloroform (50 ml). The chloroform extract was washed successively with 50 ml each of 0.1N HCl, water containing 1 percent NaHCO_3 , and water. The chloroform extract was then concentrated in vacuo to a residue, and the residue was dissolved in acetone (100 ml). The acetone solution was concentrated in vacuo to remove residual pyridine and acetic acid. This step was repeated three times, and the resulting residue was dissolved in acetone (50 ml). Water (50 ml) was added, and the resulting solution was allowed to stand at room temperature for crystallization to occur. The crystals were separated by filtration, washed with water and dried in a vacuum oven at 50°C to yield 192 mg of crystalline acetyl A80190 (m.p. $87-89^\circ\text{C}$). Molecular weight = 870 by fast-atom bombardment mass spectrometry (FABMS). IR in CHCl_3 , shown in Figure 3, shows absorption maxima at the following frequencies (cm^{-1}): 3021, 3018, 2935, 2825, 1726, 1454, 1373, 1311, 1244, 1162, 1104, 1078, 1058, 1024, 987, 976, 934 and 925.

Example 9

Preparation of Propionyl A80190

A80190 (acid form, 200 mg) was dissolved in pyridine (4 ml); propionic anhydride (4 ml) was added; and the mixture was allowed to stand at room temperature for 72 hours. Water (10 ml) was added, and the solution was extracted with chloroform (50 ml). The chloroform extract was washed successively with 50 ml each of 0.1N HCl, water containing 1 percent NaHCO_3 and water. The chloroform extract was concentrated in vacuo to a residue which was dissolved in acetone (100 ml). The acetone solution was concentrated in vacuo to remove residual pyridine and acetic acid. This step was repeated three times. The residue was dissolved in acetone (50 ml); water (50 ml) was added; and the mixture was allowed to stand at room temperature for 16-20 hours for crystallization to occur. The crystals were separated by filtration, washed with water and dried in a vacuum oven at 50°C to yield 170 mg of crystalline propionyl A80190 (m.p. $83-85^\circ\text{C}$). Molecular weight of 884 by FDMS and FABMS. IR in CHCl_3 , shown in Fig. 4, shows absorption maxima at the following frequencies (cm^{-1}): 3018, 2976, 2935, 2827, 1725, 1460, 1376, 1313, 1160, 1105, 1082, 1058, 1024, 987, 979, 945, 935 and 893.

Examples 10-12

The following A80190 ester derivatives can be prepared using the method of Examples 8-9:

n-Heptanoyl A80190

Valeryl A80190

tert-Butyryl A80190

Example 13

Preparation of A80190 4-Bromophenylurethane Derivative

A80190 (acid form, 7 g) was dissolved in benzene (100 ml), and 4-bromophenyl isocyanate (3 g) in benzene (100 ml) was added with stirring. The mixture was stirred at room temperature for 168 hours. The reaction was monitored daily by silica-gel TLC, using acetonitrile:acetone (1:1) as the developing solvent and detecting with vanillin- H_2SO_4 spray reagent. The reaction appeared to be complete by 168 hours. The precipitate which formed was separated by filtration, and the filtrate containing the derivative and some unreacted A80190 was concentrated and lyophilized to give 7.5 g of product.

This product was dissolved in acetonitrile (50 ml) and applied to a 3- x 140-cm column containing 800 ml of silica gel (Woelm, 70-150 mesh) in acetonitrile. The column was developed with acetonitrile, collecting a first fraction having a volume of 500 ml, five subsequent fractions of 100 ml each and then six fractions of 500 ml each. Elution was monitored by silica-gel TLC. Crystals formed in the fourth fraction after standing at room temperature overnight. The crystals were separated by filtration and dried under vacuum to give 689 mg of A80190 4-bromophenylurethane derivative.

The filtrate was dried. The residue was dissolved in dioxane (50 ml) and lyophilized to give an additional 477 mg of the product. The fifth through the tenth fractions were combined and concentrated to dryness. The residue was dissolved in acetonitrile (50 ml) and lyophilized to give an additional 2.3 g of amorphous A80190 4-bromophenyl urethane derivative. This compound had the following characteristics:

mp: 142-145 $^\circ\text{C}$

$[\alpha]_D^{25}$: +4° (c 10, CHCl₃)
 molecular weight: 1025 (fast-atom-bombardment mass spectrometry)

Examples 14-15

The following A80190 urethane derivatives were prepared using the method of Example 13: A80190 4-chlorophenylurethane derivative and A80190 4-nitrophenylurethane derivative.

Examples 16-23

The following urethane derivatives can be prepared using the method of Example 13:

A80190 phenylurethane
 A80190 4-methylphenylurethane
 A80190 4-iodophenylurethane
 A80190 4-fluorophenylurethane
 A80190 cyclohexylurethane
 A80190 2-phenethylurethane
 A80190 2-(phenyl)cyclopropylurethane
 A80190 4-phenoxyphenylurethane

Example 24

Preparation of A80190 Methyl Ether Derivative

A80190 in the acid form is dissolved in methanol; water ($\frac{1}{2}$ volume) is added. The solution is allowed to stand until the ether derivative is formed. The solution is evaporated under vacuum. The product is chromatographed using, for example, silica gel to give to A80190 methyl ether derivative.

Examples 25 to 27

Using a procedure like that of Example 24 and the appropriate alcohol or thiol, the following A80190 ether derivatives can be prepared:

A80190 n-Propyl Ether Derivative
 A80190 Methyl Thioether Derivative
 A80190 n-Butyl Ether Derivative

Example 28

Chromatographic Identification of A80190

I. TLC on silica gel

System: acetonitrile:acetone (1:1)
 $R_f = 0.59$

Detection: Bacillus subtilis
Vanillin-H₂SO₄ spray

II. HPLC

Adsorbent: μ Bondapak C18 (4 x 300-mm column)
 Solvent system: acetonitrile:tetrahydrofuran:H₂O (6:1:3) containing 1 percent H₃PO₄; adjusted to pH 3.0 with NH₄OH
 Detection: refractometer
 Flow rate: 3.0 ml/min
 Retention time: 9.7 min

Example 29

A80190 is prepared by the method of Example 1, but using the Actinomadura oligosporus NRRL 15877 culture.

Example 30

A80190 Modified Chick Ration for Coccidiosis Control

A balanced, high-energy ration adapted to feed chicks for rapid weight gain is prepared by the following recipe:

Ingredient	%	lbs
Ground yellow corn	50	1,000
Soybean meal, solvent-extracted dehulled, finely ground, 50 percent protein	31.09	621.8
Animal fat (beef tallow)	6.5	130
Dried fish meal, with solubles (60% protein)	5.0	100
Distillers' solubles from corn	4.0	80
Dicalcium phosphate, feed grade	1.8	36
Calcium carbonate	0.8	16

Ingredient	%	lbs
Vitamin premix (representing vitamins A, D, E, H, and B ₁₂ , choline, niacin, pantothenic acid, riboflavin, biotin, with glucose bulking agent)	0.5	10
Trace mineral premix (representing MnSO ₄ , ZnO, KI, FeSO ₄ , CaCO ₃)	0.2	4
2-Amino-4-hydroxybutyric acid (hydroxy analog of methionine)	0.1	2
A80190 (Na Salt)	0.01	0.2

These substances are mixed in accordance with standard feed-mixing techniques. Chicks fed such a ration, with water ad libitum, are protected against exposure to coccidiosis; weight gains are comparable to those of coccidiosis-free chicks fed a similar, unmedicated diet.

Example 31

A80190-Improved Beef-Cattle Ration

A balanced high-grain beef-cattle ration is prepared as follows:

Ingredient	%	lbs
Finely ground corn	67.8	1356
Ground corn cob	10	200
Dehydrated alfalfa meal, 17 percent protein	5	100
Dehulled soybean meal, solvent extracted, 50 percent protein	9.9956	199.912
Cane molasses	5	100.0
Urea	0.6	12.0
A80190 (Na salt)	0.0044	0.088
Dicalcium phosphate, feed grade	0.5	10.0
Calcium carbonate	0.5	10.0

EP 0 184 291 B1

Ingredient	%	lbs
Sodium chloride	0.3	6.0
Trace mineral premix	0.03	0.6
Vitamin A and D ₂ premix*	0.07	1.4
Vitamin E premix**	0.05	1.0
Calcium propionate	0.15	3.0

*Containing per pound: 2,000,000 I.U. of vitamin A; 227,200 I.U. of vitamin D₂ and 385.7 g of soybean feed with 1% oil added

**Corn distillers dried grains with solubles containing 20,000 I.U. of d-alpha-tocopheryl acetate per pound

The mixed feed is compressed into pellets. At an average daily ingestion rate of 15 pounds of feed per animal, this feed supplies approximately 300 mg of A80190 (Na salt) per animal per day.

Example 32A80190-Improved Swine Ration

A balanced swine farrowing ration is prepared
as follows:

<u>Ingredient</u>	<u>%</u>	<u>lbs/ton</u>
Ground yellow corn	65.10	1302
Soybean oil meal, solvent extracted dehulled	18.50	370
Dried-beet pulp	10.00	200
Dicalcium phosphate	2.90	58
Calcium carbonate	1.20	24
Swine vitamin premix ¹	1.10	22
Salt (NaCl)	0.55	11
Choline chloride, 25%	0.35	7
Trace-mineral premix ²	0.15	3
Vitamin A premix ³	0.10	2
Hydroxy analog of Methionine	<u>0.05</u>	<u>1</u>
Total	100.00	2000

¹Each kg of premix contains the following: 77,161 USP units Vitamin D₂; 2,205 I.U. of Vitamin E; 441 mg riboflavin; 1,620 mg pantothenic acid; 2,205 mg niacin; 4.4 mg Vitamin B₁₂; 441 mg Vitamin K; 19,180 mg choline; 110 mg folic acid; 165 mg pyridoxine; 110 mg thiamine; 22 mg biotin.

²Each kg of premix contains the following: 50 g of manganese as manganese sulfate; 100 g of zinc as zinc carbonate; 50 g of iron as ferrous sulfate; 5 g of copper as copper oxide; 1.5 g of iodine as potassium iodide and 150 g maximum and 130 g minimum calcium as calcium carbonate.

³Each kg of premix contains 6,6138,00 USP units Vitamin A.

For 200 pounds of this ration, a premix is prepared by adding A80190 (10 g) to a small amount of solvent-extracted soybean feed, grinding them in a mortar and pestle, and diluting the ground mixture to one pound with additional solvent-extracted soybean feed. This premix is then added to 200 lb. of the above-described swine ration, mixing by standard techniques. This medicated feed provides a level of 100 grams of A80190 per ton of basal ration. Medicated feed is fed to sows for at least one day, and preferably from seven to ten days, prior to farrowing and after farrowing for as long as is desirable.

Larger or smaller quantities of medicated ration with varying levels of A80190 are prepared by varying the quantity of A80190 in the premix and/or the quantity of basal ration.

Sows are fed at rates of from 1.0 to 10 grams of A80190 per 100 pounds of feed. The medicated feed is available ad libitum with water. Usually, sows consume about 6-8 lb of ration per day.

Example 33

A80190-Formulation for Piglets

A80190 is dissolved in a small amount of ethanol. This ethanol solution is suspended in polyethylene glycol 200. The suspension is concentrated so that each unit dose has a volume of about 0.5 to 2 ml. Such suspensions are given to young pigs at rates of 0.5 to 50 mg per lb, three times a day by gavage.

Example 34

Improved Ration for Control of Swine Dysentery

A premix is prepared by standard methods using the following ingredients:

Ingredient	Grams/Kilogram
Active Compound	150.0
Calcium Silicate	20.0
Calcium Carbonate (Oyster Shell Flour)	830.0
Total Weight	1000 gms.

This premix is added to commercial swine ration, using standard feed-mixing techniques, to give a final level of active compound of 100 grams/ton.

Demonstration of the synergistic effect with nicarbazin in combination with A80190 was obtained as follows: one-week-old broiler chicks were allotted to five-bird cages and were fed a medicated or control ration, typically for one day, prior to infection with oocysts of a coccidiosis-causing organism. The chicks were maintained on their respective rations for a period of time, typically seven days. There were four replicates per treatment.

Test 1

The chicks were inoculated with 200,000 oocysts of *Eimeria acervulina* (Lilly strain 59), 60,000 oocysts of *Eimeria maxima* (strain FS-177) and 40,000 oocysts of *Eimeria tenella* (strain FS-155).

INTESTINAL LESION SCORES A80190 (p.p.m.)						
Nicarbazin (p.p.m.)	0	4	8	12	16	20
0	9.13	8.63	5.94	3.50	1.19	0.0
20	9.13	5.13	0.19	0.13	0.0	
30	8.88	1.63	0.50	0.0		
40	8.90	0.56	0.0			
50	8.56	0.0				
125	0.08					

CECAL LESION SCORES A80190 (p.p.m.)						
Nicarbazin (p.p.m.)	0	4	8	12	16	20
0	3.44	2.63	1.75	1.19	0.19	0.0
20	3.19	2.38	0.31	0.56	0.06	
30	3.50	0.94	0.19	0.0		
40	3.17	1.00	0.58			
50	3.19	0.54				
125	0.33					

WEIGHT GAIN (grams/per chicken) A80190 (p.p.m.)						
Nicarbazin (p.p.m.)	0	4	8	12	16	20
0	147	236	265	251	272	261
20	159	268	284	266	279	
30	174	279	258	283		
40	211	258	289			
50	222	276				
125	279					

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE



Ingredient	Grams/Kilogram
Active Compound	150.0
Calcium Silicate	20.0
Calcium Carbonate (Oyster Shell Flour)	830.0
Total Weight	1000 gms.

This premix is added to commercial swine ration, using standard feed-mixing techniques, to give a final level of active compound of 100 grams/ton.

Demonstration of the synergistic effect with nicarbazin in combination with A80190 was obtained as follows: one-week-old broiler chicks were allotted to five-bird cages and were fed a medicated or control ration, typically for one day, prior to infection with oocysts of a coccidiosis-causing organism. The chicks were maintained on their respective rations for a period of time, typically seven days. There were four replicates per treatment.

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INTESTINAL LESION SCORES A80190 (p.p.m.)						
Nicarbazin (p.p.m.)	0	4	8	12	16	20
0	9.13	8.63	5.94	3.50	1.19	0.0
20	9.13	5.13	0.19	0.13	0.0	
30	8.88	1.63	0.50	0.0		
40	8.90	0.56	0.0			
50	8.56	0.0				
125	0.08					

CECAL LESION SCORES A80190 (p.p.m.)						
Nicarbazin (p.p.m.)	0	4	8	12	16	20
0	3.44	2.63	1.75	1.19	0.19	0.0
20	3.19	2.38	0.31	0.56	0.06	
30	3.50	0.94	0.19	0.0		
40	3.17	1.00	0.58			
50	3.19	0.54				
125	0.33					

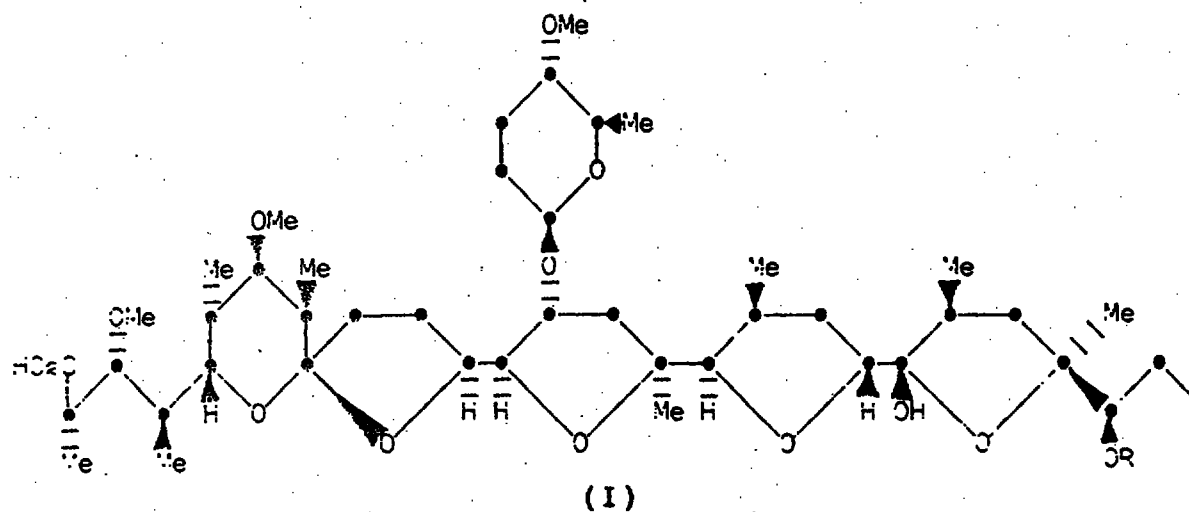
WEIGHT GAIN (grams/per chicken) A80190 (p.p.m.)						
Nicarbazin (p.p.m.)	0	4	8	12	16	20
0	147	236	265	251	272	261
20	159	268	284	266	279	
30	174	279	258	283		
40	211	258	289			
50	222	276				
125	279					

Claims

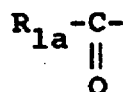
Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE



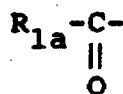
1. A compound of the formula (I):



wherein R is hydrogen or -CONHR₁ and R₁ is C₁ to C₇ alkyl, phenyl, pyridyl, furyl,
 (C₁ to C₇ alkyl)-phenyl,
 phenyl-C₁ to C₇ alkyl,
 halo = phenyl,
 nitro = phenyl,
 halo = phenyl-C₁ to C₇ alkyl,
 (C₁ to C₇ alkoxy)-phenyl,
 phenoxyphenyl,
 phenyl-C₃ to C₇ cycloalkyl optionally substituted by phenyl,
 acyl = phenyl in which acyl is a radical of the formula



wherein R_{1a} is C₁ to C₆ alkyl or hydrogen, and
 C₃ to C₇ cycloalkyl; or
 an acyl derivative thereof, in which acyl is a radical of the formula



wherein R_{1a} is C₁ to C₆ alkyl or hydrogen, and
 C₃ to C₇ cycloalkyl,
 a C₁ to C₇ alkyl ester derivative thereof, or
 an alkyl ether derivative thereof in which one or more of the hydroxyl groups has been replaced by a
 YR'' group wherein
 Y represents O or S; and
 R'' represents C₁-C₆-alkyl,
 C₁-C₄-alkoxy-C₂-C₅-alkyl,
 C₁-C₄-alkoxy-carbonyl-C₂-C₅-alkyl,
 amino-C₂-C₅-alkyl,
 mercapto-C₂-C₅-alkyl,
 hydroxyalkyl,
 haloalkyl, or
 (R')_m-phenyl(CH₂)_n,



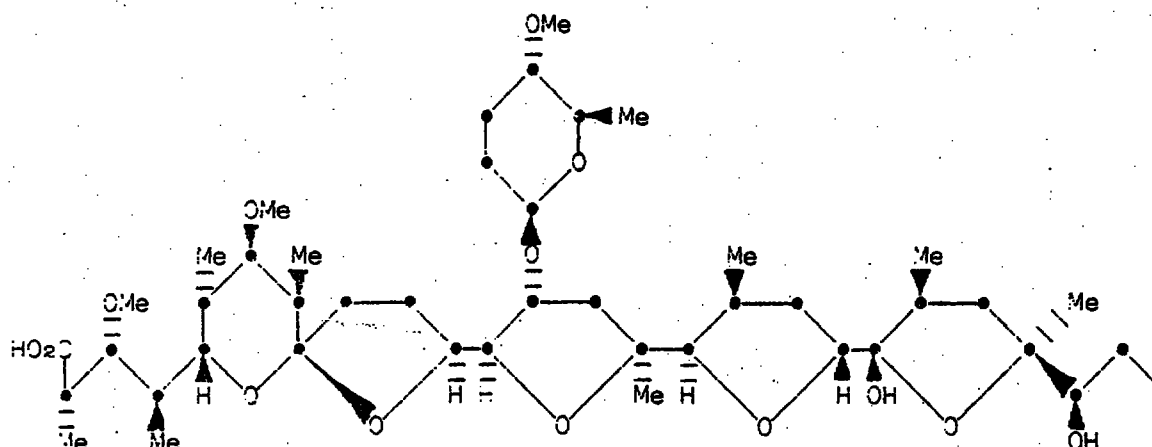
wherein R' represents C₁-C₄-alkyl, C₁-C₄-alkoxy, or hydroxy;

m represents 0-2; and

n represents 0-3, and

wherein hydroxyalkyl refers either to a monohydroxy-C₂-C₅-alkyl moiety or, when Y is 0, to the 2,3-dihydroxyprop-1-yl moiety and haloalkyl refers to a C₂-C₅-alkyl moiety having from one to three halogen substituents selected from the group consisting of bromine, chlorine and fluorine; or a salt thereof.

2. Antibiotic A80190 which has the formula:

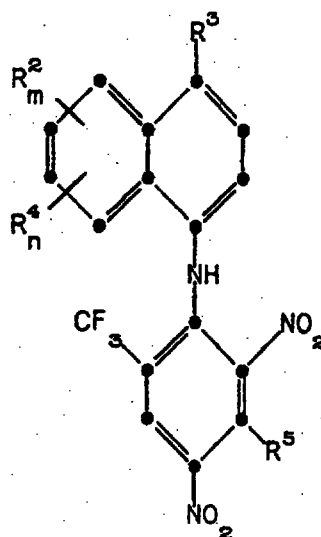


or a pharmaceutically-acceptable salt thereof.

3. A process for producing a compound as claimed in claim 1 or 2 which comprises cultivating an A80190 producing species of *Actinomadura oligospora* in a culture medium containing assimilable sources of carbon, nitrogen, and inorganic salts under submerged aerobic fermentation conditions until antibiotic A80190 is produced, and then separating A80190 from the culture medium, optionally followed by appropriate acylation, esterification, or reaction with an isocyanate of formula RNCO, where R is as defined in claim 1, to form a urethane derivative.
4. The process of Claim 3 wherein *A. oligospora* NRRL 15877 or NRRL 15878, or an A80190-producing variant, mutant or recombinant thereof, is used.
5. A compound of formula (I), or a pharmaceutically-acceptable salt thereof, for use as a veterinary or animal health product.
6. An animal feed premix comprising a compound as claimed in claim 1 or 2, or a pharmaceutically-acceptable salt thereof, as the active ingredient.
7. A process for increasing feed-utilization efficiency in ruminant animals which comprises orally administering to the animal an effective propionate-increasing amount of a compound as claimed in claim 1 or 2 or a pharmaceutically-acceptable salt thereof.
8. A compound of formula (I), or a pharmaceutically-acceptable salt thereof, for use in controlling coccidiosis in poultry.
9. A process for promoting the growth of a monogastric animal which comprises administering to the animal an effective amount of a compound as claimed in claim 1 or 2, or a pharmaceutically-acceptable salt thereof.
10. A poultry feedstuff composition comprising a first component which is a compound as claimed in claim 1 or 2, or a pharmaceutically-acceptable salt thereof, and a second component which is selected from the group consisting of a) nicarbazin, b) 4,4'-dinitrocarbanilide,



c) a naphthalenamine of the formula



wherein:

- R² is C₁-C₄ alkyl;
- R³ is halogen, C₁-C₄ fluoroalkyl, C₁-C₄ fluoroalkoxy or C₁-C₄ fluoroalkylthio;
- R⁴ is halogen;
- R⁵ is hydrogen or halogen;
- m is 0, 1 or 2; and
- n is 0 or 1;

with the proviso that, when an R⁴ substituent exists, it is at other than the 2-position;

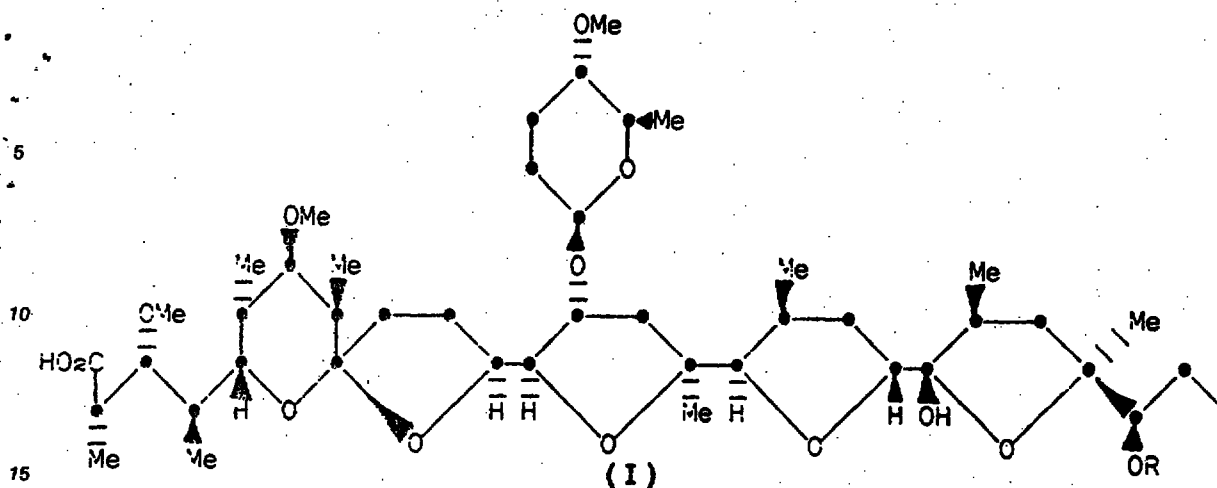
- d) a benzenamine selected from 2,4-dinitro-N-[4-(trifluoromethoxy)phenyl]-6-(trifluoromethyl)-benzenamine, 2,4-dinitro-N-[4-(1,1,2,2-tetrafluoroethoxy)phenyl]-6-(trifluoromethyl)benzenamine or 2,4-dinitro-N-[4-(pentafluoroethoxy)phenyl]-6-(trifluoromethyl)benzenamine;
 - e) metichlorpindol; or f) a pharmaceutically acceptable salt of an (a)-(e) compound;
- the components being present in the feedstuff in amounts which, in combination, are synergistic as to at least one coccidiosis-causing strain of Eimeria.

11. The composition of Claim 10 wherein the second component is nicarbazin.

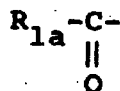
12. Actinomadura oligosporus NRRL 15878.

Claims for the following Contracting State : AT

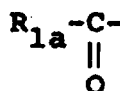
- 1. A process for preparing a compound of the formula (I):



wherein R is hydrogen or -CONHR₁ and R₁ is C₁ to C₇ alkyl, phenyl, pyridyl, furyl, (C₁ to C₇ alkyl)-phenyl, phenyl-C₁ to C₇ alkyl, halo = phenyl, nitro = phenyl, (C₁ to C₇ alkoxy)-phenyl, phenoxyphenyl, phenyl-C₃ to C₇ cycloalkyl optionally substituted by phenyl, acyl = phenyl in which acyl is a radical of the formula



wherein R_{1a} is C₁ to C₆ alkyl or hydrogen, and C₃ to C₇ cycloalkyl; or an acyl derivative thereof, in which acyl is a radical of the formula

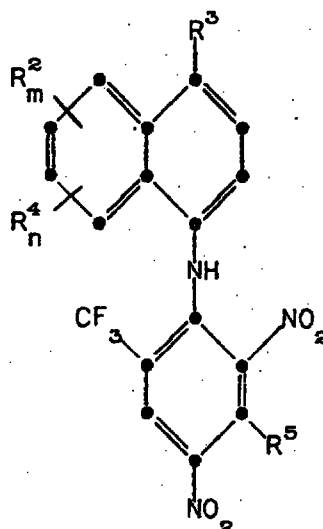


wherein R_{1a} is C₁ to C₆ alkyl or hydrogen, and C₃ to C₇ cycloalkyl, a C₁ to C₇ alkyl ester derivative thereof, or an alkyl ether derivative thereof in which one or more of the hydroxyl groups has been replaced by a YR'' group wherein Y represents O or S; and R'' represents C₁-C₆-alkyl, C₁-C₄-alkoxy-C₂-C₅-alkyl, C₁-C₄-alkoxy-carbonyl-C₂-C₅-alkyl, amino-C₂-C₅-alkyl, mercapto-C₂-C₅-alkyl, hydroxyalkyl, haloalkyl, or (R')_m-phenyl(CH₂)_n, wherein R' represents C₁-C₄-alkyl, C₁-C₄-alkoxy, or hydroxy; m represents 0-2; and n represents 0-3, and wherein hydroxyalkyl refers either to a monohydroxy-C₂-C₅-alkyl moiety or, when Y is O, to the 2,3-



dihydroxyprop-1-yl moiety and haloalkyl refers to a C₂-C₅-alkyl moiety having from one to three halogen substituents selected from the group consisting of bromine, chlorine and fluorine; or a salt thereof. which comprises cultivating an A80190 producing species of *Actinomadura oligospora* in a culture medium containing assimilable sources of carbon, nitrogen, and inorganic salts under submerged aerobic fermentation conditions until antibiotic A80190 is produced, and then separating A80190 from the culture medium, optionally followed by appropriate acylation, esterification, or reaction with an isocyanate of formula RNCO, where R is as defined above, to form a urethane derivative.

2. A process according to claim 1, wherein *A. oligospora* NRRL 15877 or NRRL 15878, or an A80190-producing variant, mutant or recombinant thereof, is used.
3. A process according to claim 1 or 2 for preparing a compound of formula (I) in which R is hydrogen, or a pharmaceutically-acceptable salt thereof.
4. A process for increasing feed-utilization efficiency in ruminant animals which comprises orally administering to the animal an effective propionate-increasing amount of a compound as claimed in claim 1 or 2.
5. The use of a compound as claimed in claim 1 or 2 for controlling coccidiosis in poultry.
6. A process for promoting the growth of a monogastric animal which comprises administering to the animal an effective amount of a compound as claimed in claim 1 or 2.
7. An animal feed premix comprising a compound as claimed in claim 1 or 2 as the active ingredient.
8. A poultry feedstuff composition comprising a first component which is a compound as claimed in claim 1 or 2 and a second component which is selected from the group consisting of a) nicarbazin, b) 4,4'-dinitrocarbanilide, c) a naphthalenamine of the formula



wherein:

- R² is C₁-C₄ alkyl;
- R³ is halogen, C₁-C₄ fluoroalkyl, C₁-C₄ fluoroalkoxy or C₁-C₄ fluoroalkylthio;
- R⁴ is halogen;
- R⁵ is hydrogen or halogen;
- m is 0, 1 or 2; and
- n is 0 or 1;

with the proviso that, when an R⁴ substituent exists, it is at other than the 2-position;



d) a benzenamine selected from 2,4-dinitro-N-[4-(trifluoromethoxy)phenyl]-6-(trifluoromethyl)-benzenamine, 2,4-dinitro-N-[4-(1,1,2,2-tetrafluoroethoxy)phenyl]-6-(trifluoromethyl)benzenamine or 2,4-dinitro-N-[4-(pentafluoroethoxy)phenyl]-6-(trifluoromethyl)benzenamine;

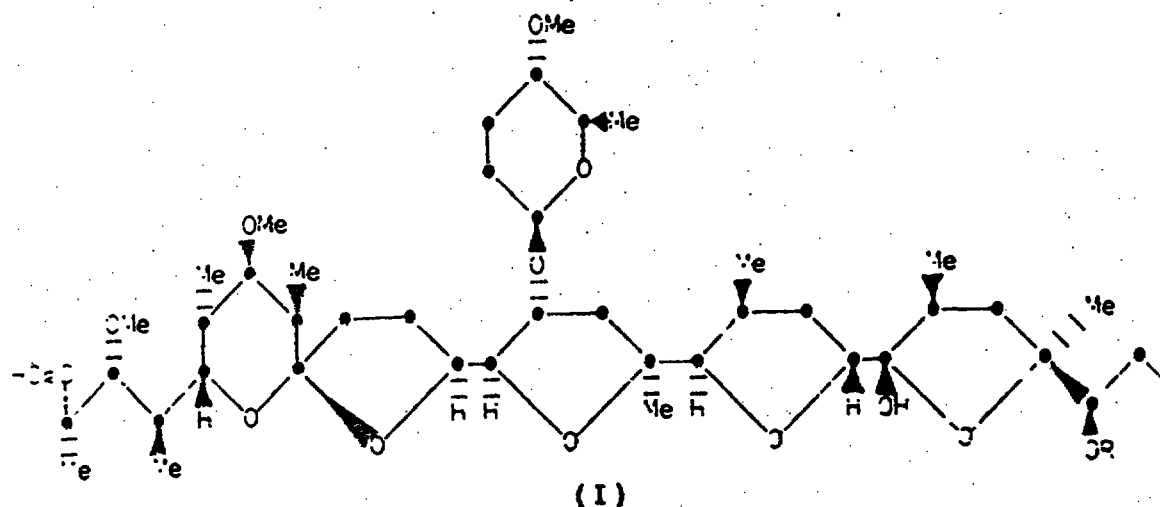
e) metichlorpindol; or f) a pharmaceutically acceptable salt of an (a)-(e) compound; the components being present in the feedstuff in amounts which, in combination, are synergistic as to at least one coccidiosis-causing strain of *Eimeria*.

9. The composition of Claim 8 wherein the second component is nicarbazin.

10 Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, TE, FR, GB, IT, LI, LU, NL, SE

1. Composé de formule (I) :



dans laquelle R représente un atome d'hydrogène ou -CONHR et

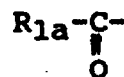
R₁ représente un groupe alkyle en C₁-C₇, un groupe phényle, un groupe pyridyle, un groupe furyle, un groupe alkyl(en C₁-C₇)phényle, un groupe phénylalkyle en C₁-C₇,

un groupe halogénophénylalkyle en C₁-C₇, un groupe alcoxy(en C₁-C₇)phényle,

un groupe phénoxyphényle,

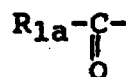
un groupe phénylcycloalkyle en C₃-C₇ éventuellement substitué par un groupe phényle, un groupe acylphényle

dans lequel le groupe acyle est un radical de formule



dans laquelle R_{1a} représente un groupe alkyle en C₁-C₆ ou un atome d'hydrogène, et un groupe cycloalkyle en C₃-C₇; ou

un de ses dérivés acylés dans lequel le groupe acyle représente un radical de formule



dans laquelle R_{1a} représente un groupe alkyle en C₁-C₆ ou un atome d'hydrogène, et un groupe cycloalkyle en C₃-C₇,

un de ses dérivés d'esters alkylés en C₁-C₇ ou un de ses dérivés d'éthers alkylés dans lequel un ou plusieurs des groupes hydroxyle ont été remplacés par un groupe YR'' dans lequel :



Y représente O ou S; et

R'' représente un groupe alkyle en C₁-C₆,

un groupe alcoxy(en C₁-C₄)alkyle en C₂-C₅,

un groupe alcoxy(en C₁-C₄)carbonylalkyle en C₂-C₅, un groupe aminoalkyle en C₂-C₅,

un groupe mercaptoalkyle en C₂-C₅,

un groupe hydroxyalkyle,

un groupe halogénalkyle

ou un groupe (R')_m-phényl(CH₂)_n,

où R' représente un groupe alkyle en C₁-C₄,

un groupe alcoxy en C₁-C₄ ou un groupe hydroxyle;

m = 0-2; et

n = 0-3,

et dans lequel l'expression "groupe hydroxyalkyle" désigne soit une fraction monohydroxyalkyle en C₂-

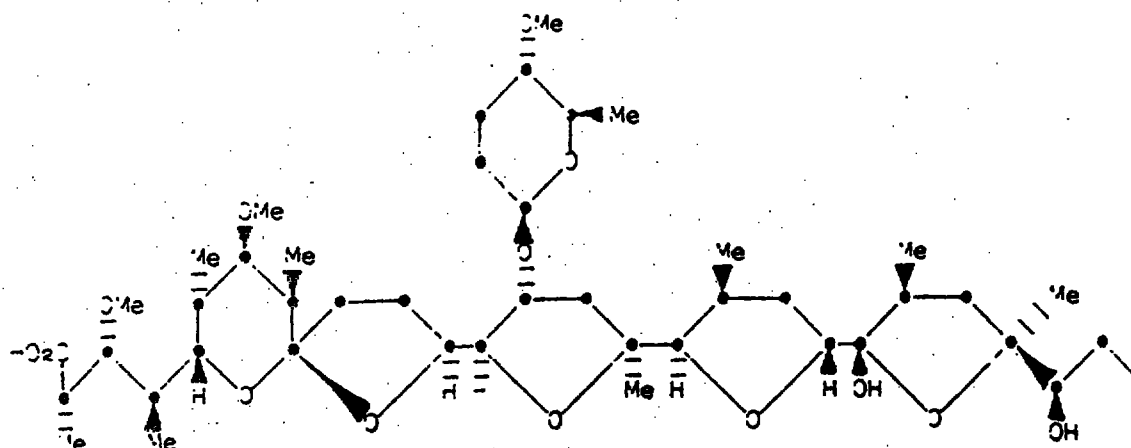
C₅, soit, lorsque Y = O, la fraction 2,3-dihydroxyprop-1-yle et l'expression "groupe halogénalkyle"

désigne une fraction alkyle en C₂-C₅ contenant de 1 à 3 substituants halogénés choisis parmi le

groupe comprenant le brome, le chlore et le fluor;

ou un de ses sels.

2. Antibiotique A80190 répondant à la formule



ou un de ses sels pharmaceutiquement acceptables.

3. Procédé pour préparer un composé selon la revendication 1 ou 2, qui consiste à cultiver une espèce de *Actinomadura oligospora* productrice de A80190 dans un milieu de culture contenant des sources assimilables de carbone, d'azote, ainsi que des sels inorganiques, dans des conditions de fermentation aérobie submergée, jusqu'à ce que l'on obtienne une production de l'antibiotique A80190, et ensuite, à séparer A80190 du milieu de culture, cette séparation étant éventuellement suivie d'une acylation, d'une estérification ou d'une réaction appropriée avec un isocyanate de formule RNCO où R est tel que défini dans la revendication 1, pour obtenir un dérivé d'uréthane.

4. Procédé selon la revendication 3, dans lequel on utilise *A. oligospora* NRRL 15877 ou NRRL 15878 ou encore un de ses variants, mutants ou recombinants producteur de A80190.

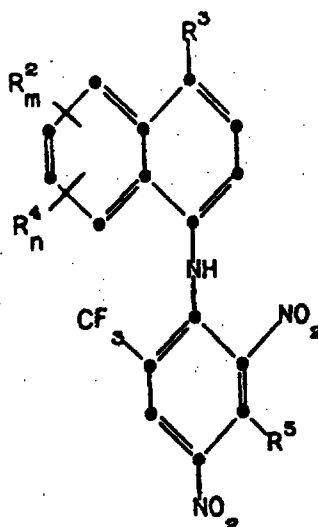
5. Composé de formule (I) ou un de ses sels pharmaceutiquement acceptable, destiné à être utilisé comme produit vétérinaire ou destiné à préserver la santé des animaux.

6. Prémélange d'aliments pour animaux comprenant un composé selon la revendication 1 ou 2, ou un de ses sels pharmaceutiquement acceptable, comme ingrédient actif.

7. Procédé destiné à améliorer le rendement alimentaire chez des animaux ruminants, qui consiste à administrer à l'animal, par voie orale, une quantité efficace augmentant la teneur en propionates, d'un composé selon la revendication 1 ou 2 ou d'un de ses sels pharmaceutiquement acceptable.



8. Composé de formule (I) ou d'un de ses sels pharmaceutiquement acceptable, destiné à être utilisé pour maîtriser la coccidiose dans la volaille.
9. Procédé pour favoriser la croissance d'un animal monogastrique, qui consiste à administrer à l'animal, une quantité efficace d'un composé selon la revendication 1 ou 2 ou d'un de ses sels pharmaceutiquement acceptable.
10. Composition d'aliments pour volailles, comprenant un premier composant, à savoir un composé selon la revendication 1 ou 2 ou un de ses sels pharmaceutiquement acceptable et un second composant choisi parmi le groupe comprenant :
- la nicarbazine;
 - le 4,4'-dinitrocarbanilide;
 - une naphthalénamine de formule :



dans laquelle :

- R^2 représente un groupe alkyle en C_1-C_4 ;
- R^3 représente un atome d'halogène, un groupe fluoroalkyle en C_1-C_4 , un groupe fluoroalcoxy en C_1-C_4 ou un groupe fluoroalkyl(en C_1-C_4)thio;
- R^4 représente un atome d'halogène;
- R^5 représente un atome d'hydrogène ou un atome d'halogène;
- m est égal à 0, 1 ou 2; et
- n est égal à 0 ou 1;

avec cette réserve que, lorsqu'un substituant de R^4 existe, il se trouve à une position différente de la position 2;

d) une benzénamine choisie parmi la 2,4-dinitro-N-[4-(trifluorométhoxy)phényl]-6-(trifluorométhyl)-benzénamine; la 2,4-dinitro-N-[4-(1,1,2,2-tétrafluoréthoxy)phényl]-6-(trifluorométhyl)benzénamine ou la 2,4-dinitro-N-[4-(pentafluoréthoxy)phényl]-6-(trifluorométhyl)benzénamine;

e) du métichloropindol; ou

f) un sel pharmaceutiquement acceptable d'un composé (a)-(e);

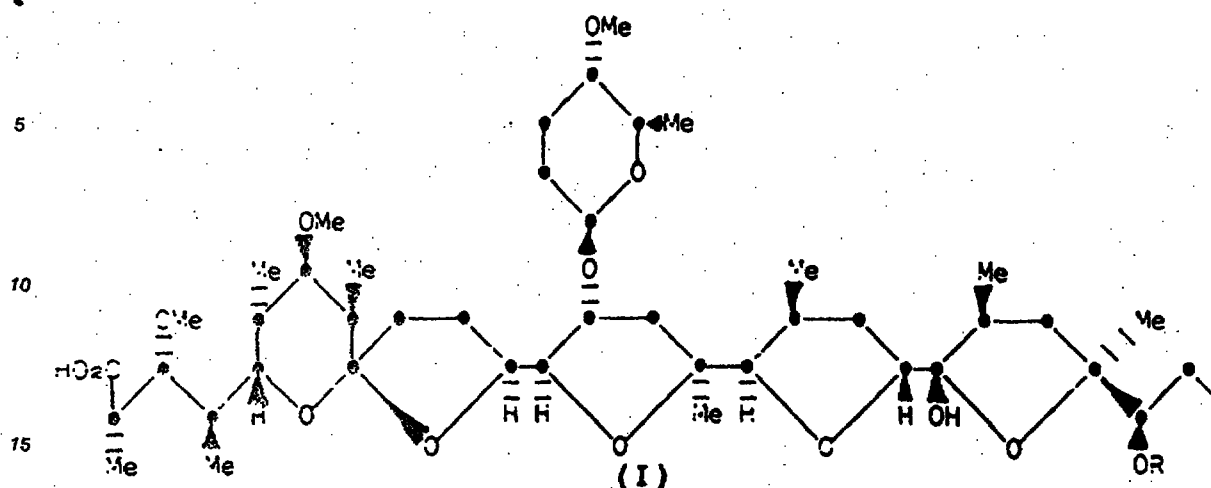
les composants étant présents dans l'aliment dans des quantités qui, en combinaison, ont un effet synergique vis-à-vis d'au moins une souche de *Elmeria* provoquant la coccidiose.

11. Composition selon la revendication 10, dans laquelle le second composant est la nicarbazine.

12. *Actinomadura oligosporus* NRRL 15878.

Revendications pour l'Etat contractant suivant : AT

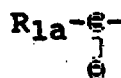
1. Procédé pour préparer un composé de formule (I) :



dans laquelle R représente un atome d'hydrogène ou -CONHR et

R₁ représente un groupe alkyle en C₁-C₇, un groupe phényle, un groupe pyridyle, un groupe furyle, un groupe alkyl(en C₁-C₇)phényle, un groupe phénylalkyle en C₁-C₇, un groupe halogénophénylalkyle en C₁-C₇, un groupe alcoxy(en C₁-C₇)phényle, un groupe phénoxyphényle, un groupe phénylcycloalkyle en C₃-C₇ éventuellement substitué par un groupe phényle, un groupe acylphényle

dans lequel le groupe acyle est un radical de formule



dans laquelle R_{1a} représente un groupe alkyle en C₁-C₆ ou un atome d'hydrogène,

et un groupe cycloalkyle en C₃-C₇; ou

un de ses dérivés acylés dans lequel le groupe acyle représente un radical de formule



dans laquelle R_{1a} représente un groupe alkyle en C₁-C₆ ou un atome d'hydrogène,

et un groupe cycloalkyle en C₃-C₇,

un de ses dérivés d'esters alkylés en C₁-C₇ ou un de ses dérivés d'éthers alkylés dans lequel un ou plusieurs des groupes hydroxyle ont été remplacés par un groupe YR'' dans lequel :

Y représente O ou S; et

R'' représente un groupe alkyle en C₁-C₆,

un groupe alcoxy(en C₁-C₄)alkyle en C₂-C₅,

un groupe alcoxy(en C₁-C₄)carbonylalkyle en C₂-C₅, un groupe aminoalkyle en C₂-C₅,

un groupe mercaptoalkyle en C₂-C₅,

un groupe hydroxyalkyle,

un groupe halogénalkyle

ou un groupe (R')_m-phényl(CH₂)_n,

où R' représente un groupe alkyle en C₁-C₄,

un groupe alcoxy en C₁-C₄ ou un groupe hydroxyle;

m = 0-2; et

n = 0-3,

et dans lequel l'expression "groupe hydroxyalkyle" désigne soit une fraction monohydroxyalkyle en C₂-C₅, soit, lorsque Y = O, la fraction 2,3-dihydroxyprop-1-yle et l'expression "groupe halogénalkyle"

désigne une fraction alkyle en C₂-C₅ contenant de 1 à 3 substituants halogénés choisis parmi le groupe comprenant le brome, le chlore et le fluor;
ou d'un de ses sels,

qui consiste à cultiver une espèce de *Actinomadura oligospora* productrice de A80190 dans un milieu de culture contenant des sources assimilables de carbone, d'azote, ainsi que des sels inorganiques, dans des conditions de fermentation aérobie submergée, jusqu'à ce que l'on obtienne une production de l'antibiotique A80190, et ensuite, à séparer A80190 du milieu de culture, cette séparation étant éventuellement suivie d'une acylation, d'une estérification ou d'une réaction appropriée avec un isocyanate de formule RNCO où R est tel que défini ci-dessus, pour obtenir un dérivé d'uréthane.

2. Procédé selon la revendication 1, dans lequel on utilise *A. oligospora* NRRL 15877 ou NRRL 15878 ou encore un de ses variants, mutants ou recombinants producteur de A80190.

3. Procédé selon la revendication 1 ou 2 pour préparer un composé de formule (I) dans laquelle R représente un atome d'hydrogène, ou un de ses sels pharmaceutiquement acceptable.

4. Procédé destiné à améliorer le rendement alimentaire chez des animaux ruminants, qui consiste à administrer à l'animal, par voie orale, une quantité efficace augmentant la teneur en propionates, d'un composé selon la revendication 1 ou 2.

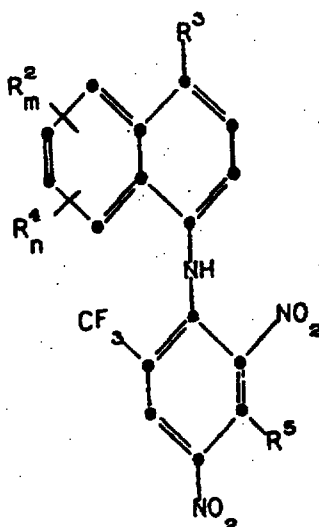
5. Utilisation d'un composé selon la revendication 1 ou 2 pour maîtriser la coccidiose dans la volaille.

6. Procédé pour favoriser la croissance d'un animal monogastrique, qui consiste à administrer à l'animal, une quantité efficace d'un composé selon la revendication 1 ou 2.

7. Prémélange d'aliments pour animaux comprenant un composé selon la revendication 1 ou 2, comme ingrédient actif.

8. Composition d'aliments pour volailles, comprenant un premier composant, à savoir un composé selon la revendication 1 ou 2 et un second composant choisi parmi le groupe comprenant :

- a) la nicarbazine;
- b) le 4,4'-dinitrocarbanilide;
- c) une naphthalénamine de formule :



dans laquelle :

- R² représente un groupe alkyle en C₁-C₄;
- R³ représente un atome d'halogène, un groupe fluoroalkyle en C₁-C₄, un groupe fluoroalcoxy en C₁-C₄ ou un groupe fluoroalkyl(en C₁-C₄)thio;



- R^4 représente un atome d'halogène;
 R^5 représente un atome d'hydrogène ou un atome d'halogène;
 m est égal à 0, 1 ou 2; et
 n est égal à 0 ou 1;

avec cette réserve que, lorsqu'un substituant de R^4 existe, il se trouve à une position différente de la position 2;

d) une benzénamine choisie parmi la 2,4-dinitro-N-[4-(trifluorométhoxy)phényl]-6-(trifluorométhyl)-benzénamine; la 2,4-dinitro-N-[4-(1,1,2,2-tétrafluoréthoxy)phényl]-6-(trifluorométhyl)benzénamine ou la 2,4-dinitro-N-[4-(pentafluoréthoxy)phényl]-6-(trifluorométhyl)benzénamine;

e) du métichloropindol; ou

f) un sel pharmaceutiquement acceptable d'un composé (a)-(e);

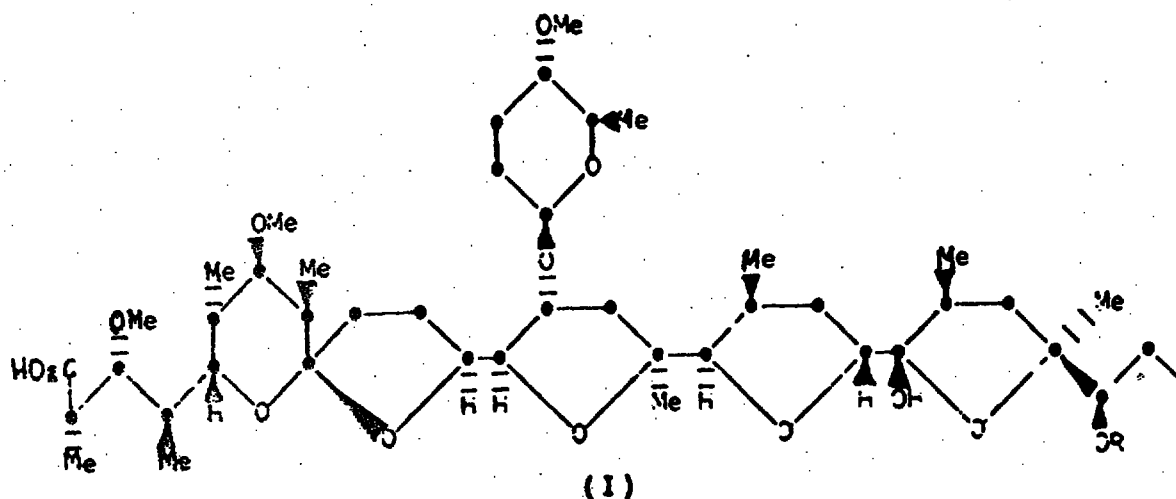
les composants étant présents dans l'aliment dans des quantités qui, en combinaison, ont un effet synergique vis-à-vis d'au moins une souche de *Eimeria* provoquant la coccidiose.

9. Composition selon la revendication 8, dans laquelle le second composant est la nicarbazine.

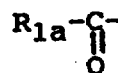
Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

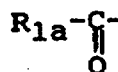
1. Verbindung der Formel (I):



worin R Wasserstoff oder -CONHR₁ ist und R₁ ein C₁-C₇-Alkyl-, Phenyl-, Pyridyl-, Furyl-, (C₁-C₇-Alkyl)-phenyl-, Phenyl-C₁-C₇-Alkyl-, Halogenphenyl-, Nitrophenyl-, Halogenphenyl-C₁-C₇-alkyl-, (C₁-C₇-Alkoxy)-phenyl-, Phenoxyphenylrest, ein gegebenenfalls mit einem Phenylrest substituierter Phenyl-C₃-C₇-cycloalkylrest, ein Acylphenylrest, worin die Acylgruppe ein Rest der Formel



ist, worin R_{1a} ein C₁-C₆-Alkylrest oder Wasserstoff ist, und ein C₃-C₇-Cycloalkylrest ist; oder ein Acylderivat davon, worin der Acylrest ein Rest der Formel

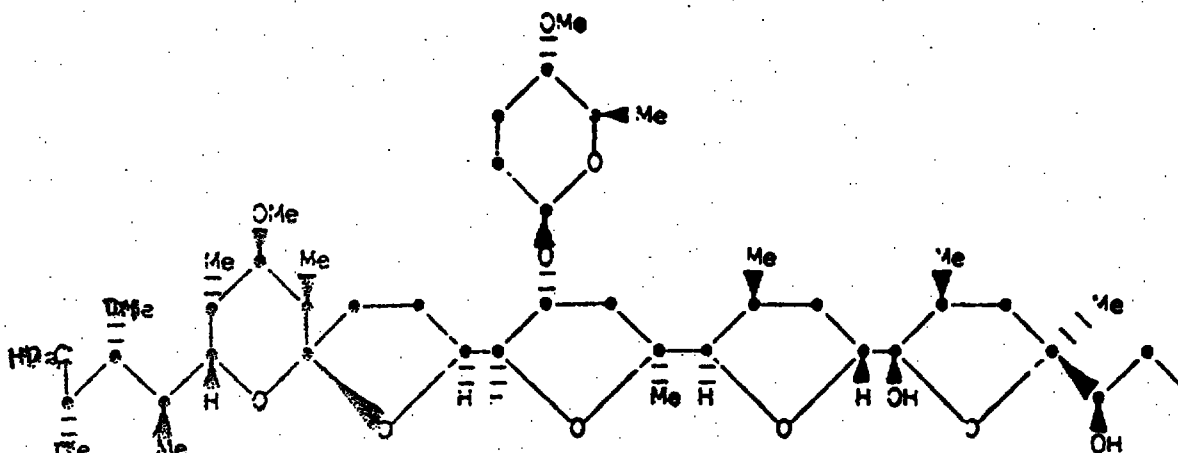


ist, worin R_{1a} ein C₁-C₆-Alkylrest oder Wasserstoff ist, und ein C₃-C₇-Cycloalkylrest ist, ein C₁-C₇-Alkylesterderivat davon oder



ein Alkyletherderivat davon, worin eine oder mehrere der Hydroxylgruppen ersetzt wurden durch eine YR"-Gruppe, worin Y O oder S bedeutet und R" einen C₁-C₆-Alkyl-, C₁-C₄-Alkoxy-C₂-C₅-alkyl-, C₁-C₄-Alkoxycarbonyl-C₂-C₅-alkyl-, Amino-C₂-C₅-alkyl-, Mercapto-C₂-C₅-alkyl-, Hydroxyalkyl-, Halogenalkylrest bedeutet oder (R')_m-Phenyl(CH₂)_n, worin R' einen C₁-C₄-Alkyl-, C₁-C₄-Alkoxy- oder Hydroxyrest bedeutet; m 0 bis 2 bedeutet und n 0 bis 3 bedeutet und worin sich der Ausdruck Hydroxyalkylrest entweder auf einen Monohydroxy-C₂-C₅-alkylrest oder, wenn Y O ist, auf einen 2,3-Dihydroxyprop-1-yl-rest bezieht und der Ausdruck Halogenalkylrest sich auf einen C₂-C₅-Alkylrest, der 1 bis 3 Halogensubstituenten ausgewählt aus der Gruppe bestehend aus Brom, Chlor und Fluor aufweist, bezieht, oder ein Salz davon.

2. Antibiotikum A80190 mit der Formel



oder ein pharmazeutisch annehmbares Salz davon.

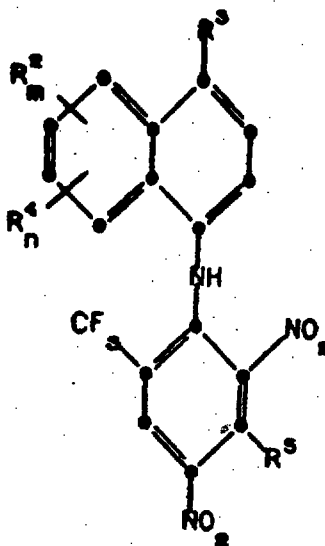
3. Verfahren zur Herstellung einer Verbindung nach Anspruch 1 oder 2, umfassend, daß man eine A80190 produzierende Art von Actinomadura oligospora in einem Kulturmedium, das assimilierbare Quellen für Kohlenstoff, Stickstoff und anorganische Salze enthält, unter aeroben Submersfermentationsbedingungen züchtet, bis das Antibiotikum A80190 produziert wird und dann A80190 aus dem Kulturmedium abtrennt und gegebenenfalls anschließend in geeigneter Weise acyliert, verestert oder mit einem Isocyanat der Formel RNCO umsetzt, worin R wie in Anspruch 1 definiert ist, unter Bildung eines Urethanderivats.
4. Verfahren nach Anspruch 3, worin oligospora NRRL 15877 oder NRRL 15878 oder eine A80190 produzierende Variante, Mutante oder Rekombinante davon verwendet wird.
5. Verbindung der Formel (I) oder ein pharmazeutisch annehmbares Salz davon zur Verwendung als gesundheitsförderndes Produkt im Veterinärbereich oder für Tiere.
6. Tierfutturvormischung, umfassend eine Verbindung nach Anspruch 1 oder 2 oder ein pharmazeutisch annehmbares Salz davon als aktiven Inhaltsstoff.
7. Verfahren zur Erhöhung der Futterverwertungseffizienz bei Wiederkäuern, umfassend, daß man dem Tier eine wirksame propionaterhöhende Menge einer Verbindung nach Anspruch 1 oder 2 oder ein pharmazeutisch annehmbares Salz davon oral verabreicht.
8. Verbindung der Formel (I) oder ein pharmazeutisch annehmbares Salz davon zur Verwendung zur Kontrolle der Coccidiose bei Geflügel.
9. Verfahren zur Förderung des Wachstums eines monogastrischen Tiers, umfassend, daß man dem Tier



eine wirksame Menge einer Verbindung nach Anspruch 1 oder 2 oder ein pharmazeutisch annehmbares Salz davon verabreicht.

10. Geflügelfutterzusammensetzung, umfassend eine erste Komponente, die eine Verbindung nach Anspruch 1 oder 2 oder ein pharmazeutisch annehmbares Salz davon ist, und eine zweite Komponente ausgewählt aus der Gruppe bestehend aus

- a) Nicarbazin,
- b) 4,4'-Dinitrocarbanilid,
- c) einer Naphthalinaminverbindung der Formel



worin

- R² ein C₁-C₄-Alkylrest ist;
- R³ Halogen, ein C₁-C₄-Fluoralkyl-, C₁-C₄-Fluoralkoxy- oder C₁-C₄-Fluoralkylthioest ist;
- R⁴ Halogen ist;
- R⁵ Wasserstoff oder Halogen ist;
- m 0, 1 oder 2 ist; und
- n 0 oder 1 ist;

mit dem Vorbehalt, daß dann, wenn ein R⁴-Substituent existiert, er an einer anderen als der 2-Position ist;

d) einem Benzolamin ausgewählt aus 2,4-Dinitro-N-[4-(trifluormethoxy)phenyl]-6-(trifluormethyl)benzolamin; 2,4-Dinitro-N-[4-(1,1,2,2-tetrafluorethoxy)phenyl]-6-(trifluormethyl)benzolamin oder 2,4-Dinitro-N-[4(pentafluorethoxy)phenyl]-6-(trifluormethyl)benzolamin;

e) Metichlorpindol; oder

f) einem pharmazeutisch annehmbaren Salz einer der Verbindungen (a) bis (e);

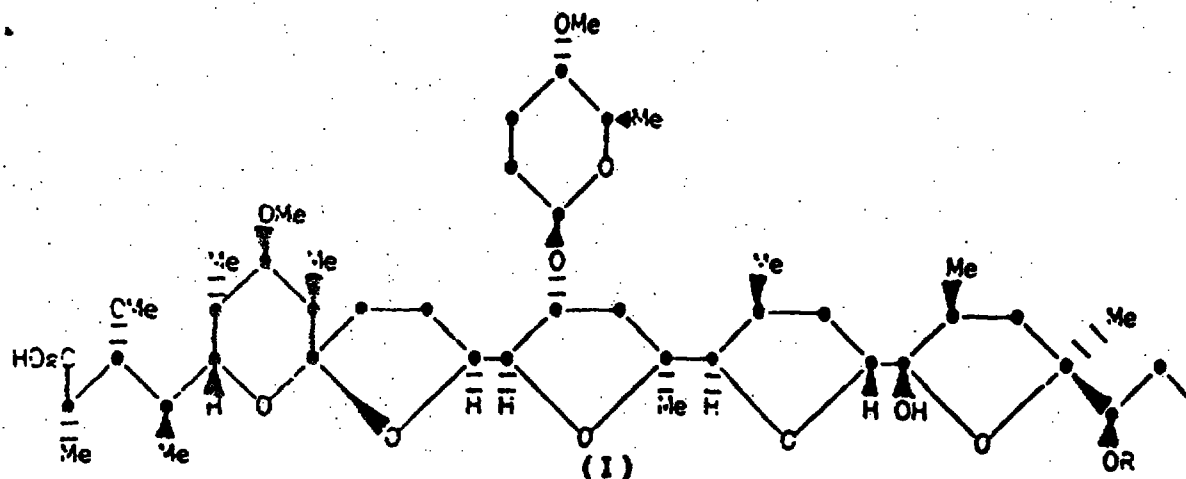
wobei die Komponenten in dem Futter in solchen Mengen vorhanden sind, die in Kombination synergistisch sind gegenüber mindestens einem Coccidiose verursachenden Stamm von Eimeria.

11. Zusammensetzung nach Anspruch 10, worin die zweite Komponente Nicarbazin ist.

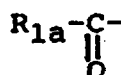
12. Actinomadura oligosporus NRRL 15878.

Patentansprüche für folgenden Vertragsstaat : AT

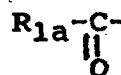
1. Verfahren zur Herstellung einer Verbindung der Formel (I):



worin R Wasserstoff oder -CONHR₁ ist und R₁ ein C₁-C₇-Alkyl-, Phenyl-, Pyridyl-, Furyl-, (C₁-C₇-Alkyl)-phenyl-, Phenyl-C₁-C₇-Alkyl-, Halogenphenyl-, Nitrophenyl-, Halogenphenyl-C₁-C₇-alkyl-, (C₁-C₇-Alkoxy)-phenyl-, Phenoxyphenylrest, ein gegebenenfalls mit einem Phenylrest substituierter Phenyl-C₃-C₇-cycloalkylrest, ein Acylphenylrest, worin die Acylgruppe ein Rest der Formel



ist, worin R_{1a} ein C₁-C₆-Alkylrest oder Wasserstoff ist, und ein C₃-C₇-Cycloalkylrest ist; oder ein Acylderivat davon, worin der Acylrest ein Rest der Formel

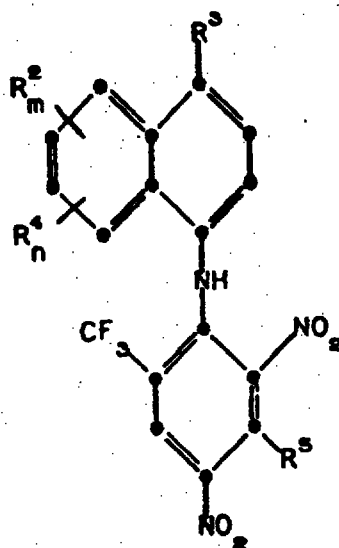


ist, worin R_{1a} ein C₁-C₆-Alkylrest oder Wasserstoff ist, und ein C₃-C₇-Cycloalkylrest ist, ein C₁-C₇-Alkylesterderivat davon oder ein Alkyletherderivat davon, worin eine oder mehrere der Hydroxylgruppen ersetzt wurden durch eine YR''-Gruppe, worin Y O oder S bedeutet und R'' einen C₁-C₆-Alkyl-, C₁-C₄-Alkoxy-C₂-C₅-alkyl-, C₁-C₄-Alkoxy-carbonyl-C₂-C₅-alkyl-, Amino-C₂-C₅-alkyl-, Mercapto-C₂-C₅-alkyl-, Hydroxyalkyl-, Halogenalkylrest bedeutet oder (R')_m-Phenyl(CH₂)_n-, worin R' einen C₁-C₄-Alkyl-, C₁-C₄-Alkoxy- oder Hydroxyrest bedeutet; m 0 bis 2 bedeutet und n 0 bis 3 bedeutet und worin sich der Ausdruck Hydroxyalkylrest entweder auf einen Monohydroxy-C₂-C₅-alkylrest oder, wenn Y O ist, auf einen 2,3-Dihydroxyprop-1-yl-rest bezieht und der Ausdruck Halogenalkylrest sich auf einen C₂-C₅-Alkylrest, der 1 bis 3 Halogensubstituenten ausgewählt aus der Gruppe bestehend aus Brom, Chlor und Fluor aufweist, bezieht, oder eines Salzes davon, umfassend, daß man eine A80190 produzierende Art von Actinomyces oligospora in einem Kulturmedium, das assimilierbare Quellen für Kohlenstoff, Stickstoff und anorganische Salze enthält, unter aeroben Submersfermentationsbedingungen züchtet, bis das Antibiotikum A80190 produziert wird und dann A80190 aus dem Kulturmedium abtrennt und gegebenenfalls anschließend in geeigneter Weise acyliert, verestert oder mit einem Isocyanat der Formel RNCO umgesetzt, worin R wie in Anspruch 1 definiert ist, unter Bildung eines Urethanderivats.

2. Verfahren nach Anspruch 1, worin A. oligospora NRRL 15877 oder NRRL 15878 oder eine A80190 produzierende Variante, Mutante oder Rekombinante davon verwendet wird.
3. Verfahren nach Anspruch 1 oder 2 zur Herstellung einer Verbindung der Formel (I), worin R Wasserstoff ist, oder eines pharmazeutisch annehmbaren Salzes davon.



4. Verfahren zur Erhöhung der Futterverwertungseffizienz bei Wiederkäuern, umfassend, daß man dem Tier eine wirksame propionaterhöhende Menge einer Verbindung nach Anspruch 1 oder 2 oral verabreicht.
5. Verwendung einer Verbindung nach Anspruch 1 oder 2 zur Kontrolle von Coccidiose bei Geflügel.
6. Verfahren zur Förderung des Wachstums eines monogastrischen Tiers, umfassend, daß man dem Tier eine wirksame Menge einer Verbindung nach Anspruch 1 oder 2 verabreicht.
7. Tierfuttermischung, umfassend eine Verbindung nach Anspruch 1 oder 2 als aktiven Inhaltsstoff.
8. Geflügelfutterzusammensetzung, umfassend eine erste Komponente, die eine Verbindung nach Anspruch 1 oder 2 ist, und eine zweite Komponente ausgewählt aus der Gruppe bestehend aus
 - a) Nicarbazin,
 - b) 4,4'-Dinitrocarbanilid,
 - c) einer Naphthalinaminverbindung der Formel:



worin

- R^2 ein C_1 - C_4 -Alkylrest ist;
 R^3 Halogen, ein C_1 - C_4 -Fluoralkyl-, C_1 - C_4 -Fluoralkoxy- oder C_1 - C_4 -Fluoralkylthioest ist;
 R^4 Halogen ist;
 R^5 Wasserstoff oder Halogen ist;
 m 0, 1 oder 2 ist; und
 n 0 oder 1 ist;

mit dem Vorbehalt, daß dann, wenn ein R^4 -Substituent existiert, er an einer anderen als der 2-Position ist;

d) einem Benzolamin ausgewählt aus 2,4-Dinitro-N-[4-(trifluormethoxy)phenyl]-6-(trifluormethyl)-benzolamin; 2,4-Dinitro-N-[4-(1,1,2,2-tetrafluorethoxy)phenyl]-6-(trifluormethyl)benzolamin oder 2,4-Dinitro-N-[4-(pentafluorethoxy)phenyl]-6-(trifluormethyl)benzolamin;

e) Metichlorpindol; oder

f) einem pharmazeutisch annehmbaren Salz einer der Verbindungen (a) bis (e);

wobei die Komponenten im dem Futter in solchen Mengen vorhanden sind, die in Kombination synergistisch sind gegenüber mindestens einem Coccidiose verursachenden Stamm von Eimeria.

9. Zusammensetzung nach Anspruch 8, worin die zweite Komponente Nicarbazin ist.