

PATENT SPECIFICATION

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- (72) Inventors GEORG ALBERS-SCHONBERG
 RICHARD WILLIAM BURG
 THOMAS WILLIAM MILLER
 ROBERT EUGENE ORMOND and
 HYMAN WALLICK

(54) CYCLIC ETHERS

(71) We, MERCK & CO. INC., a corporation duly organized and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention is concerned with cyclic ethers. The compounds of the present invention, which are collectively identified as C-076, are produced in accordance with the invention by the aerobic fermentation of an aqueous nutrient medium with a strain of the microorganism *Streptomyces avermitilis*, viz. NRRL 8165, or a mutant thereof, and recovering the C-076. The nutrient medium contains assimilable sources of carbon and nitrogen, as well as inorganic salts.

It has been found that compounds in accordance with the present invention have significant and broad spectrum antiparasitic activity. At least eight distinct but closely related novel compounds are produced by *Streptomyces avermitilis*. They are described herein as C-076 A1a, A1b, A2a, A2b, B1a, B1b, B2a and B2b. All have significant antiparasitic activity.

The microorganisms capable of producing C-076 compounds are of a new species of the genus *Streptomyces*, which has been named *Streptomyces avermitilis*. One such culture, isolated from soil, is designated MA-4680 in the culture collection of Merck & Co., Inc., Rahway, New Jersey. A C-076-producing sample of this culture has been deposited in the permanent culture collection of the Fermentation Section of the Northern Utilization Research Branch, U.S. Department of Agriculture at Peoria, Illinois, and has been assigned the accession number NRRL 8165. A sample of NRRL 8165 has also been deposited, without restriction as to availability, in the permanent culture collection of the American Type Culture Collection at 12301 Parklawn Drive, Rockville, Maryland 20852, and has been assigned the accession number ATCC 31,267.

The morphological and cultural characteristics of *Streptomyces avermitilis* are set forth below:

Morphology: Sporophores form spirals as side branches on aerial mycelia. Spirals are compact but become more open as culture ages. Spores are in chains of more than 15 spores and are usually spherical to oval at 970x magnification. Sporulation is observed on oatmeal agar, glycerol-asparagine agar, salts-starch agar and egg albumin agar. Spore surface is smooth as seen by electron microscopy.

Oatmeal agar

Vegetative growth: Reverse—very dark brown
 Aerial mycelium: Powdery, brownish gray (4li)* mixed with white
 Soluble pigment: Brown.

Czapek Dox agar (sucrose nitrate agar)

Vegetative growth: Poor, colorless
 Aerial mycelium: Scant, grayish
 Soluble pigment: Light grayish tan.

	Egg albumin agar	
	Vegetative growth: Tan	
	Aerial mycelium: Moderate, light grayish-yellow-brown (3ge)* mixed with white	
5	Soluble pigment: Light yellowish tan.	5
	Glycerol asparagine agar	
	Vegetative growth: Reverse—yellowish brown	
	Aerial mycelium: Powdery, brownish gray (4li)* mixed with white	
	Soluble pigment: Light, yellowish brown.	
10	Inorganic salts-starch agar	10
	Vegetative growth: Reverse—grayish yellowish brown	
	Aerial mycelium: Powdery, light brownish gray (4ig)* edged with darker brownish gray (4li)*	
	Soluble pigment: Light yellowish brown.	
15	Yeast extract-dextrose+salts agar	15
	Vegetative growth: Reverse—dark brown	
	Aerial mycelium: Moderate, brownish white	
	Soluble pigment: Brown.	
20	Yeast extract-malt extract agar	20
	Vegetative growth: Reverse—dark brown	
	Aerial mycelium: Moderate, brownish white	
	Soluble pigment: Brown.	
25	Peptone-iron-yeast extract agar	25
	Vegetative growth: Dark brown	
	Aerial mycelium: None	
	Soluble pigment: Dark brown to black	
	Melanin: Positive	
	HS production: Positive.	
30	Nutrient agar	30
	Vegetative growth: Tan	
	Aerial mycelium: Sparse, grayish	
	Soluble pigment: Light brown.	
35	Nutrient starch agar	35
	Vegetative growth: Tan	
	Aerial mycelium: Sparse, grayish white	
	Soluble pigment: Light brown	
	Hydrolysis of starch: Good.	
40	Potato plug	40
	Vegetative growth: Tan	
	Aerial mycelium: Brown mixed with grayish white	
	Soluble pigment: Grayish brown.	
45	Loeffler's Blood serum	45
	Vegetative growth: Grayish tan	
	Aerial mycelium: None	
	Soluble pigment: Some browning of medium	
	Liquefaction: None.	
50	Nutrient tyrosine agar	50
	Vegetative growth: Reverse—dark brown to black	
	Aerial mycelium: Sparse, grayish	
	Soluble pigment: Dark brown	
	Decomposition of tyrosine: None.	
	Carbon utilization	
	Pridham-Gottlieb basal medium + 1% carbon source; +=growth; no growth as compared to negative control (no carbon source)	

3.	1,573,955	3.
	Glucose +	
	Arabinose +	
	Cellulose -	
	Fructose +	
5	Inositol +	5
	Lactose +	
	Maltose +	
	Mannitol +	
	Mannose +	
10	Raffinose +	10
	Rhamnose +	
10	Sucrose +	
	Xylose +	
	Nutrient gelatin agar	
15	Vegetative growth: Tan	15
	Aerial mycelium: Sparse, grayish white	
15	Soluble pigment: Light brown	
	Liquefaction of gelatin: Good.	
	Gelatin stabs	
20	Vegetative growth: Brown ring	20
	Aerial mycelium: None	
20	Soluble pigment: Greenish brown	
	Liquefaction of gelatin: Complete	
	Skim milk agar	
25	Vegetative growth: Dark brown	25
	Aerial mycelium: None	
25	Soluble pigment: Dark brown	
	Hydrolysis of casein: Good.	
	Litmus milk	
30	Vegetative growth: Dark brown growth ring	30
	Aerial mycelium: None	
30	Color: Dark brown	
	Coagulation and/or peptonization: Complete peptonization; becoming alkaline (pH 8.1).	
35	Skim milk	35
	Vegetative growth: Dark brown growth ring	
35	Aerial mycelium: None	
	Soluble pigment: Dark brown	
	Coagulation and/or peptonization: Complete peptonization; becoming alkaline (pH 8.0).	
40	Temperature range: (Yeast extract-dextrose+salts agar)	
40	28°C—Good vegetative growth and aerial mycelia	
	37°C—Good vegetative growth and aerial mycelia	
	50°C—No growth.	
45	Oxygen requirement: (Stab culture in yeast extract-dextrose+salts agar)	45
	Aerobic.	
45	All readings taken after three weeks at 28°C unless noted otherwise. pH of all media approximately neutral (6.8—7.2).	
	Color number designations (*) taken from Color Harmony Manual, 1958, 4th Edition Container Corporation of America, Chicago, Illinois.	
50		50
50	A careful comparison of the foregoing data with published descriptions including Bergey's Manual of Determinative Bacteriology (Eighth Edition) of known microorganisms reveals significant differences that indicate that the microorganism should be classified as a new species. On this basis, it was designated <i>Streptomyces avermitilis</i> .	
55		55

The above description is illustrative of a strain of *Streptomyces avermitilis* that can be used in the production of the C-076 compounds described herein. However, the present invention can also use mutants of the above described microorganism, for example, those C-076-producing mutants obtained by natural selection or by mutating agents including X-ray irradiation, ultraviolet irradiation and nitrogen mustard.

One example of such an organism is a strain of *Streptomyces avermitilis* MA 4848 which was isolated after irradiation with ultraviolet light of *Streptomyces avermitilis* MA 4680. A lyophilized tube and a frozen vial of this culture has been deposited in the permanent culture collection of the American Type Culture Collection, and they have been assigned the accession numbers 31272 and 31271 respectively. Slightly higher fermentation yields of C-076 have been obtained using this frozen stock as inoculum.

In the specific description and Examples, it should be noted that the following words are trade marks: 'Cerelease', 'Varian', 'Solketal', 'Super-cel', 'Porasil' and 'Sephadex'.

Aqueous media such as those used for the production of many antibiotic substances are suitable for use in the process of the present invention for the preparation of C-076. Such nutrient media contain sources of carbon and nitrogen assimilable by the microorganism and generally low levels of inorganic salts. In addition, the fermentation media may contain traces of metals necessary for the growth of the microorganisms. These are usually present in sufficient concentration in the complex sources of carbon and nitrogen which may be used as nutrient sources, but can, of course, be added separately to the medium if desired.

In general, carbohydrates such as sugars, for example dextrose, sucrose, maltose, lactose, dextran and Cerelease, and starches are suitable sources of assimilable carbon in the nutrient media. The exact quantity of the carbon source which is utilized in the medium will depend, in part, upon the other ingredients in the medium but it is usually found that an amount of carbon source such as carbohydrate of from 0.5 to 5% by weight of the medium is satisfactory. These carbon sources can be used individually or several such carbon sources may be combined in the same medium.

Various nitrogen sources, such as yeast hydrolysates, yeast autolysate, soybean meal, casein hydrolysates, yeast extracts, corn steep liquors, distillers solubles, cottonseed meal and meat extract, are readily assimilable by *Streptomyces avermitilis* in the production of the C-076 compounds. The various sources of nitrogen can be used alone or in combination in amounts ranging from 0.2 to 6% by weight of the medium.

Among the nutrient inorganic salts that can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, magnesium, ammonium, calcium, phosphate, sulfate, chloride, and carbonate ions. Also included are trace amounts of metals such as cobalt, manganese and iron.

It should be noted that the media described hereinbelow and in the Examples are merely illustrative of the wide variety of suitable media and are not intended to be limitative.

The following are Examples of media suitable for growing strains of *Streptomyces avermitilis* for producing the C-076 compounds.

Medium A

Corn meal	20.0 g
Distiller's solubles	10.0 g
Soybean meal	15.0 g
Sodium citrate	4.0 g
CaCl ₂ ·2H ₂ O	0.5 g
Polyglycol P2000	2.5 ml
MgSO ₄ ·7H ₂ O	0.1 g
CoCl ₂ ·6H ₂ O	0.01 g
FeSO ₄ ·7H ₂ O	0.01 g
Distilled water	1000 ml
pH 6.5	

Medium B

Soluble starch	20.0 g
Corn steep liquor	15.0 g
Cerelease	5.0 g

Ingredient	Amount	Notes
Soybean meal	4.0 g	
(NH ₄) ₂ SO ₄	1.0 g	
Corn meal	2.5 ml	
Soybean oil	0.3 g	
KH ₂ PO ₄	6.0 g	
CaCO ₃	1000 ml	
Distilled water		
pH 6.7		
Medium C		
Tomato paste	40.0 g	
Oat flour	15.0 g	
Distilled water	1000 ml	
pH 6.0		
Medium D		
Oat flour	20.0 g	
Tomato paste	20.0 g	
Distilled water	1000 ml	
pH 5.5		
Medium E		
Dextrose	10.0 g	
Peptone (Available from Difco Laboratories, Detroit, Michigan)	5.0 g	
Yeast autolysate (Available as Ardamine pH from Yeast Products Inc., Paterson, New Jersey)	3.0 g	
NaCl	12.7 g	
KCl	0.72 g	
FeSO ₄ (NH ₄) ₂ SO ₄ · 6H ₂ O	0.035 g	
MgCl ₂ · 6H ₂ O	5.32 g	
CaCl ₂ · 2H ₂ O	0.73 g	
Distilled water	1000 ml	
pH 7.4		

The fermentation with the C-076-producing microorganisms can be conducted at temperatures ranging from 20° to 40°C. For optimum results, it is most convenient to conduct these fermentations at a temperature in the range of from 24° to 30°C, preferably 27—28°C. The pH of the nutrient medium suitable for producing the C-076 compounds can vary from 5.0 to 9.0 with a preferred range of from 6.0 to 7.5.

Small-scale fermentations are conveniently carried out by placing suitable quantities of nutrient medium in a flask using known sterile techniques, inoculating the flask with either spores or vegetative cellular growth of a C-076 producing strain of *Streptomyces avermitilis*, loosely stoppering the necks of the flask with cotton, and permitting the fermentation to proceed in a constant room temperature of about 28°C on a rotary shaker for from 3 to 10 days. For larger-scale work, it is preferable to conduct the fermentation in suitable tanks provided with an agitator and a means of aerating the fermentation medium. The nutrient medium is made up in the tank and after sterilization is inoculated with a suitable source of vegetative cellular growth of a C-076-producing strain of *Streptomyces avermitilis*. The fermentation is allowed to continue for from 1 to 8 days while agitating and/or aerating the nutrient medium at a temperature in the range of from 24 to 37°C. The degree of aeration is dependent upon several factors such as the size of the fermentor and agitation speed. Generally, the larger scale fermentation media are agitated at 95 to 150 RPM, with introduction of 2 to 20 cubic feet per minute of air.

The novel substances of this invention, which are generically referred to herein as C-076, are found primarily in the mycelium on termination of the *Streptomyces avermitilis* fermentation, and may be recovered and separated from one another as described below. Four major and four minor components of the C-076 as elaborated by *Streptomyces avermitilis* have been isolated. The eight different compounds are identified herein as C-076 A1a, A1b, A2a, A2b, B1a, B1b, B2a and B2b. The major components have been given the suffix "a" in our identification terminology and the minor components the suffix "b". The structural difference

between the "a" and "b" compounds is believed to be the same for each of the four pairs.

As might be expected, even the major C-076 compounds are not produced in equal amounts by the fermentations described herein. In general, it has been found that the A1 compounds comprise 20 to 30% by weight of the total C-076 complex produced, the A2 compounds 1—20% and the B1 and B2 compounds each 25—35%. The weight ratio of the "a" series of compounds to the "b" series is from 85:15 to 99:1.

The separation of the C-076 series of compounds from the whole fermentation broth and the recovery of the individual components is carried out by solvent extraction and application of chromatographic fractionations with various chromatographic techniques and solvent systems.

The C-076 compounds have slight solubility in water, but are soluble in organic solvents. This property may be conveniently used to recover them from the fermentation broth. Thus in one recovery method, the whole fermentation broth is filtered and the aqueous filtrate discarded. The wet mycelial cake is then extracted with an appropriate organic solvent. While any organic solvent may be used, it is preferable to use a water-miscible solvent such as acetone, methanol or ethanol. Generally, several extractions are desirable to achieve maximum recovery. The solvent removes the C-076 active components as well as other substances lacking the antiparasitic activity of C-076. If the solvent is water-miscible, the water is also removed from the wet mycelia. The extracted mycelia may be discarded. The solvent extracts are evaporated to remove the organic solvent and extracted several times with a second solvent. When the first extraction involves a water-miscible solvent, the second extraction preferably involves a water-immiscible organic solvent such as chloroform, methylene chloride, carbon tetrachloride, ethyl acetate, methylethyl ketone or methylisobutyl ketone. These latter extracts are dried and concentrated using known techniques to afford a residue comprising C-076 admixed with other materials. This fraction is then conveniently chromatographed in order to separate the active C-076 compounds from other material and also to separate and isolate the individual C-076 compounds. The chromatographic techniques for purifying the C-076 compounds are generally known. Examples of such techniques are column chromatography, using such media as silica gel, aluminium oxide and dextran gels, and elution of such columns with various solvents, and/or a combination of two or more solvents, in varying ratios. Liquid chromatography is used for the detection of the C-076 compounds, and high pressure liquid chromatography may be used to isolate purified fractions containing one or more of such compounds. Likewise, thin-layer chromatography may be used to detect the presence of and to isolate the individual C-076 compounds. The use of the foregoing techniques, as well as others known to those skilled in this art, will afford purified compositions comprising the C-076 compounds as well as the individual C-076 compounds themselves. The presence of the active C-076 compounds is determined by analysing the various chromatographic fractions for antiparasitic activity and also by the spectral characteristics (such as ultraviolet and infrared) of the said compounds as described below.

The spectral and other physical-chemical characteristics of the individual C-076 compounds are set forth in tabular form in Table I. These compounds are very soluble in most common organic solvents, and are of minimal solubility in water.

The ultraviolet spectral data in Table I were obtained on a Cary Model 15 Ultraviolet Spectrometer in methanolic solutions in 1 cm quartz cells. The concentration of the compound was approximately 25 µg/ml. The ultraviolet absorption, while represented as that of a particular "a series" compound, is actually the absorption of the "a series" compound containing a minor amount of a "b series" compound. The "a" and "b" series differ only by a —CH₂— residue in a lower alkyl substituent, which difference is not associated with the chromophore. Ultraviolet absorption primarily characterizes the degree and nature of unsaturation present in the particular compound. The optical rotations were determined using standard techniques with a Karl Zeiss polarimeter. The concentration factor (c) is given as a percent of the compound in the stated solvent.

TABLE I

Molecular Formula	<i>Ala</i> $C_{49}H_{74}O_{14}$	<i>Alb</i> $C_{48}H_{72}O_{14}$	<i>A2a</i> $C_{49}H_{76}O_{15}$	<i>A2b</i> $C_{48}H_{74}O_{15}$	<i>B1a</i> $C_{48}H_{72}O_{14}$	<i>B1b</i> $C_{47}H_{70}O_{14}$	<i>B2a</i> $C_{48}H_{74}O_{15}$	<i>B2b</i> $C_{47}H_{72}O_{15}$
Optical Rotation $[\alpha]_D^{25}(CHCl_3)$	$+68.5^{\circ} \pm 2^{\circ}$ ($C=0.77$)		$+48.8^{\circ} \pm 2^{\circ}$ ($C=1.64$)		$+55.7^{\circ} \pm 2^{\circ}$ ($C=1.06$)		$+38.3^{\circ} \pm 2^{\circ}$ ($C=0.87$)	
Molecular Weight (Determined by mass spectrometry)	886	872	904	890	872	858	890	876
Ultraviolet Absorption Spectrum	237 (28,700; 4,458)	237 (28,800; 4,459)	237 (28,800; 4,459)	237 (28,800; 4,459)	237 (29,120; 4,464)	237 (29,120; 4,464)	237 (27,580; 4,441)	237 (27,580; 4,441)
λ -max (m μ)	243 (31,275; 4,495)	243 (31,740; 4,501)	243 (31,740; 4,501)	243 (31,740; 4,501)	243 (31,850; 4,503)	243 (31,850; 4,503)	243 (30,590; 4,486)	243 (30,590; 4,486)
(ϵ ; log ϵ)	252 (20,290; 4,307)	252 (20,425; 4,310)	252 (20,425; 4,310)	252 (20,425; 4,310)	252 (20,510; 4,431)	252 (20,510; 4,431)	252 (20,060; 4,302)	252 (20,060; 4,302)

¹³C nuclear magnetic resonance spectral data for C-076 *A1a*, *A2a*, *B1a* and *B2a* are set forth in Table II below. The spectra are obtained from a Varian Nuclear Magnetic Spectrometer Model CFT-20 in deuterated chloroform solution using tetramethylsilane as an internal standard. The solution volume and the concentration of sample are given for each case, followed by the chemical shifts relative to tetramethyl silane for each compound given in parts per million (ppm). The chemical shifts are given for a single carbon atom unless otherwise indicated in a parenthetical expression following the chemical shift.

TABLE II

TABLE II

10	A1 (0.6 ml 16%)	10	
	12.0, 13.0, 15.1, 16.4, 17.7, 18.4, 19.9, 20.3, 27.5, 30.6, 34.3 (3C), 35.2, 36.6, 39.7, 40.5, 45.7, 56.4 (2C), 57.7, 67.3, 68.2 (2C), 68.4 (2C), 74.9, 76.1, 77.0, 77.5, 78.3, 79.4, 80.6 (2C), 82.0, 95.0, 95.8, 98.5, 118.4 (2C), 119.7, 124.9, 127.8, 135.2, 136.0, 136.1, 137.6, 139.9, 173.8.		
15	A2 (0.6 ml 16%)	15	
	11.8, 12.4, 13.8, 15.1, 17.7, 18.4, 19.9, 20.3, 27.3, 34.3 (3C), 35.2, 35.8, 36.5, 39.8, 40.8, 41.2, 45.7, 56.4 (2C), 57.7, 67.3, 67.7, 68.2 (3C), 69.9, 70.8, 76.1, 77.0, 77.6, 78.3, 79.4, 80.6, (2C), 81.8, 94.9, 98.6, 99.7, 117.7, 118.4, 119.7, 124.9, 135.7, 136.1, 137.6, 140.0, 173.7.		
20	B1 (0.3 ml 16%)	20	
	12.0, 12.9, 15.1, 16.4, 17.7, 18.4, 19.9, 20.2, 27.5, 30.6, 34.3, (3C), 35.2, 36.6, 39.8, 40.5, 45.7, 56.4 (2C), 67.3, 67.8, 68.2 (2C), 68.4 (2C), 75.0, 76.1, 78.3, 79.4 (2C), 80.5 (2C), 82.0, 95.0, 95.8, 98.5, 118.1, 118.4, 120.4, 124.8, 127.9, 135.2, 136.2, 137.9 (2C), 139.7, 173.6.		
25	B2 (0.6 ml 16%)	25	
	11.8, 12.4, 13.8, 15.1, 17.7, 18.4, 19.9, 20.2, 27.3, 34.4 (3C), 35.2, 35.8, 36.5, 39.8, 40.8, 41.2, 45.8, 56.4 (2C) 67.3, 67.7 (2C), 68.3 (3C), 69.9, 70.9, 76.1, 78.3, 79.4 (2C), 80.5(2C), 81.8, 94.9, 98.6, 99.7, 117.7, 118.0, 120.4, 124.8, 135.7, 138.0 (2C), 139.8, 173.5.		
30	The characteristic mass spectral peaks of eight C-076 compounds are given in Table III. The first line of Table III represents the mass-to-charge ratio (m/e) of the molecular ion of each respective compound, and the remaining numbers record the mass to charge ratio of the principal fragments of each compound. Mass-to-charge ratios found on the same horizontal row indicate analogous fragments in each compound. The mass-to-charge ratios given as whole numbers were obtained from an LKB Mass Spectrometer Model LKB-9000. The mass-to-charge ratios given to the fourth decimal place were obtained from a High Resolution Varian Mass Spectrometer Model MAT-731.		30
35			35

TABLE III

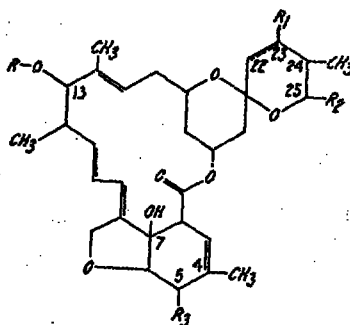
40	Characteristic Mass Spectral Peaks								40
	<i>A1a</i>	<i>A1b</i>	<i>A2a</i>	<i>A2b</i>	<i>B1a</i>	<i>B1b</i>	<i>B2a</i>	<i>B2b</i>	
	886.5072	872	904	890	872	858	890	876	
	742.4229	728	760	746	728	714	746	732	
	580.3406	566.3265	598	584	566	552	584	570	
45	548.3136	534	566	552	548.3131	534	566	552	45
	456.2886	442	456	442	456	442	456	442	
	305.2120	291.1962	323.2225	309.2070	305	291	323	309	
			305	291			305	291	
	275.1292	275	275	275	261.1139	261	261	261	
50	257.1382	257	257	257	257	257	257	257	50
	221.1527	207	239.1637	225	221	207	239	225	
	193.1587	179.1429	211.1691	197.1542	193	179	211	197	
	199.1101	199	199	199	199	199	199	199	
	169.1226	155	179.1076	179	169	155	179	179	
55	145.0867	145	145	145	145	145	145	145	55

TABLE III (contd.)
Characteristic Mass Spectral Peaks

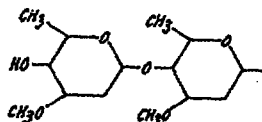
<i>A1a</i>	<i>A1b</i>	<i>A2a</i>	<i>A2b</i>	<i>B1a</i>	<i>B1b</i>	<i>B2a</i>	<i>B2b</i>
137.0954	137	137	137	137	137	137	137
127.0754	127	127	127	127	127	127	127
113.0604	113	113	113	113	113	113	113
95.0496	95	95	95	95	95	95	95
87.0444	87	87	87	87	87	87	87

The attached Figures 1 to 8 are accurate reproductions of infrared and proton-nuclear magnetic resonance spectra of four of the C-076 compounds. Figures 1—4 are infrared spectra for C-076 *A1a*, *A2a*, *B1a* and *B2a* respectively. Figures 5 to 8 are proton-nuclear magnetic spectra for C-076 *A1a*, *A2a*, *B1a* and *B2a* respectively. The infrared spectra were obtained on a Perkin-Elmer Infrared Spectrometer Model 421 in chloroform solution. The proton-nuclear magnetic resonance spectra were obtained in deuterated chloroform solution in a Varian Nuclear Magnetic Resonance Spectrometer Model Ha-100 and the spectra show chemical shifts given in parts per million (ppm) relative to tetramethyl silane as an internal standard.

Experimental data, including the studies and measurements described herein, indicate that the C-076 compounds have the following planar structural formula:



where R is the α - L - oleandrosyl - α - L - oleandroside of the structure:



and the broken line indicates that the 22(23) linkage is a single or a double bond; R_1 is hydroxy and is present only when the broken line indicates a single bond.

R_2 is iso-propyl or sec-butyl; and

R_3 is methoxy or hydroxy.

In the foregoing structural formula, the individual compounds are as set forth in Table IV.

TABLE IV

	R_1	R_2	R_3
<i>A1a</i>	Double bond	sec-butyl	—OCH ₃
<i>A1b</i>	Double bond	iso-propyl	—OCH ₃
<i>A2a</i>	—OH	sec-butyl	—OCH ₃
<i>A2b</i>	—OH	iso-propyl	—OCH ₃
<i>B1a</i>	Double bond	sec-butyl	—OH
<i>B1b</i>	Double bond	iso-propyl	—OH
<i>B2a</i>	—OH	sec-butyl	—OH
<i>B2b</i>	—OH	iso-propyl	—OH

As might be expected, the compounds of this invention in which R_2 is sec-butyl (the "a series" of compounds) and the corresponding compound wherein R_2 is iso-propyl (the "b series"), act similarly in most recovery procedures such as those involving solvent extraction. In each pair of "a" and "b" compounds the "a" compound is found in greater amount and generally makes up about 85 to 99% of a mixture of the "a" and "b" compounds. The presence of the iso-propyl

compound is verified in the mass spectra of the compounds, in which the mass peaks representing the fragments containing the sec-butyl group have companion peaks with a mass 14 units (or one $-\text{CH}_2$ group) lower. In addition, high pressure liquid chromatography has been used to separate the *A1b* component from the *A1a* component, and the mass spectrum of the *A1b* component has been verified by high resolution mass spectrometry (see Table III).

The novel compounds of this invention have significant parasitocidal activity as anthelmintics, insecticides and acaricides, in human and animal health and in agriculture.

The disease or group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes widespread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Nematodirus*, *Cooperia*, *Ascaris*, *Bunostomum*, *Oesophagostomum*, *Chabertia*, *Trichuris*, *Strongylus*, *Trichonema*, *Dictyocaulus*, *Capillaria*, *Heterakis*, *Toxocara*, *Ascaridia*, *Oxyuris*, *Ancylostoma*, *Uncinaria*, *Toxascaris* and *Parascaris*. Certain of these, such as *Nematodirus*, *Cooperia*, and *Oesophagostomum* attack primarily the intestinal tract while others, such as *Haemonchus* and *Ostertagia*, are more prevalent in the stomach while still others such as *Dictyocaulus* are found in the lungs. Still other parasites may be located in other tissues and organs of the body such as the heart and blood vessels and subcutaneous and lymphatic tissue. The parasitic infections known as helminthiasis lead to anemia, malnutrition, weakness, weight loss and severe damage to the walls of the intestinal tract and other tissues and organs and, if left untreated, may result in death of the infected host. The C-076 compounds of this invention have unexpectedly high activity against these parasites, and in addition are also active against *Dirofilaria* in dogs, *Nematospiroides*, *Syphacia* and *Aspiculuris* in rodents, and arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas and blowfly, in sheep *Lucilia sp.*, biting insects and such migrating dipterous larvae as *Hypoderma sp.* in cattle, *Gastrophilus* in horses, and *Cuterebra sp.* in rodents.

The compounds of the present invention are also useful against parasites that infect humans. The most common genera of parasites of the gastro-intestinal tract of man are *Ancylostoma*, *Necator*, *Ascaris*, *Strongyloides*, *Trichinella*, *Capillaria*, *Trichuris*, and *Enterobius*. Other medically important genera of parasites which are found in the blood or other tissues and organs outside the gastro-intestinal tract are the filarial worms such as *Wuchereria*, *Brugia*, *Onchocerca* and *Loa*, *Dracunculus* and extra-intestinal stages of the intestinal worms *Strongyloides* and *Trichinella*. The compounds are also of value against arthropods parasitizing man, biting insects and other dipterous pests causing annoyance to man.

The compounds are also active against household pests such as the cockroach, *Blattella sp.*, clothes moth, *Tineola sp.*, carpet beetle, *Attagenus sp.* and the housefly *Musca domestica*.

The compounds are also useful against insect pests of stored grains such as *Tribolium sp.*, *Tenebrio sp.* and of agricultural plants such as spider mites, (*Tetranychus sp.*), aphids, (*Acyrtiosiphon sp.*); and against migratory orthopterans such as locusts and immature stages of insects living on plant tissue. The compounds are useful as a nematocide for the control of soil nematodes and plant parasites such as *Meloidogyne spp.* which may be of importance in agriculture.

The present invention provides compositions containing an inert carrier incorporating one or more C-076 compounds. These compounds may be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench where used as an anthelmintic in mammals. The drench is normally a solution, suspension or dispersion of the active ingredient, usually in water together with a suspending agent such as bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contain from 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight. The capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or di-calcium phosphate.

Where it is desired to administer the C-076 compounds in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active

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15	15	15
20	20	20
25	25	25
30	30	30
35	35	35
40	40	40
45	45	45
50	50	50
55	55	55
60	60	60
65	65	65

compound are used. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate and vegetable gums. Such unit dosage formulations may be varied widely with respect to their total weight and content of the antiparasitic agent depending upon factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or optionally fed separately. Alternatively, the antiparasitic compounds of the invention may be administered to animals parenterally, for example, by intraruminal, intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil or cotton seed oil. Other parenteral vehicles, such as organic preparations using Solketal and glycerol, and aqueous parenteral formulations are also used. The active C-076 compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

Although the antiparasitic agents of this invention find their primary use in the treatment and/or prevention of helminthiasis, they are also useful in the prevention and treatment of diseases caused by other parasites, for example, arthropod parasites such as ticks, lice, fleas, mites and other biting insects in domesticated animals and poultry. They are also effective in treatment of parasitic diseases that occur in other animals including humans. The optimum amount for best results will, of course, depend upon the particular compound, the species of animal to be treated and the type and severity of parasitic infection or infestation. Generally, good results are obtained with the novel compounds by the oral administration of from 0.001 to 10 mg per kg of animal body weight, the said total dose being given at one time or in divided doses over a relatively short period of time such as 1-5 days. With the preferred compounds of the invention, excellent control of such parasites is obtained in animals by administering from 0.025 to 0.5 mg per kg of body weight in a single dose. Repeat treatments are given as required to combat re-infections and are dependent upon the species of parasite and the husbandry techniques. The techniques for administering these materials to animals are known to those skilled in the veterinary field.

When the compounds described herein are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the antiparasitic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits and crushed limestone. The active C-076 compounds are intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from 0.005 to 2.0% by weight of the active compound are particularly suitable as feed premixes. Feed supplements, which are fed directly to the animal, contain from 0.0002 to 0.3% by weight of the active compounds.

Such supplements are added to the animal feed in an amount to give the finished feed the concentration of active compound desired for the treatment and control of parasitic diseases. Although the desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular C-076 compound, the compounds of this invention are usually fed at concentrations of 0.00001 to 0.002% in the feed in order to achieve the desired antiparasitic result.

In using the compounds of this invention, the individual C-076 components may be isolated and purified and used in that form. Alternatively, mixtures of two

or more of the individual C-076 components may be used. It is not necessary to completely separate the various C-076 compounds obtained from the purification of the fermentation broth. Generally, there is obtained a mixture containing two or more of the C-076 compounds, but having other unrelated compounds excluded therefrom, and such mixtures may be used for the prevention and treatment of parasitic diseases as described herein. Such a mixture generally will contain unequal proportions of the C-076 compounds, however, all of the compounds have substantial activity and the antiparasitic activity of the mixture can be accurately determined. In particular it may not be necessary to separate the "b" components from the related "a" component. Such compounds differ only in the length of the 25 side-chain. Separation of these closely related compounds is generally not practised since the "b" compound is present only in a very small percentage by weight.

In addition, where the C-076 compounds are to be added to an animal's feed, it is possible to utilize the dried mycelial cake from the fermentation broth. The mycelia contain a preponderance of the activity and since the level of the activity of the mycelia can be determined, it can be added directly to the animal's feed.

The compounds of this invention have a broad spectrum of activity against many internal parasites at low dosage levels and in many different animals. At levels of about 2.5 mg per kg of animal body weight, concentrated mixtures of C-076 compounds are fully active in sheep against *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Cooperia* spp., and *Oesophagostomum columbianum*. Similarly in cattle at dosages as low as 0.043 mg/kg, C-076 B2 is fully active against *Ostertagia ostertagi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Oesophagostomum radiatum* and *Dictyocaulus viviparus*. In addition, a horse infected with bots (*Gastrophilus intestinalis* and *Gastrophilus haemorrhoidalis*), large and small strongyles and *Oxyuris* was successfully treated with 10 mg/kg (about 1% active compounds by weight) of a mixed concentrate of C-076 compounds, and a dog infected with the microfilarial stage of heartworm (*Dirofilaria immitis*) was successfully treated with a single oral dose at 10 mg/kg (about 1% active compounds by weight) of a mixed concentrate of C-076 compounds. In rodents, such as mice, infections of *Syphacia*, *Nematospiroides* and *Aspiculuris* have been successfully treated by the oral administration of the C-076 compounds or of the concentrates obtained from the extraction of the mycelia.

The C-076 compounds of this invention are also useful in combating agricultural pests that inflict damage upon crops while they are growing or while in storage. The compounds are applied using known techniques such as sprays, dusts or emulsions to the growing or stored crops to effect protection from such agricultural pests.

The anthelmintic activity of C-076 may be determined by orally administering via the feed, a sample of C-076 individual compound, a mixture of C-076 compounds or a concentrated extract to a mouse that had been infected 3 days earlier with *Nematospiroides dubius*. At 11, 12 and 13 days after the initiation of the medication, the feces of the mouse are examined for *N. dubius* eggs, and on the next day the mouse is sacrificed and the number of worms present in the proximal portion of the small intestine are determined. An active compound is observed when there is a significant reduction of egg and worm counts when compared to infected, unmedicated controls.

The following examples are being provided in order that the invention may be more fully understood. Such examples are not to be construed as being limitative of the invention.

EXAMPLE 1

The contents of a lyophilized tube of *Streptomyces avermitilis* MA-4680 are transferred aseptically to a 250 ml Erlenmeyer flask containing 50 ml of Medium 1. The inoculated flask is incubated for 3 days at 28°C on a rotary shaking machine at a speed of 220 RPM in a 2 inch radius circular orbit. At the end of this time, a 250 ml Erlenmeyer flask containing 50 ml of Medium 2 is inoculated with a 2 ml sample from the first flask. This flask is incubated for 3 days at 28°C on a rotary shaking machine at a speed of 220 RPM in a 2 inch diameter circular orbit. 50 ml of the resulting fermentation broth containing C-076 is effective against an *N. dubius* infection in mice.

Composition of Media

Medium 1

Dextrose	20 g
Peptone	5 g
Meat extract	5 g
Primary yeast	3 g
NaCl	5 g
CaCO ₃ (after pH adjustment)	3 g
Distilled water	1000 ml
pH 7.0	

Medium 2

Tomato paste	20 g
Modified starch	20 g
Primary yeast	10 g
CoCl ₂ ·6H ₂ O	0.005 g
Distilled water	1000 ml
pH 7.2—7.4	

EXAMPLE 2

A lyophilized tube of *Streptomyces avermitilis* MA-4680 is opened aseptically and the contents suspended in 50 ml of Medium 1 in a baffled 250 ml Erlenmeyer flask. This flask is shaken for 3 days at 28°C on a rotary shaking machine at a speed of 220 RPM with a 2 inch diameter circular orbit. 0.2 ml of this seed medium is used to inoculate a Slant of Medium 3. The inoculated slant medium is incubated at 28°C for 10 days and stored at 4°C until used to inoculate 4 more slants of Medium 3. These slants are incubated in the dark for 8 days. One of these slants is used to inoculate 3 baffled 250 ml Erlenmeyer flasks containing 50 ml of No. 4 Seed Medium. The seed flasks are shaken for 2 days at 27 to 28°C on a rotary shaking machine at 220 RPM with a 2 inch diameter circular orbit. The contents of these flasks are pooled and used to inoculate (5% inoculum) baffled 250 ml Erlenmeyer flasks containing 40 ml of various production media. Flasks containing media 2, 5 and 6 are incubated for 4 days at 28°C on a rotary shaking machine at 220 RPM with a 2 inch diameter circular orbit. The resulting broth containing C-076 is then harvested and tested for anthelmintic activity. In all cases 62 ml of whole broth and the solids obtained from centrifuging 25 ml of whole broth are fully active against *N. dubius* helminth infections in mice.

Medium 3 (Slant Medium)

Dextrose	10.0 g
Bacto Asparagine	0.5 g
K ₂ HPO ₄	0.5 g
Bacto agar	15.0 g
Distilled water	1000 ml
pH 7.0	

Medium 4 (Seed Medium)

Soluble starch	10.0 g
Ardamine pH	5.0 g
NZ Amine E	5.0 g
Beef extract	3.0 g
MgSO ₄ ·7H ₂ O	0.5 g
Cerelose	1.0 g
Na ₂ HPO ₄	0.190 g
KH ₂ PO ₄	0.182 g
CaCO ₃	0.5 g
Distilled water	1000 ml
pH 7.0—7.2	

Medium 5

Tomato paste	40.0 g
Oat flour	10.0 g
Cerelose	10.0 g
Corn steep liquor	5.0 g
Trace element mix	10.0 ml
Distilled water	1000 ml
pH 6.8	

	Trace Element Mix		
	FeSO ₄ · 7H ₂ O	1000 mg	
	MnSO ₄ · 4H ₂ O	1000 mg	
	CuCl ₂ · 2H ₂ O	25.0 mg	
	CaCl ₂	100.0 mg	5
	H ₂ BO ₃	56.0 mg	
	(NH ₄) ₂ MoO ₄ · 4H ₂ O	10.0 mg	
	ZnSO ₄ · 7H ₂ O	200.0 mg	
	Distilled water	1000 ml	
	pH		10
	Medium 6		
	CPC Industrial Starch Modified		
	(Available from CPC Corp.)	40.0 g	
	Distiller's Solubles	7.0 g	
15	Autolyzed Yeast (Ardamine		15
	pH available from Yeast		
	Products Inc.	5.0 g	
	CoCl ₂ · 6H ₂ O	50.0 mg	
20	Distilled water	1000 ml	20
	pH 7.3		

EXAMPLE 3

A 0.5×1.0 cm loop of one of the four slants of Medium 3 prepared as in Example 2 is used to inoculate a baffled 250 ml Erlenmeyer flask containing 50 ml of Seed Medium No. 4. The seed flask is shaken for 1 day at 27 to 28°C on a rotary shaking machine at 220 RPM with a 2 inch diameter circular orbit. The seed flask is then stored stationary at 4°C until it is ready to be used. The contents of this flask are then used to inoculate (5% inoculum) 20 unbaffled 250 ml Erlenmeyer flasks containing 40 ml of Medium No. 2. After 4 days incubation at 28°C on a rotary shaking machine at 220 RPM with a 2 inch diameter circular orbit, 19 of the flasks are harvested and pooled. The combined fermentation broths containing C-076 are filtered affording 500 ml of filtrate and 84 g of mycelia. 78 of mycelia is extracted with 150 ml of acetone for 1/2 hour with stirring and the mixture filtered. The filter cake is washed with 50 ml of acetone and the filtrate and washings are combined and concentrated to 46.5 ml. 30 ml of the concentrate is adjusted to pH 4 with dilute hydrochloric acid and extracted 3 times with 30 ml portions of chloroform. The extracts are dried by filtering through dry Infusorial Earth (Super-Cell), combined and concentrated to dryness *in vacuo*. The oily residue of C-076, weighing 91.4 mg, is dissolved in chloroform sufficient to make 3 ml of solution which represents 1% of broth volume. The C-076 obtained in this recovery procedure is fully active against *N. dubius* infections in mice. In addition, the chloroform extraction achieved a 70-fold purification of C-076 from the whole broth.

EXAMPLE 4

A seed culture is prepared by inoculating 50 ml of Medium 4 in a 250 ml baffled Erlenmeyer flask with a 0.5×1.0 cm loop from one of the four slants of Medium 3 as prepared in Example 2. The flask is incubated at 28°C on a rotary shaking machine at 220 RPM with a 2 inch diameter circular orbit for 2 days. The seed culture is used to inoculate a 2 liter Erlenmeyer production flask containing 250 ml of Medium 2. The inoculum volume is 5.0 ml (2%). The production flask is incubated at 28°C on a rotary shaker at 220 RPM for 4 days. At the end of this time the whole broth containing C-076 is harvested. 6 ml of this whole broth when tested in a mouse infected with *N. dubius* is found to be fully active.

EXAMPLE 5

Step A
 A 250 ml baffled Erlenmeyer flask containing 50 ml of Medium 7 is inoculated with a frozen vial of *Streptomyces avermitilis* MA-4680. The flask is incubated at 28°C on a rotary shaking machine at 160 RPM with a 2 inch diameter circular orbit at 160 RPM for 24 hours.

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Medium 7

Dextrose	1 gm
CPC Industrial Starch Modified	10 gm
Meat extract	3 gm
NZ Amine E	5 gm
Autolysed Yeast (Ardamine pH)	5 gm
MgSO ₄ · 7H ₂ O	0.05 gm
Na ₂ HPO ₄	0.19 gm
KH ₂ PO ₄	0.182 gm
CaCO ₃	0.5 gm
Distilled water	1000 ml
pH 7.0—7.2	

Step B

Two 2 liter baffled Erlenmeyer flasks each containing 500 ml of Medium 7 are inoculated with 10 ml of the flask contents of Step A. The media are incubated at 28°C on a rotary shaking machine at 160 RPM with a 2 inch diameter circular orbit for 24 hours.

Step C

To a 756 liter stainless steel fermentor containing 467 liters of Medium 8 is added 1 liter of the whole fermentation media from Step B. The fermentor is stirred at 28°C at 130 RPM for 96 hours and with aeration at an air flow of 10 cubic feet per minute.

Medium 8

Tomato paste	20 gm/l
Primary yeast N.F.	10 gm/l
Starch, modified, CPC	20 gm/l
CoCl ₂ · 6H ₂ O	5 mg/l
Polyglycol 2000	0.321 ml/l
Distilled water	q.s.
pH 7.2—7.4	

At the end of this time 15.5 l of the whole broth is filtered and the mycelia containing C-076 washed with water. The wet mycelia (2268 g) are extracted with 3 liters of acetone with stirring. The mixture is filtered and the filtrate concentrated to 1550 ml and adjusted to pH 4.0 with dilute HCl. This solution is extracted 3 times with equal volumes of chloroform. The chloroform extracts are dried by filtering through dry Infusorial Earth (SuperCel), combined and concentrated to dryness *in vacuo*. The residual oil of C-076 weighs 5.12 g. 3.3 mg is fully active against *N. dubius* in mice.

4.69 g of this oil is dissolved in 142 ml of chloroform and chromatographed on a column containing 90 g of silica gel packed in chloroform. The column is developed with 1400 ml of chloroform. The column is then eluted with chloroform/ethanol (49:1), collecting 145 cuts of 5 ml each. Following this the column is eluted with chloroform/ethanol (19:1), collecting fractions 146—226 of 5 ml each. Fractions 49—72 are combined and evaporated to dryness affording 200 mg of an oil (A). Fractions 79—184 are likewise combined and evaporated affording 291 mg of an oil (B). 400 µg of each fraction is fully active against *N. dubius* in mice. These two fractions (A and B) are analysed separately on silica-gel thin-layer chromatographic plates (Quanta/Gram QIF plates, available from Quanta/Gram Inc., Fairfield, New Jersey). The plates are developed with chloroform/methanol (19:1). The spots are analysed for their ultraviolet activity and one spot of each fraction has the characteristic ultraviolet absorption for the C-076 compounds (see Table I). From fraction A, the spot with an R_f of 0.83 and from fraction B the spot with an R_f of 0.57 have such absorption. These spots represent the C-076 A compounds and the C-076 B compounds respectively.

198 mg of the oil (A) above is chromatographed on 80 g of silica gel packed in chloroform, eluting with chloroform/methanol (199:1) until 520 ml is collected, followed by chloroform/methanol (99:1), collecting fractions of 10 ml each. The fraction from 630 to 720 ml affords 30.4 mg; the fraction from 730 to 950 ml affords 78.4 mg and the fraction from 950 to 1040 ml affords 20 mg of an oily material. Fractions 1 and 2 containing C-076 A components when tested in mice against *N. dubius* at levels of 1.0, 0.5 and 0.25 mg are fully active.

EXAMPLE 6

Step A

A 250 ml baffled Erlenmeyer flask containing 50 ml of Medium 8 is inoculated with a frozen vial of *Streptomyces avermitilis* MA 4680. The flask is incubated at 28°C on a rotary shaking machine at 160 RPM with a 2 inch diameter circular orbit for 24 hours.

Step B

A 2 liter baffled Erlenmeyer flask containing 500 ml of Medium 8 is inoculated with 10 ml of the flask contents of Step A. The medium is incubated at 28°C on a rotary shaking machine at 160 RPM with a 2 inch diameter circular orbit for 24 hours.

Step C

To a 189 liter stainless steel fermentor containing 160 liters of Medium 9 is added 500 ml of inoculum from Step B. The fermentor is incubated at 28°C with stirring at 150 RPM for 24 hours aerating at a rate of 3 cubic feet per minute.

Medium 9

Dextrose	1 gm/l
Corn starch	10 gm/l
Meat extract	3 gm/l
Autolysed Yeast (Ardamine pH)	5 gm/l
MgSO ₄ · 7H ₂ O	0.05 gm/l
Na ₂ HPO ₄	0.10 gm/l
KH ₂ PO ₄	0.182 g/l
CaCO ₃	0.5 gm/l
Distilled water	q.s.
pH 7.0—7.2	

Step D

To a 756 liter stainless steel fermentor containing 467 liters of Medium 6 is added 43 liters of inoculum from Step C. The fermentor is incubated at 28°C with stirring at 130 RPM for 144 hours and with aeration at an air flow rate of 10 cubic feet per minute.

Step E

At the end of this time, the whole broth is filtered and the filter cake containing C-076 is washed with water. The filter cake is slurried in 120 liters of acetone for 30 minutes and then filtered, and the solids are washed with 30 liters of acetone. The acetone washings are combined and evaporated under reduced pressure to a volume of 40 liters. The concentrate is adjusted to pH 4.0 with dilute hydrochloric acid. The concentrate is extracted 3 times with equal volumes of chloroform. The chloroform extracts are dried by filtering through a pad of dry Infusorial Earth (Super-Cel). The extracts are passed through the Super-Cel, then combined. The combined extracts are concentrated under reduced pressure to a volume of 4 liters. The chloroform concentrate is filtered and passed through a column of 2.4 kg of silica gel in chloroform. The column is eluted with chloroform, collecting eight 3.5 liter fractions. The column is then eluted with chloroform/methanol (49:1), collecting eight more 3.5 liter fractions (fractions 9—16). Fraction number 3 is concentrated to dryness, affording 76 g of an oily material containing a preponderance of the C-076 materials.

97% of this material is dissolved in 685 ml of methylene chloride and chromatographed through 800 g of silicic acid (Mallinckrodt Chemical Co., 100 mesh sieved again through an 80 mesh screen). The column (7.62 cm diameter, 36 cm length) is developed with methylene chloride/benzene (7:3), about 7.5 l, followed by 5% isopropanol in methylene chloride/benzene (7:3), 2.25 l. The fraction eluted with the 5% isopropanol in methylene chloride/benzene, which contains a strongly colored band, contains virtually all of the C-076 material, as determined by thin-layer chromatography (as described in Example 5). This fraction (500 ml) is evaporated and rechromatographed on 105 g of silicic acid (column 3.7 cm diameter, 18 cm length) in methylene chloride. The column is developed with 100 ml portions of methylene chloride containing 5, 10 and 20% ether. Further elution with 20% ether in methylene chloride produces 2 colored

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bands. The fractions between the two bands contained virtually all of the C-076 material as determined by thin layer chromatography.

The C-076 containing fraction is chromatographed on 59 g of silicic acid (column 3.7 cm diameter, 11 cm length) in methylene chloride. The column is developed with 10% ether in methylene chloride. After the first material begins to elute, a fraction of 70 ml is taken, followed by 26 fractions of 5—6 ml each. Fractions 3—26 are combined, affording 1.35 g of material, and analysed by thin-layer chromatography (silica gel plates-Analtech GF 254, developed with 5% isopropanol in methylene chloride). The material with an Rf of 0.28 in this system is C-076 A1.

The column is then eluted with 20% ether in methylene chloride (200 ml), followed by 50% ether in methylene chloride (800 ml). A small amount of C-076 A1/A2 mixture is eluted, followed by all C-076 A2. The total residue of the C-076 A2 fraction is 800 mg.

Further elution with 5% isopropanol in methylene chloride affords C-076 B1 (135 mg). The separation is followed by observing the ultraviolet absorption of the eluent. C-076 B1 and A2 have very similar Rf values on silica gel thin layer chromatography plates (Analtech GF 254) in 5% isopropanol in methylene chloride. However, the two components are clearly distinguishable on the same plates developed with 15% isopropanol in hexane.

The entire C-076 A1 fraction is applied to 14 silica gel plates (Analtech Hf 254, 20x20 cm 500 μ thick). The plates are developed in 10% isopropanol in hexane. The band containing the C-076 A1 is removed from the plates, extracted with ether, evaporated and reapplied to 6 more plates and developed 5 times with 5% isopropanol in hexane. The C-076 A1 is removed from the plates and again chromatographed, developing with pure ether, affording 270 mg of substantially pure C-076 A1. The infrared and nuclear magnetic resonance spectra for this sample are reproduced as Figures 1 and 5 and Table II respectively.

The C-076 A2 fraction is chromatographed on 10 silica gel (Analtech HF 254) plates, developing 5 times with 15% isopropanol in hexane, affording 265 mg of substantially pure C-076 A2. The infrared and nuclear magnetic resonance spectra for this sample are reproduced in Figures 2 and 6 respectively, and Table II.

The C-076 B1 fraction is chromatographed on 2 plates (as above) in 15% isopropanol in hexane, affording 55 mg of substantially pure C-076 B1. The nuclear magnetic resonance spectrum of this sample is reproduced in Figure 7, and Table II.

EXAMPLE 7

The fermentation described in Example 6 is repeated twice and the whole broths are combined. The fermentation broth is worked up as described in Example 6, recovering 3.3 l of an initial chloroform extract which contains 60 mg/ml of total solids and is estimated to be 0.5% C-076 by thin layer chromatographic analysis.

3 liters of this chloroform solution is chromatographed on 2400 g of silica gel (Davidson Grade 62) packed in chloroform. The column (9.5x122 cm) is developed with eight 3800 ml portions of chloroform (fractions 1—8), followed by eight 3800 ml portions of chloroform/methanol (49:1 fractions 9—16). The individual fractions are analysed by thin layer chromatography (silica gel plates, Quanta/Gram QIF) developed with chloroform/methanol 19:1. Fractions 9—11, 12—13 and 14 are each evaporated to dryness affording 6.63 g of solids containing the C-076 A components in fractions 9—11, 24.91 g of solids containing C-076 B components in fractions 12—13, and 4.71 g of solids also containing the C-076 B components in fraction 14.

The materials from fractions 12—14 are combined (29.62 g), dissolved in 100 ml of methylene chloride and chromatographed on 400 g of silica gel (Davidson Grade 62) in methylene chloride. The column is eluted with 1500 ml of methylene chloride/2-propanol (99:1); 1500 ml of methylene chloride/2-propanol (49:1); 2000 ml of methylene chloride/2-propanol (19:1); and 1000 ml of methylene chloride/2-propanol (9:1). The eluent volumes between 5500—6000 ml (2.56 g) and 6000—6500 ml (5.03 g) are combined in 25 ml of methylene chloride and chromatographed on 60 g of silica gel in hexane. Foreruns of 70 ml of hexane and 100 ml of hexane/diethyl ether (4:1) are taken and the column then developed with 600 ml of hexane/diethyl ether (1:4), taking 200 ml cuts, and finally eluting with 700 ml of ether, taking 100 ml cuts. Column eluent volumes from 400 to 600 ml affords 2.035 g of solids containing C-076 B1 components; volumes 600—1100 contained 0.881 g of solids containing mixed C-076 B1/B2 components; and volumes 1100—1500 ml contained 0.381 g of solids containing C-076 B2 components.

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5 The mixed C-076 B1/B2 components are then dissolved in 4.2 ml of methyl alcohol/water (4:1) and chromatographed on C18 Porasil (Bondapak-37-75 micron size) in the same solvent. The reverse phase high pressure column (more polar components eluted first) is 1.2 meters by 16 mm and is eluted at a rate of 800 ml per hour taking 21.3 ml fractions. The presence of C-076 components is monitored by observing the ultraviolet absorption of the fractions. C-076 B2 is recovered in fractions 24 to 37 and C-076 B1 is recovered in fractions 51—70, recovering 195.4 mg of C-076 B2 and 137 mg of C-076 B1.

10 Each sample is then separately chromatographed on 4 g columns of silic agel (Davidson Grade 62) in methylene chloride. The columns are eluted with 35 ml of methylene chloride/methanol (9:1). The last 20 ml of eluent from each column is collected and evaporated to dryness, affording 155.3 mg of C-076 B2 and 90 mg of C-076 B1 respectively.

15 Then 50 mg of C-076 B1 and 100 mg of C-076 B2 are chromatographed on preparative silica gel plates (Analtech Hf 254), developed with 12% isopropanol in hexane, followed by development with ether, recovering C-076 B1 and C-076 B2 which are substantially pure. The infrared absorption spectrum of the thus recovered C-076 B1 and B2 is as shown in Figures 3 and 4 respectively. The nuclear magnetic spectrum of the thus recovered C-076 B2 is as shown in Figure 8.

20 EXAMPLE 8

A 250 ml baffled Erlenmeyer flask containing 50 ml of the following medium:

	Lactose	2.0%	
	Distiller's solubles	1.5%	
25	Autolysed yeast, Ardamine pH	0.5%	
	pH—before sterilization	7.0	25

is inoculated with the contents of one frozen vial of *Streptomyces avermitilis* MA 4848 and incubated on a rotary shaker at 28°C for 24 hours at 150 RPM.

30 10 ml of the above fermentation medium is used to inoculate 500 ml of the same medium as above in a 2 liter baffled Erlenmeyer flask. The fermentation medium is incubated at 150 RPM on a rotary shaker at 28°C for 24 hours.

All of the foregoing medium is used to inoculate 467 liters of the following medium in a 756 liter stainless steel fermentor:

	Lactose	2.0%	
	Distiller's solubles	1.5%	
35	Autolysed yeast, Ardamine pH	0.5%	
	Polyglycol 2000	0.32 ml/liter	
	pH—before sterilization	7.0	35

40 The fermentation medium is incubated at 28°C for 40 hours with an air flow of 10 cubic feet per minute and an agitation rate 130 RPM.

230 liters of the above medium is used to inoculate 4310 liters of the following medium in a 5670 liter stainless steel fermentor:

	Dextrose	4.5%	
	Peptonized milk	2.4%	
45	Autolysed yeast, Ardamine pH	0.25%	
	Polyglycol 2000	2.5 ml/liter	
	pH—before sterilization	7.0	45

The fermentation continues for 144 hours at 26°C with an air flow rate of 54.3 cubic feet per minute and agitation of 120 RPM.

50 The fermentation medium is filtered and the mycelial filter cake washed with about 550 liters of water. The filtrate and washings are discarded. The filter cake is agitated with about 1500 liters of acetone for about one hour and filtered. The filter cake is washed with a mixture of about 150 liters of acetone and 40 liters of deionized water, affording about 2000 liters of extract.

55 The foregoing fermentation and extraction is repeated on the same scale, affording a further 2000 liters of acetone extract which is combined with the first extract and evaporated to a volume of about 800 liters. The pH of the concentrate is adjusted to about 4.7 with concentrated hydrochloric acid and combined with about 800 liters of methylene chloride. The combined solvents are agitated for about 4 hours and separated. The aqueous layer is combined with an additional 800 liters

	18	19	1,573,955	19
nethyl micron polar ml per red by red in 195.4	5	5	of methylene chloride and agitated for about 4 hours. The layers are separated and each methylene chloride extract separately treated with about 10 kilograms of Super-Cel and filtered. Both extracts are evaporated to a combined volume of about 60 liters.	5
c agel ml of mn is mg of	10	10	EXAMPLE 9 The 60 liter solution of C-076 in methylene chloride of the previous example is concentrated to dryness <i>in vacuo</i> and the residue is combined 3 times with 60 liter portions of methanol and evaporated to dryness to remove any residual methylene chloride. The final methanol concentrate volume is approximately 36 liters. The methanol solution is stored overnight and filtered. The filter cake is washed with 40 liters of fresh methanol and the methanol filtrates and washings are combined. The methanol solution is combined with 95 liters of ethylene glycol and 130 liters of heptane. The two-layer solution is agitated for 5 minutes and the lower layer (ethylene glycol and methanol) is separated. The heptane solution is washed with a mixture of 20 liters of ethylene glycol and 6.3 liters methanol. After five minutes of agitation, the lower layer is separated and combined with the first ethylene glycol/methanol extract. An equal volume of water (approximately 150 liters) containing 79 g of salt per liter is added to the ethylene glycol/methanol extracts. This solution is extracted with 150 liters of ethyl ether with agitation for 5 minutes. The ethereal layer is washed with 75 liters of water (1/2 volume) and agitated for 5 minutes and the layers separated. This procedure is repeated an additional 2 times (the final water wash contains 20 g of salt per liter), affording a final ethereal layer volume of 110 liters. The ethereal layer is concentrated <i>in vacuo</i> , to a minimum volume, keeping the temperature less than 25°C. 40 liters of methylene chloride is added to the residue and the solution is evaporated to dryness. This procedure is repeated and the final residue concentrated <i>in vacuo</i> at 50°C to dryness.	10
ed on nol in 76 B2 thus uclear 8.	15	15		15
dium:	20	20		20
	25	25		25
s MA			EXAMPLE 10 A 30 centimeter diameter column is prepared with a layer of 34 kilograms of activated alumina followed by a layer of 34 kilograms of activated carbon in a solution of methylene chloride. The residue from the previous example is dissolved in methylene chloride to a volume of 34 liters and applied to the column and eluted with 34 liters of methylene chloride. These fractions are discarded. A 3% solution of isopropanol and methylene chloride (20.8 liters of isopropanol and 660 liters of methylene chloride) is applied to the column and eluted in approximately 200 liter fractions. The combined isopropanol and methylene chloride fractions are evaporated <i>in vacuo</i> at a bath temperature of about 60°C to a volume of about 20 liters. The bath temperature is reduced to about 45°C and the extract is evaporated to dryness <i>in vacuo</i> . The residue is dissolved in 10 parts methylene chloride, 10 parts hexane and one part methanol to a final volume of 15 liters. This solution is applied directly to the Sephadex LH-20 column of the next example.	
of the ation owing:	30	30		30
	35	35		35
of 10	40	40		40
owing	45	45	EXAMPLE 11 A 30 centimeter diameter column is prepared in methanol with 36 kilograms of Sephadex LH-20 (available from Pharmacia Fine Chemicals, 800 Centennial Avenue, Piscataway, New Jersey 08854) and washed with a solvent consisting of 10 parts methylene chloride, 10 parts hexane and one part methanol. One-fourth of the C-076 solution of Example 10 is applied to the column and the column eluted at a rate of 250 ml per minute. Two 20 liter forecuts are collected and discarded, followed by 20 two-liter rich cuts (identified as fractions 1—20), followed by a single 20 liter tail cut, which is discarded. Fractions 1—8 are found to contain the C-076 A compounds and fractions 9—20 are found to contain the C-076 B compounds.	45
cubic	50	50		50
l with ake is filter rs of	55	55	EXAMPLE 12 The process of Example 11 is repeated on the same scale three more times and all of the fractions containing the C-076 B components (fractions 9—20) are combined and evaporated to dryness, affording 818 g of crude mixed C-076 B components. The sample is found to contain 55% C-076 B1 and 39% of C-076 B2. 680.5 g of this sample is dissolved in 2 liters of methylene chloride and placed in a 22 liter three-neck round bottom flask, followed by the addition of 13.6 liters of methanol. The methylene chloride is removed by distillation. 13.6 liters of ethylene glycol is added as the methanol is being distilled under reduced pressure. The rate of distillation is maintained such that the temperature of the solution does not go	55
scale, first strate with about liters	60	60		60

below 65°C. When the addition of the ethylene glycol is complete, the solution is allowed to cool at 5°C for sixteen hours. The crystals are filtered and washed with 1 liter of cold ethylene glycol. The crystals are then redissolved in 2 liters of methylene chloride and the solution placed in a 22 liter three-necked round bottom flask. The procedure described above is repeated twice. The first time 12.5 liters each of methanol and ethylene glycol is used and the second time 13.6 liters each of methanol and ethylene glycol is used. The final crystals are washed with 1 liter of cold ethylene glycol and 1 liter of water. The crystals are dissolved in 4 liters of water and dried by filtering through sodium sulfate. The benzene solution is concentrated to a volume of 2 liters and lyophilized affording 241.2 gm of a white powder consisting of 98% C-076 B1 and 1% of C-076 B2.

The mother liquors (22 liters) from the first two crystallizations above are combined and diluted with 22 liters of water. The aqueous solution is extracted with 60 liters of toluene and again with 15 liters of toluene. The toluene extract is then washed with 48 liters of water. The organic phase is filtered through Super-Cel to remove any residual water and evaporated, affording 336 gm of solid material consisting of 79% C-076 B2 and 16% C-076 B1 compounds.

EXAMPLE 13

In the four Sephadex LH-20 columns of the procedure of Example 11, fractions 1—8 contain the C-076 A compounds and are combined. By HPLC analysis the mixture is found to contain 252 g of C-076 A2a, 16 g of A2b, 94 g of A1a and 24 g of A1b. The material is dissolved in a solvent system consisting of hexane:toluene:methanol in the proportion of 6:1:1 and applied to the Sephadex LH-20 column of the same dimensions as the one used in Example 11 in the above solvent. Fractions are collected at the rate of 250 ml per minute and a 20 liter forecut is collected and discarded. Further elution affords 2 additional 20 liter forecuts, which are also discarded, and 50 four liter rich cuts, which contain C-076 A compounds. Fractions 3—8 are found to contain predominantly C-076 A1 components (40.2 g A1a and 6.7 g A1b), and fractions 29—36 are found to contain C-076 A2 compounds (117.2 g A2a and 7.35 g of A2b). Fractions 9—28 contain a mixture of C-076 A1 and A2 compounds.

EXAMPLE 14

A sample of 150 g of C-076 B1 from Example 12 is dissolved in 3 liters of a solvent mixture of hexane:toluene:methanol in the ratio of 3:1:1. The solution is passed through a column of Sephadex LH-20 (of the same dimensions as the one used in Example 11) in the above solvent, taking fractions at the rate of 250 ml per minute. After two 20 liter portions of the solvent mixture are collected and discarded, a forecut of 10 liters is taken and discarded. Then 30 rich cuts of 2 liters each are taken. Fractions 1—13 and 25—30 are discarded. Fractions 14—16 are combined and contain 80 g of predominately C-076 B1a. Fractions 22—24 are combined and contain 6.7 g of predominately C-076 B1b. Fractions 17—21 contain a mixture of C-076 B1a and B1b.

Fractions 17—21 above are combined and concentrated and passed through a Sephadex LH-20 column with the same solvent system as above. Three 20 liter forecuts are taken and discarded. Rich cuts are then taken as follows: 5 cuts of 2 liters each (fractions 1—5); 20 cuts of 1 liter each (fractions 6—25); and 10 cuts of 2 liters each (fractions 26—35). Fractions 1—15 are discarded; fractions 16—21 contain 13.5 g of C-076 B1a and 0.4 g of C-076 B1b; fractions 22—26 contain 44 g of C-076 B1a and 0.13 g of C-076 B1b; fractions 27—30 contain 10.2 g of C-076 B1a and 0.8 g of C-076 B1b.

EXAMPLE 15

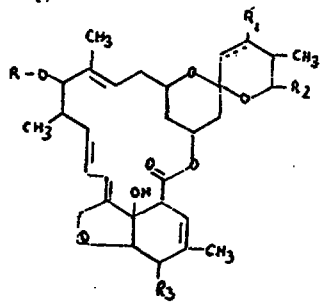
A mixture of all 8 C-076 components is chromatographed on a high-pressure liquid chromatography column 4 mmx30 cm packed with 10 microns μ Bondapak C₁₈ silica gel (available from Waters Associates Inc., Maple Street, Milford, Massachusetts 01757), eluting with 85:15 (v/v) methanol:water at a constant 40°C. At a flow rate of 1.2 ml per minute all eight compounds are separated and the elution volumes, which under the foregoing constant conditions are characteristic of the individual compounds are as follows:

Elution Volume (V _e) ml	
C-076 B2b	5.90
C-076 B2a	6.52
C-076 A2b	7.12
C-076 A2a	7.88
C-076 B1b	8.36
C-076 B1a	9.60
C-076 A1b	10.24
C-076 A1a	11.88

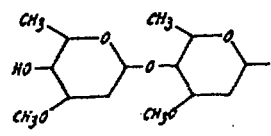
The separation of C-076 "b" components from the respective "a" components is accomplished using techniques such as high-pressure liquid chromatography. An absolute methanolic solution of 30 microliters of a mixture of C-076 A1a and A1b, estimated to contain 30 micrograms of C-076 A1b, is placed on a 3x250 mm high-pressure liquid chromatography column containing Spherisorb 5 micron ODS (available from Spectra Physics) as packing. The column is eluted with 85:15 methanol-water at a rate of 0.15 ml/min. The elution of the products is followed by observing the ultraviolet absorption of the eluent and collecting the individual components at the outlet of the UV monitor. 30 micrograms of C-076 A1b is recovered and analysed in a mass spectrometer. The mass spectrum of this sample is recorded in the second column of Table III.

WHAT WE CLAIM IS:—

1. A process for the preparation of C-076 as herein defined, that comprises fermenting, with *Streptomyces avermitilis* NRRL 8165 (ATCC 31267) or a mutant thereof, an aqueous nutrient medium containing an assimilable source of carbon, an assimilable source of nitrogen and inorganic salts under aerobic conditions and recovering C-076 from the resulting fermentation broth.
2. A process as claimed in Claim 1 in which the fermentation is conducted at a temperature of from 20 to 40°C and a pH of from 5.0 to 9.0.
3. A process as claimed in Claim 2 in which the temperature is from 24 to 30°C and the pH is from 6.0 to 7.5.
4. A process as claimed in any preceding claim in which the nutrient medium contains 0.5 to 5% by weight of carbon sources and 0.2 to 6% by weight of nitrogen sources.
5. A process as claimed in any preceding claim in which the fermentation is carried out for from 1 to 8 days.
6. A process as claimed in any one of Claims 1 to 5, in which the mutant is *Streptomyces avermitilis* ATCC 31271.
7. A process as claimed in any one of Claims 1 to 5, in which the mutant is *Streptomyces avermitilis* ATCC 31272.
8. A process as claimed in any preceding claim in which the C-076 is recovered by filtering the fermentation medium.
9. A process as claimed in Claim 8 including the further steps of extracting the resulting mycelium with a water-miscible organic solvent, evaporating the organic solvent and extracting the residue with a water-immiscible organic solvent.
10. A compound having the formula:



where R is:



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Elution Volume (Ve) ml

C-076 B2b	5.90
C-076 B2a	6.52
C-076 A2b	7.12
C-076 A2a	7.88
C-076 B1b	8.36
C-076 B1a	9.60
C-076 A1b	10.24
C-076 A1a	11.88

The separation of C-076 "b" components from the respective "a" components is accomplished using techniques such as high-pressure liquid chromatography. An absolute methanolic solution of 30 microliters of a mixture of C-076 A1a and A1b, estimated to contain 30 micrograms of C-076 A1b, is placed on a 3x250 mm high-pressure liquid chromatography column containing Spherisorb 5 micron ODS (available from Spectra Physics) as packing. The column is eluted with 85:15 methanol-water at a rate of 0.15 ml/min. The elution of the products is followed by observing the ultraviolet absorption of the eluent and collecting the individual components at the outlet of the UV monitor. 30 micrograms of C-076 A1b is recovered and analysed in a mass spectrometer. The mass spectrum of this sample is recorded in the second column of Table III.

WHAT WE CLAIM IS:—

1. A process for the preparation of C-076 as herein defined, that comprises fermenting, with *Streptomyces avermitilis* NRRL 8165 (ATCC 31267) or a mutant thereof, an aqueous nutrient medium containing an assimilable source of carbon, an assimilable source of nitrogen and inorganic salts under aerobic conditions and recovering C-076 from the resulting fermentation broth.

2. A process as claimed in Claim 1 in which the fermentation is conducted at a temperature of from 20 to 40°C and a pH of from 5.0 to 9.0.

3. A process as claimed in Claim 2 in which the temperature is from 24 to 30°C and the pH is from 6.0 to 7.5.

4. A process as claimed in any preceding claim in which the nutrient medium contains 0.5 to 5% by weight of carbon sources and 0.2 to 6% by weight of nitrogen sources.

5. A process as claimed in any preceding claim in which the fermentation is carried out for from 1 to 8 days.

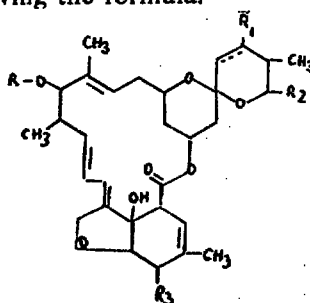
6. A process as claimed in any one of Claims 1 to 5, in which the mutant is *Streptomyces avermitilis* ATCC 31271.

7. A process as claimed in any one of Claims 1 to 5, in which the mutant is *Streptomyces avermitilis* ATCC 31272.

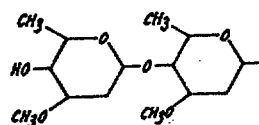
8. A process as claimed in any preceding claim in which the C-076 is recovered by filtering the fermentation medium.

9. A process as claimed in Claim 8 including the further steps of extracting the resulting mycelium with a water-miscible organic solvent, evaporating the organic solvent and extracting the residue with a water-immiscible organic solvent.

10. A compound having the formula:



where R is:



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and the broken line indicates that the 22 (23) linkage is a single or a double bond; R_1 is hydroxy and is present only when the broken line indicates a single bond.

R_2 is iso-propyl or sec-butyl; and

R_3 is methoxy or hydroxy.

5 11. A compound claimed in Claim 10 in which the broken line indicates a double bond, R_2 is iso-propyl and R_3 is methoxy. 5

12. The compound claimed in Claim 10 in which the broken line indicates a double bond, R_2 is iso-propyl and R_3 is hydroxy.

10 13. The compound claimed in Claim 10 in which the broken line indicates a double bond, R_2 is sec-butyl and R_3 is methoxy. 10

14. The compound claimed in Claim 10 in which the broken line indicates a double bond, R_2 is sec-butyl and R_3 is hydroxy.

15. The compound claimed in Claim 10 in which the broken line indicates a single bond, R_1 is hydroxy, R_2 is iso-propyl and R_3 is methoxy.

15 16. The compound claimed in Claim 10 in which the broken line indicates a single bond, R_1 is hydroxy, R_2 is iso-propyl and R_3 is hydroxy. 15

17. The compound claimed in Claim 10 in which the broken line indicates a single bond, R_1 is hydroxy, R_2 is sec-butyl and R_3 is methoxy.

20 18. The compound claimed in Claim 10 in which the broken line indicates a single bond, R_1 is hydroxy, R_2 is sec-butyl and R_3 is hydroxy. 20

19. A compound of the class of C-076 *A1a*, *A2a*, *B1a* and *B2a*;

25 C-076 *A1a* having the empirical formula $C_{49}H_{74}O_{14}$, a molecular weight of 886, an infrared absorption spectrum as shown in Figure 1, an ultraviolet absorption maximum at 237, 243 and 252 $m\mu$, a nuclear magnetic resonance spectrum as shown in Figure 5, and principal mass spectrometry peaks occurring at m/e 87, 95, 113, 127, 137, 145, 169, 199, 221, 257, 275, 305, 456, 548, 580, 742 and 886; 25

30 C-076 *A2a* having the empirical formula $C_{49}H_{76}O_{15}$, a molecular weight of 904, an infrared absorption spectrum as shown in Figure 2, an ultraviolet absorption maximum at 237, 243 and 252 $m\mu$, a nuclear magnetic resonance spectrum as shown in Figure 6, and principal mass spectrometry peaks occurring at m/e 87, 95, 113, 127, 137, 145, 179, 199, 211, 239, 257, 275, 305, 323, 456, 566, 598, 760 and 904; 30

35 C-076 *B1a* having the empirical formula $C_{49}H_{74}O_{14}$, a molecular weight of 872, and infrared absorption spectrum as shown in Figure 3, an ultraviolet absorption maximum at 237, 243 and 252 $m\mu$, a nuclear magnetic resonance spectrum as shown in Figure 7, and principal mass spectrometry peaks occurring at m/e 87, 95, 113, 127, 137, 145, 169, 199, 193, 221, 257, 261, 305, 456, 548, 566, 728 and 872; 35

40 C-076 *B2a* having the empirical formula $C_{49}H_{76}O_{15}$, a molecular weight of 890, an infrared absorption spectrum as shown in Figure 4, an ultraviolet absorption maximum at 237, 243 and 252 $m\mu$, a nuclear magnetic resonance spectrum as shown in Figure 8, and principal mass spectrometry peaks occurring at m/e 87, 95, 113, 127, 137, 145, 179, 199, 211, 239, 257, 261, 305, 323, 456, 566, 584, 746 and 890. 40

20. A composition useful for the prevention and treatment of parasitic diseases which comprises an inert carrier having one or more C-076 compounds incorporated therein.

45 21. A composition as claimed in Claim 20, in the form of a capsule, bolus, tablet or liquid drench. 45

22. A composition as claimed in Claim 20, in the form of an animal feed.

23. A composition as claimed in Claim 20, in a liquid injectable form suitable for veterinary use.

50 24. A composition as claimed in Claim 20, in the form of a premix or supplement suitable for addition to an animal feed. 50

25. A composition as claimed in Claim 22 or 24, in which the C-076 compound(s) is/are used in the form of a dried mycelial cake from a fermentation broth.



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26. A method of producing C-076 (as hereinbefore defined), substantially as hereinbefore described in any one of the Examples.

27. C-076 produced by a process or method as claimed in any one of Claims 1 to 9 and 26.

For the Applicants
D. YOUNG & CO.,
Chartered Patent Agents,
9 & 10 Staple Inn,
London WC1V 7RD.

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