(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 214 731**
B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **02.01.91**

(21) Application number: **86305604.0**

(22) Date of filing: **22.07.86**

(51) Int. Cl.[5]: **C 07 D 493/22**, C 07 H 17/04, A 61 K 31/365, A 61 K 31/70, C 12 P 17/08, A 23 K 1/17, A 01 N 63/02, A 01 N 43/22, C 12 P 19/62 // (C07D493/22, 313:00, 311:00, 311:00, 307:00),(C12P17/08, C12R1:465),(C12P19/62, C12R1:465)

(54) **Antiparasitic avermectin and milbemycin derivatives and process for their preparation.**

(30) Priority: **27.07.85 GB 8518999**
09.08.85 GB 8520069
24.04.86 GB 8610063
02.05.86 GB 8610862

(43) Date of publication of application:
18.03.87 Bulletin 87/12

(45) Publication of the grant of the patent:
02.01.91 Bulletin 91/01

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(56) References cited:
EP-A-0 001 689
EP-A-0 002 615
EP-A-0 184 173
FR-A-2 570 390

The file contains technical information submitted after the application was filed and not included in this specification

(73) Proprietor: **Pfizer Limited**
Ramsgate Road
Sandwich Kent CT13 9NJ (GB)
(84) **GB**

(73) Proprietor: **Pfizer Corporation**
Calle 15 1/2 Avenida Santa Isabel
Colon (PA)
(84) **BE CH DE FR IT LI LU NL SE AT**

(72) Inventor: **Gibson, Stephen Paul**
25, George V Avenue
Westbrook Margate Kent (GB)
Inventor: **Holdom, Kelvin Scott**
9, Tothill Street
Minster Ramsgate Kent (GB)
Inventor: **Goudie, Alexander Crossan**
1, Hildersham Close
St. Peters Broadstairs Kent (GB)
Inventor: **Bu'lock, John Desmond**
205, Stockport Road
Marple Manchester (GB)

(74) Representative: **Moore, James William, Dr. et al**
Pfizer Limited Ramsgate Road
Sandwich Kent CT13 9NJ (GB)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European patent convention).

Courier Press, Leamington Spa, England.

EP 0 214 731 B1

Description

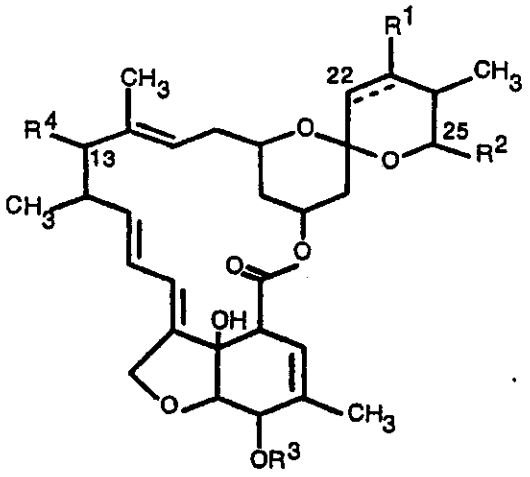
This invention relates to antiparasitic agents and in particular to compounds related to the avermectins and milbemycins but having a novel substituent group at the 25-position and to a process for their preparation.

The avermectins are a group of broad spectrum antiparasitic agents referred to previously as the C—076 compounds. They are produced by fermenting a strain of the microorganisms *Streptomyces avermitilis* ATCC 31267, 31271 or 31272 under aerobic conditions in an aqueous nutrient medium containing inorganic salts and assimilable sources of carbon and nitrogen. The morphological and cultural properties of the strains ATCC 31267, 31271 and 31272 are described in detail in British Patent Specification no. 1573955 which also describes the isolation and the chemical structure of the eight individual components which make up the C—076 complex. The milbemycins are structurally related macrolide antibiotics lacking the sugar residues at the 13-position. They are produced by fermentation, for example as described in British Patent Specification no. 1390336 and European Patent Application publication no. 0170006.

We have now discovered that by adding certain specified carboxylic acids, or derivatives thereof, to the fermentation of an avermectin producing organism it is possible to obtain novel compounds, related to the avermectics but having an unnatural substituent group at the 25-position in place of the isopropyl or sec-butyl group which is normally present. The novel compounds are highly active antiparasitic agents having particular utility as anthelmintics, ectoparasiticides, insecticides and acaricides.

Thus, according to one aspect of the invention there is provided a process for producing a novel avermectic derivative having an unnatural substituent group at the 25-position which comprises adding a carboxylic acid, or a salt, ester or amide thereof or oxidative precursor therefor, to a fermentation of an avermectic producing strain of the organism *Streptomyces avermitilis* and isolating the novel avermectic derivative.

Conventional chemical transformation reactions can be used to prepare further derivatives from these compounds. Thus, according to a further aspect of the invention there are provided compounds having the formula:



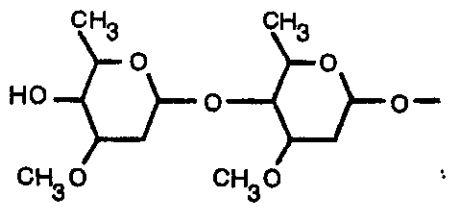
(I)

wherein the broken line at the 22—23 position represents an optional double bond and wherein R^1 is H or OH and the double bond is absent, or, the double bond is present and R^1 is absent;

R^2 is an alpha-branched C_3—C_8 alkyl, alkenyl, alkoxyalkyl or alkylthioalkyl group; an alpha-branched C_4—C_8 alkynyl group; a (C_5—C_8 cycloalkyl)alkyl group wherein the alkyl group is an alpha-branched C_2—C_5 alkyl group; a C_3—C_8 cycloalkyl or C_5—C_8 cycloalkenyl group, either of which may optionally be substituted by methylene or one or more C_1—C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C_1—C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;

R^4 is H or a 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group of the formula:



with the proviso that when R^2 is alkyl it is not isopropyl or sec-butyl, and when R^4 is H, R^2 is not 2-buten-2-yl, 2-penten-2-yl or 4-methyl-2-penten-2-yl.

In the above definition, alkyl groups containing 3 or more carbon atoms may be straight or branched chain. Halo means fluoro, chloro, bromo or iodo. Alpha-branched means that the carbon atom attached to the 25-ring position is a secondary carbon atom linked to two further carbon atoms, or in the case where R^2 is alkoxyalkyl or alkylthioalkyl, it is linked to a further carbon atom and an oxygen or sulphur atom. When R^2 is alkyl of 5 or more carbon atoms, the remainder of the alkyl chain may be straight or branched chain.

Preferred compounds of the formula I are those wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy. Also preferred are compounds of the formula I wherein R^2 is a C_5 or C_6 cycloalkyl or cycloalkenyl group which may optionally be substituted by one or more C_1—C_4 alkyl groups, cyclopentyl being particularly preferred. In another group of preferred compounds R^2 is cyclobutyl. In another group of preferred compounds R^2 is cyclobutyl. In a further group of preferred compounds R^2 is a 5 or 6 membered oxygen or sulphur containing heterocyclic ring, particularly a 3-thienyl or 3-furyl ring, which may optionally be substituted by one or more C_1—C_4 alkyl groups or halogen atoms. In a yet further group of preferred compounds, R^2 is a C_3—C_8 alkylthioalkyl group, particularly a 1-methylthioethyl group.

In accordance with the invention the compounds of formula I wherein R^1 is OH and the double bond is absent or wherein the double bond is present and R^1 is absent and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy are prepared by fermenting an avermectin producing organism, such as a strain of the organism *Streptomyces avermitilis* ATCC 31267, 31271 or 31272, in the presence of the appropriate carboxylic acid of the formula R^2CO_2H, wherein R^2 is as previously defined, or a salt, ester, or amide thereof or oxidative precursor therefor. The acid is added to the fermentation either at the time of inoculation or at intervals during the fermentation. Production of the compounds of formula (I) may be monitored by removing samples from the fermentation, extracting with an organic solvent and following the appearance of the compound of formula (I) by chromatography, for example using high pressure liquid chromatography. Incubation is continued until the yield of the compound of formula (I) has been maximised, generally for a period of from 4 to 6 days.

A preferred level of each addition of the carboxylic acid or derivative thereof is between 0.05 and 1.0 grams per litre. The best yields of the compounds of formula (I) are obtained by gradually adding the acid to the fermentation, for example by daily additions of the acid or derivative thereof over a period of several days. The acid is preferably added as a salt, such as the sodium or ammonium salt, but may be added as an ester, such as the methyl or ethyl ester or as an amide. Alternative substrates which may be used in the fermentation are derivatives which are oxidative precursors for the carboxylic acids; thus, for example suitable substrates would be aminoacids of the formula $R^2CH(NH_2)CO_2H$, glyoxylic acids of the formula R^2COCO_2H, methylamine derivatives of the formula $R^2CH_2NH_2$, substituted lower alkanoic acids of the formula $R^2(CH_2)_nCO_2H$ wherein n is 2, 4 or 6, methanol derivatives of the formula R^2CH_2OH or aldehydes of the formula R^2CHO, wherein R^2 is as previously defined. The media used for the fermentation may be a conventional complex media containing assimilable sources of carbon, nitrogen and other trace elements. However we have found that for better results a strain of the organism derived from *Streptomyces avermitilis* ATCC 31271 which gives improved yields of a compound of formula I when cultured in a semi-defined medium may be used and this has the advantage that crude solvent extracts contain significantly less unwanted material which greatly simplifies the subsequent isolation and purification stages. Such a strain has been deposited with the National Collection of Industrial Bacteria (NCIB) on 19th July, 1985 under the accession number NCIB 12121. The morphological and cultural characteristics of this strain are otherwise generally as described in British Patent specification no. 1573955 for strain ATCC 31267.

After fermentation for a period of several days at a temperature preferably in the range of from 24 to 33°C, the fermentation broth is centrifuged or filtered and the mycelial cake is extracted with acetone or methanol. The solvent extract is concentrated and the desired product is then extracted into a water-immiscible organic solvent, such as methylene chloride, ethyl acetate, chloroform, butanol or methyl isobutyl ketone. The solvent extract is concentrated and the crude product containing the compounds of formula (I) is further purified as necessary by chromatography, for example using preparative reverse phase, high pressure liquid chromatography.

The product is generally obtained as a mixture of the compounds of formula (I) wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy, R^1 is OH and the double bond absent or R^1 is absent and the double bond is present and wherein R^3 is H or CH_3; however the proportions can vary depending on the particular carboxylic acid employed and the conditions used.

We have found that a broad range of carboxylic acids as defined by R^2CO_2H may be added to the fermentation to yield avermectins having a novel substituent group at the 25-position. Examples of particular acids which may be employed include the following:

2-methylvaleric acid
2-methylpent-4-enoic acid
2-methylthiopropionic acid
2-cyclopropyl propionic acid
cyclobutane carboxylic acid
cyclopentane carboxylic acid
cyclohexane carboxylic acid

cycloheptane carboxylic acid
2-methylcyclopropane carboxylic acid
3-cyclohexene-1-carboxylic acid
and thiophene-3-carboxylic acid

In one particular and preferred aspect of the invention, the fermentation is performed in the presence of cyclopentane carboxylic acid sodium salt to yield predominantly the compound of formula (I) wherein R^1 is OH, the double bond is absent, R^2 is cyclopentyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-4-oleandrosyloxy.

In another preferred aspect of the invention, the fermentation is performed in the presence of thiophene-3-carboxylic acid sodium salt to yield predominantly the compound of formula (I) wherein R^1 is OH, the double bond is absent, R^2 is thien-3-yl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-4-oleandrosyloxy.

In a further preferred aspect of the invention the fermentation is performed in the presence of 2-methylthiopropionic acid sodium salt to yield predominantly the compound of formula (I) wherein R^1 is OH, the double bond is absent, R^2 is 1-methylthioethyl, R_3 is CH^3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-4-oleandrosyloxy.

Compounds of the formula (I) wherein the double bond is present and R^1 is absent may alternatively be prepared from the corresponding compound of formula (I) wherein R^1 is OH and the double bond is absent by a dehydration reaction. The reaction is performed by first selectively protecting the hydroxyl groups at the 5 and 4'' positions, e.g. as the t-butyldimethylsilyloxy acetyl derivative, then reacting with a substituted thiocarbonyl halide, such as (4-methylphenoxy)thiocarbonyl chloride, followed by heating in a high boiling point solvent, e.g. trichlorobenzene, to effect the dehydration. The product is finally deprotected to give the unsaturated compound. These steps together with appropriate reagents and reaction conditions are described in United States patent 4328335.

The compounds of formula I wherein R^3 is H may also be prepared from the corresponding compounds wherein R^3 is CH_3 by demethylation. This reaction is achieved by treating the 5-methoxy compound, or a suitably protected derivative thereof, with mercuric acetate and hydrolysing the resulting 3-acetoxy enol ether with dilute acid to give the 5-keto compound. This is then reduced using, for example, sodium borohydride to yield the 5-hydroxy derivative. Appropriate reagents and reaction conditions for these steps are described in United States patent 4423209.

The compounds of formula I wherein R^1 is H and the double bond is absent can be prepared from the corresponding compound wherein the double bond is present and R^1 is absent, by selective catalytic hydrogenation using an appropriate catalyst. For example the reduction may be achieved using tris(triphenylphosphine)rhodium (I) chloride as described in European patent application publication no. 0001689.

The compounds of formula (I) wherein R^4 is H are prepared from the corresponding compounds wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy by removing the 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrose group by mild hydrolysis with an acid in an aqueous organic solvent to yield the aglycone having a hydroxy group at the 13-position; this is then halogenated, for example by reaction with a benzene sulphonyl halide, to yield the 13-deoxy-13-halo derivative which is finally selectively reduced, for example using tributyltin hydride. In order to avoid unwanted side reactions it is desirable to protect any other hydroxy groups which may be present, for example using a *tert*-butyldimethylsilyl group. This is then readily removed after the halogenation or reduction step by treatment with methanol containing a trace of acid. All these steps together with appropriate reagents and reaction conditions for their performance are described in European patent application publication no. 0002615.

Compounds of the formula (I) wherein R^4 is H, R^1 is either H or OH and the double bond is absent, may also be prepared by adding the appropriate carboxylic acid, or a salt, ester or amide thereof or oxidative precursor therefor, to a fermentation of a milbemycin producing organism, and isolating the desired milbemycin derivative having an unnatural substituent group at the 25-position. Examples of milbemycin producing organisms include for instance *Streptomyces hygroscopicus* strain NRRL 5739 as described in British Patent Specification no. 1390336, *Streptomyces cyaneogriseus* subsp. noncyanogenus NRRL 15773 as described in European patent application publication no. 0170006 and *Streptomyces thermoarchaenis* NCIB 12015 as described in GB 2166436A.

The compounds of the invention are highly active antiparasitic agents having particular utility as anthelmintics, ectoparasiticides, insecticides and acaricides.

Thus the compounds are effective in treating a variety of conditions caused by endoparasites including, in particular, helminthiasis which is most frequently caused by a group of parasitic worms described as nematodes and which can cause severe economic losses in swine, sheep, horses and cattle as well as affecting domestic animals and poultry. The compounds are also effective against other nematodes which affect various species of animals including, for example, *Dirofilaria* in dogs and various parasites which can infect humans including gastro-intestinal parasites such as *Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, Enterobius* and parasites which are found in the blood or other tissues and organs such as filiarial worms and the extra intestinal stages of *Strongyloides* and *Trichinella.*

The compounds are also of value in treating ectoparasite infections including in particular arthropod

ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, biting insects and migrating dipterous larvae which can affect cattle and horses.

The compounds are also insecticides active against household pests such as the cockroach, clothes moth, carpet beetle and the housefly as well as being useful against insect pests of stored grain and of agricultural plants such as spider mites, aphids, caterpillars and against migratory orthopterans such as locusts.

The compounds of formula (I) are administered as a formulation appropriate to the specific use envisaged and to the particular species of host animal being treated and the parasite or insect involved. For use as an anthelmintic the compounds may be administered orally in the form of a capsule, bolus, tablet or preferably a liquid drench, or alternatively, they may be administered by injection or as an implant. Such formulations are prepared in a conventional manner in accordance with standard veterinary practice. Thus capsules, boluses or tablets may be prepared by mixing the active ingredient with a suitable finely divided diluent or carrier additionally containing a disintegrating agent and/or binder such as starch, lactose, talc, magnesium stearate etc. A drench formulation may be prepared by dispersing the active ingredient in an aqueous solution together with dispersing or wetting agents etc. and injectable formulations may be prepared in the form of a sterile solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. These formulations will vary with regard to the weight of active compound depending on the species of host animal to be treated, the severity and type of infection and the body weight of the host. Generally for oral administration a dose of from about 0.001 to 10 mg per Kg of animal body weight given as a single dose or in divided doses for a period of from 1 to 5 days will be satisfactory but of course there can be instances where higher or lower dosage ranges are indicated and such are within the scope of this invention.

As an alternative the compounds may be administered with the animal feedstuff and for this purpose a concentrated feed additive or premix may be prepared for mixing with the normal animal feed.

For use as an insecticide and for treating agricultural pests the compounds are applied as sprays, dusts, emulsions and the like in accordance with standard agricultural practice.

The invention is illustrated by the following Examples in which Examples 1 to 19 are Examples of the preparation of compounds of the formula (I), Example 20 is an example of a drench formulation and Examples 21 and 22 illustrate the antiparasitic and insecticidal activity of the compounds.

Example 1

25-Cyclopentyl-avermectin A2

A suspension of a slope culture of *S. avermitilis* NCIB 12121 was inoculated into 600 mls of a medium containing lactose (12.0 g), distillers solubles (8.0 g) and yeast extract (3.0 g), contained in a 3 litre flask, and incubated at 28°C for 3 days. The inoculum was used to inoculate 16 litres of a medium containing soluble starch (640 g), ammonium sulphate (32 g), dipotassium hydrogen phosphate (16 g), sodium chloride (16 g), magnesium sulphate $7H_2O$ (16 g), calcium carbonate (32 g), soluble yeast extract (6.4 g), ferrous sulphate $7H_2O$ (0.016 g), zinc sulphate $7H_2O$ (0.016 g) and manganese chloride $4H_2O$ (0.016 g), contained in a 20 litre fermenter. The fermentation was incubated at 28°C, with agitation at 250 r.p.m. and aerated at 15 litres per minute. Cyclopentane carboxylic acid sodium salt (1.6 g) was added after 24 hours and again after 48 and 72 hours incubation and the fermentation was continued for 120 hours. After this time the mycelium was removed by filtration and extracted with acetone: 1N-hydrochloric acid (100:1; 3 × 7 litres). The extract was concentrated to approximately 2 litres under reduced pressure and extracted with methylene chloride (2 × 5 litres). The methylene chloride extract was concentrated to dryness to give the crude product as a mobile oil which was dissolved in diethyl ether and added to a column of silica gel (1 kg). The column was eluted with diethyl ether collecting 100 ml fractions. Fractions 20—40 were combined and the solvent evaporated to yield partially purified material. The product was dissolved in a mixture of methanol and water (4:1) and chromatographed on a C18 Micro-Bondapack column (50 mm × 50 cm) in a Waters Prep 500 high pressure liquid chromatograph using the same solvent at a flow rate of 100 ml per minute. Fractions 35 to 50 containing the desired product were combined and rechromatographed on a C18 Zorbax ODS (Trademark, Dupont) column (21 mm × 25 cm) eluting with a mixture of methanol and water (4:1) at a flow rate of 9 mls. per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R^1 is OH, the double bond is absent, R^2 is cyclopentyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy as a white powder. m.p. 150.5—151°C. The structure of the product was confirmed by mass spectrometry and by C13 nuclear magnetic resonance spectroscopy as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 939 (theoretical 939).

Electron impact mas spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 335, 317, 275, 257, 251, 233, 205, 181, 179, 145, 127, 113, 111, 95 and 87.

The 13C nuclear magnetic resonance spectral data were obtained on a Brucker Model WM—250 spectrometer with a sample concentration of 20 mg/ml in deuterochloroform. The chemical shifts in parts per million relative to tetramethylsilane were: 14.1, 15.3, 17.8, 18.5, 19.9, 20.3, 24.6, 25.9, 26.2, 29.3, 34.4 (2C), 34.7, 36.7, 37.8, 39.8, 40.5, 41.0, 41.3, 45.8, 56.4, 56.6, 57.8, 67.4; 67.6, 68.0, 68.3, 68.7, 69.9, 70.5, 76.0,

77.6 (2C), 78.3, 79.5, 80.7 (2C), 81.8, 94.9, 98.7, 99.8, 117.7, 118.5, 119.8, 125.0, 135.8, 136.3, 137.8, 140.1 and 173.8.

Example 2

A suspension of a slope culture of *S. avermitilis* ATCC 31271 was inoculated into 50 mls of a medium containing lactose (1.0 g), distillers solubles (0.75 g) and yeast extract (0.25 g), contained in a 350 ml flask, and incubated at 28°C for 3 days. This inoculum (4 mls) was used to inoculate each of 50 flasks containing 50 mls of medium containing corn starch (2.0 g), soya flour (0.35 g) and yeast extract (0.25 g) contained in a 350 ml flask, and the flasks were incubated at 28°C.

After 24 hours, cyclopentane carboxylic acid sodium salt (5 mg) was added to each flask and incubation was continued for a further 5 days. After this time the contents of the flasks were bulked and the mycelium separated by centrifugation. The mycelium was extracted with acetone:1N-hydrochloric acid (100:1) and the acetone extract concentrated to dryness. The extract was analysed high pressure liquid chromatography and was shown to contain a product identical with the product of Example 1.

Example 3

An inoculum was prepared as described in Example 1 and used to inoculate 50 mls of the medium as used in Example 1, contained in 350 ml flasks. After incubation for 24 hours, 2-aminocyclopentyl acetic acid (cyclopentylglycine) (5 mg) was added and the fermentation was continued for a further 5 days. The product was recovered by extraction of the mycelium with acetone and methylene chloride. The extract was analysed by HPLC which indicated that the product contained a compound identical to the product of Example 1.

Example 4

The conditions of Example 3 were followed except that cyclopentyl methanol was used as substrate with similar results.

Example 5

The conditions of Example 3 were followed except that the methyl ester of cyclopentane carboxylic acid, dissolved in methanol, was used as substrate with similar results.

Example 6

The conditions of Example 3 were followed except that cyclopentane carboxylic acid, dissolved in methanol was used as substrate with similar results.

Example 7

25-(Thien-3-yl)avermectin

A suspension of a slope culture of *S. avermitilis* NCIB 12121 was inoculated into 600 mls of a medium containing lactose (12.0 g), distillers solubles (8.0 g) and yeast extract (3.0 g), contained in a 3 litre flask, and incubated at 28°C for 3 days. The inoculum was used to inoculate 16 litres of a medium containing soluble starch (640 g), ammonium sulphate (32 g), dipotassium hydrogen phosphate (16 g), sodium chloride (16 g), magnesium sulphate $7H_2O$ (16 g), calcium carbonate (32 g), soluble yeast extract (6.4 g), ferrous sulphate $7H_2O$ (0.016 g), zinc sulphate $7H_2O$ (0.016 g) and manganese chloride $4H_2O$ (0.016 g), contained in a 20 litre fermenter. The fermentation was incubated at 28°C, with agitation at 250 r.p.m. and aerated at 15 litres per minute. Thiophene-3-carboxylic acid sodium salt (1.6 g) was added after 24 hours and again after 48 and 72 hours incubation and the fermentation was continued for 120 hours. After this time the mycelium was removed by filtration and extracted with acetone: 1N-hydrochloric acid (100:1; 3 × 7 litres). The extract was concentrated to approximately 2 litres under reduced pressure and extracted with methylene chloride (2 × 5 litres). The methylene chloride extract was concentrated to dryness to give the crude product as a mobile oil which was dissolved in diethyl ether and added to a column of silica gel (1 kg). The column was eluted with diethyl ether collecting 200 ml fractions. Fractions 32—45 were combined and the solvent evaporated to yield partially purified material. The product was dissolved in a mixture of methanol and water (3:1) and chromatographed on a C18 Micro-Bondapack column (50 mm × 50 cm) in a Waters Prep 500 high pressure liquid chromatograph using the same solvent at a flow rate of 100 ml per minute. Fractions 27 to 36 containing the desired product were combined and rechromatographed on a C18 Zorbax ODS (Trademark, Dupont) column (21 mm × 25 cm) eluting with a mixture of methanol and water (3:1) at a flow rate of 9 mls. per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R^1 is OH, the double bond is absent, R^2 is thien-3-yl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy as a white powder. m.p. 167°C. The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectroscopy was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 953 (theoretical 953).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 349, 331, 275, 265, 257, 247, 237, 219, 195, 145, 127, 113, 95 and 87.

Example 8

A vegetative cell suspension of *S. avermitilis* NCIB 12121, held at −60°C in 10% v/v aqueous (2 mls) glycerol was inoculated into 50 ml of medium containing lactose (1.0 g), distillers solubles (0.75 g) and yeast extract (0.25 g) contained in a 300 ml conical flask and incubated at 28°C for 24 hours, with shaking. The inoculum was then added to 600 ml of the above medium contained in a 3 litre flask and the mixture was incubated at 28°C for 24 hours with shaking. The product was used to inoculate 10 litres of the above medium contained in a 16 litre fermenter which was incubated at 28°C for 24 hours at an agitation speed of 350 r.p.m. with aeration at 10 litres of air per minute. This fermentation (600 ml) was used to inoculate 16 litres of a medium containing partially hydrolysed starch (640 g) ammonium sulphate (32 g), dipotassium hydrogen phosphate (16 g), sodium chloride (16 g) magnesium sulphate $7H_2O$ (16 g), calcium carbonate (32 g), soluble yeast extract (6.4 g), ferrous sulphate $7H_2O$ (0.016 g), zinc sulphate $7H_2O$ (0.016 g), and manganese chloride $4H_2O$ (0.016 g), contained in a 20 litre fermenter. The fermentation was incubated at 28°C, with agitation at 350 r.p.m. and aerated at 15 litres per minute. Cyclobutane carboxylic acid sodium salt (1.6 g) was added after 24 hours and again after 48 and 72 hours incubation and the fermentation was continued for 120 hours. After this time the mycelium was removed by filtration and extracted with acetone (3 × 7 litres). The extract was concentrated to approximately 2 litres under reduced pressure and extracted with methylene chloride (2 × 5 litres). The methylene chloride was concentrated to dryness to give the crude product as a mobile oil. This was taken up in iso-octane (150 ml) and the solution extracted with a mixture of methanol (95 ml) and water (5 ml). Evaporation of the methanolic extract gave partially purified material which was separated into its individual components by high pressure liquid chromatography as follows: The residue was dissolved in a little methanol and chromatographed in a C18 Micro-Bondapack column (50 mm × 50 cm) in a Waters Prep 500 high pressure liquid chromatograph using a mixture of methanol/water (4:1) at a flow rate of 100 ml per minute. Fractions 1 to 4 were combined and used in Example 9, fractions 5 to 9 were combined and used in Example 10, fractions 10 to 19 were combined and used in Example 11 and fractions 20 to 35 were combined and used in Example 12.

Example 9

25-Cyclobutyl-avermectin B2 (R^1 = OH, R^3 = H)

The combined fractions 1 to 4 from Example 8 were evaporated to dryness and the residue was re-chromatographed on a C18 Zorbax ODS (Trademark, Dupont) column (21 mm × 25 cm) eluting with a mixture of methanol and water (3:1) at a flow rate of 9 mls per minute. The relevant fractions were combined, the solvent evaporated and the product subjected to a final purification on a Silica Spherisorb 5 micron (Trademark, HPLC Technology) column (10.5 mm × 25 cm) eluting with a mixture of methylene chloride and methanol (98:2) at a flow rate of 4 mls per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R^1 is OH, the double bond is absent, R^2 is cyclobutyl, R^3 is H and R^4 is 4′-(alpha-L-oleandrosyl)-L-oleandrosyloxy, as a white powder, m.p. 110—112°C. The structure of the product was confirmed by mass spectrometry as follows:—

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride, $(M + Na)^+$ observed at m/e 911 (theoretical 911).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 321, 303, 261, 257, 237, 219, 209, 191, 179, 167, 145, 127, 113, 111, 95 and 87.

Example 10

25-Cyclobutyl-avermectin A2 (R^1 = OH, R^3 = CH_3)

The combined fractions 5 to 9 from Example 8 were evaporated to dryness and the residue was re-chromatographed twice on a C18 Zorbax ODS (Trademark, Dupont) column, (21 mm × 25 cm) eluting with a methanol and water mixture (77:23) at a flow rate of 9 mls per minute. Suitable fractions were combined and evaporated to yield the compound of formula (I) wherein R_1 is OH, the double bond is absent, R^2 is cyclobutyl, R^3 is CH_3 and R^4 is 4′-(alpha-L-oleandrosyl)-L-oleandrosyloxy as a white powder m.p. 135—140°C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 925 (theoretical 925).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 596, 454, 321, 303, 275, 237, 219, 209, 191, 179, 167, 145, 127, 113, 111, 95 and 87.

Example 11

25-Cyclobutyl-avermectin B1 (22,23-Double bond present, R^3 = H)

The combined fractions 10 to 19 from Example 8 were evaporated to dryness and the residue dissolved in methanol and chromatographed on a C18 Zorbax ODS (Trademark, Dupont) column (21 mm × 25 cm) eluting with a mixture of methanol and water (4:1) at a flow rate of 9 mls per minute. The relevant fractions were combined and the solvent evaporated to give a product which was re-chromatographed on a Silica Zorbax SIL (Trademark, Dupont) column (21 mm × 25 cm) eluting with a mixture of dichloromethane and

methanol (98.5:1.5) at a flow rate of 9 mls per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R^1 is absent, the double bond is present, R^2 is cyclobutyl, R^3 is H and R^4 is 4'-(alpha-L-oleandrosyl)-L-oleandrosyloxy, as a white powder m.p. 135—138°C. The structure of the product was confirmed by mass spectrometry as follows:—

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 893 (theoretical 893).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 303, 261, 257, 219, 191, 167, 145, 127, 113, 111, 95 and 87.

Example 12

25-Cyclobutyl-avermectin A1 (22,23-Double bond present, $R^3 = CH_3$)

The combined fractions 20 to 35 from Example 8 were evaporated to dryness and the residue chromatographed on a C18 Zorbax ODS (Trademark, Dupont) column (21 mm × 25 cm) at a flow rate of 9 mls per minute. The relevant fractions were combined, the solvent evaporated and the product was re-chromatographed on a Silica Sperisorb 5 micron (Trademark, HPLC Technology) column (10.5 mm × 25 cm) eluting with a mixture of dichloromethane and methanol (98.5:1.5) at a flow rate of 4 mls per minute. Combination of the relevant fractions followed by evaporation gave the compound of formula (I) wherein R^1 is absent, the double bond is present, R^2 is cyclobutyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-L-oleandrosyloxy as a white powder m.p. 120—124°C. The structure of the product was confirmed by mass spectrometry as follows:—

Fast atoms bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 907 (theoretical 907).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 578, 303, 275, 257, 219, 191, 167, 145, 127, 113, 111, 95 and 87.

Example 13

25-(Cyclohex-3-enyl)avermectin A2

The medium and conditions of Example 1 were followed except that 3-cyclohexenoic acid sodium salt was used as the substrate to yield the compound of formula I wherein R^1 is OH, the double bond is absent, R^2 is cyclohex-3-enyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy as a white powder mpt. 131—5°C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 951 (theoretical 951).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 624, 480, 347, 329, 275, 263, 245, 235, 217, 205, 193, 179, 145, 127, 113, 111, 95 and 87.

Example 14

25-Cyclohexyl avermectin A2

The medium and conditions of Example 1 were followed except that cyclohexane carboxylic acid sodium salt was used as the substrate to yield the compound of formula I wherein R^1 is OH, R^2 is cyclohexyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy as a white powder mpt. 112—117°C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 953 (theoretical 953).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 624, 482, 349, 331, 275, 265, 247, 237, 219, 207, 195, 179, 145, 127, 113, 111, 95 and 87.

Example 15

25-(1-Methylthioethyl)avermectin A2

The medium and conditions of Example 1 were followed except that 2-methylthiopropionic acid sodium salt was used as the substrate to yield the compound of formula I wherein R_1 is OH, R^2 is 1-methylthioethyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-oleandrosyloxy as a white powder, m.p. 134—138°C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070F mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 945 (theoretical 945).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 341, 323, 275, 263, 257, 239, 211, 187, 179, 145, 127, 113, 111, 95 and 87.

Example 16

25-(2-Methylcyclopropyl)avermectin A2

The medium and conditions of Example 1 were followed except that 2-methylcyclopropane carboxylic acid sodium salt was used as the substrate to yield the compound of formula I wherein R^1 is OH, R^2 is 2-methylcyclopropyl, R^3 is CH_3 and R_4 is 4'-(alpha-L-oleandrosyl)-oleandrosyloxy, as a white powder, m.p. 147—150°C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070F mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M + Na)^+$ observed at m/e 925 (theoretical 925).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 596, 454, 303, 275, 237, 219, 209, 191, 179, 167, 145, 127, 113, 111, 95 and 87.

Example 17

The procedure of Example 1 was followed but using the sodium salt of the following carboxylic acids as substrate instead of cyclopentane carboxylic acid to yield the appropriate 25-substituted avermectins of formula (I) wherein R^1 is OH and the double bond is absent or wherein the double bond is present and R^1 is absent, R^3 is H or OH and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy:

2-methylvaleric acid
2,3-dimethylbutyric acid
2-methylhexanoic acid
2-methylpent-4-enoic acid
2-methylpentanoic acid
2-cyclopropyl propionic acid
cycloheptane carboxylic acid
4,4-difluorocyclohexane carboxylic acid
4-methylenecyclohexane carboxylic acid
3-methylcyclohexane carboxylic acid
cyclopentene-1-carboxylic acid
1-cyclohexene carboxylic acid
tetrahydropyran-4-carboxylic acid
thiophene-2-carboxylic acid
3-furoic acid

and 2-chloro-thiophene-4-carboxylic acid.

Example 18

25-Cyclobutyl-22,23-dihydro-avermectin B1

The product of Example 11 in benzene is hydrogenated in the presence of tris(triphenylphosphine)-rhodium (I) chloride according to the procedure of EP—A—0001689 to yield the corresponding compound of formula (I) wherein R^1 is H and the double bond is absent:

Example 19

13-Deoxy-25-cyclopentyl-avermectin A2-aglycone

The product of Example 1 is treated with dilute sulphuric acid at room temperature and the resulting aglycone product is isolated and reacted with t-butyldimethylsilylchloride in dimethylformamide to provide the 23-O-t-butyldimethylsilyl aglycone derivative. This is dissolved in methylene chloride containing 4-dimethylaminopyridine and diisopropylethylamine, cooled in ice and treated dropwise with 4-nitrobenzenesulphonylchloride to yield the 13-chloro-13-deoxy product. This is finally dehalogenated by reaction with tributyltinhydride and deprotected with methanol containing a trace of para-toluene sulphonic acid following the procedures described in EP—A—0002615 to provide the compound of the formula I wherein R^1 and R^4 are each H, R^3 is OH, the double bond is absent and R^2 is cyclopentyl.

Example 20

Drench Formulation

The product of any one of the preceding Examples was dissolved in polyethylene glycol (average molecular weight 300) to give a solution containing 400 micrograms/ml for use as a drench formulation.

Example 21

Anthelmintic Activity

Anthelmintic activity was evaluated against *Caenorhabditis elegans* using the *in vitro* screening test described by K. G. Simpkin and G. L. Coles in Parisitology, 1979, *79,* 19. The products of Examples 1, 7 and 9—16 all killed 100% of the worms at a well concentration of 0.1 micrograms per ml.

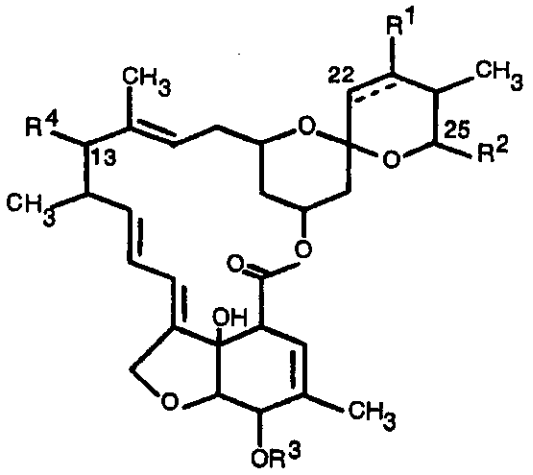
Example 22

Insecticidal Activity

Activity against adult house fly *Musca domestica* is demonstrated using a standard test procedure in which the flies are anaesthetised under carbon dioxide and 0.1 microlitres of acetone containing the test compound is deposited on the thorax of female flies. The product of Examples 1, 7 and 9—16 all killed 100% of the treated flies at a dose of 0.01 micrograms per fly.

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

1. A compound having the formula:



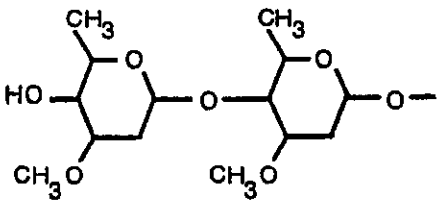
(I)

wherein the broken line at the 22—23 position represents an optional double bond and wherein R^1 is H or OH and the double bond is absent, or, the double bond is present and R^1 is absent;

R^2 is an alpha-branched C_3—C_8 alkyl, alkenyl, alkoxyalkyl or alkylthioalkyl group; an alpha-branched C_4—C_8 alkynyl group; a (C_5—C_8 cycloalkyl)alkyl group wherein the alkyl group is an alpha-branched C_2—C_5 alkyl group; a C_3—C_8 cycloalkyl or C_5—C_8 cycloalkenyl group, either of which may optionally be substituted by methylene or one or more C_1—C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C_1—C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;

R^4 is H or a 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group of the formula:



with the proviso that when R^2 is alkyl it is not isopropyl or sec-butyl, and when R^4 is H, R^2 is not 2-buten-2-yl, 2-penten-2-yl or 4-methyl-2-penten-2-yl.

2. A compound of the formula I as claimed in claim 1 wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy.

3. A compound as claimed in claim 2 wherein R^2 is a C_5 or C_6 cycloalkyl or cycloalkenyl group which may optionally be substituted by one or more C_1—C_4 alkyl groups.

4. A compound as claimed in claim 3 wherein R^2 is cyclopentyl.

5. A compound as claimed in claim 3 wherein R^2 is cyclohexyl.

6. A compound as claimed in claim 2 wherein R^2 is cyclobutyl.

7. A compound as claimed in claim 2 wherein R^2 is a 5 or 6 membered oxygen or sulphur containing heterocyclic ring which may optionally be substituted by one or more C_1—C_4 alkyl groups or halogen atoms.

8. A compound as claimed in claim 7 wherein R^2 is 3-thienyl.

9. A compound as claimed in claim 2 wherein R^2 is C_3—C_8 alkylthioalkyl group.

10. A compound as claimed in claim 9 wherein R^2 is 1-methylthioethyl.

11. A process for producing a novel avermectin derivative having an unnatural substituent group at the 25-position which comprises adding a carboxylic acid, or a salt, ester or amide thereof or oxidative precursor therefor, to a fermentation of an avermectin producing strain of the organism *Streptomyces avermitilis* and isolating the novel avermectin derivative.

12. A process for producing a compound of the formula (I) as claimed in claim 1 which comprises fermenting an avermectin producing strain of the organism *Streptomyces avermitilis* in the presence of a carboxylic acid of the formula R^2CO_2H wherein R^2 is as defined in claim 1, or a salt, ester or amide thereof or oxidative precursor therefor, and isolating the compound of formula (I) wherein R^1 is OH and the double bond is absent or wherein the double bond is present and R^1 is absent and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy and, if desired, reducing the compound wherein the double bond is present and R^1 is absent to obtain the compound of formula (I) wherein R^1 is H and the double bond is absent or, if desired, removing the 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group by hydrolysis followed by halogenation and reduction to yield the compound of formula (I) wherein R^4 is H.

13. A process as claimed in claim 12 wherein the organism is *Streptomyces* avermitilis NCIB 12121.

14. A composition for the treatment and prevention of parasitic infections in humans and animals, including ectoparasiticidal, insecticidal, acaricidal and anthelmintic compositions, which comprises a compound of the formula (I) as claimed in any one of claims 1 to 10 together with an inert diluent or carrier.

15. A composition as claimed in claim 14 in the form of a liquid drench or an oral or injectable formulation.

16. A composition as claimed in claim 14 in the form of an animal feedstuff or in the form of a premix or supplement for addition to animal feed.

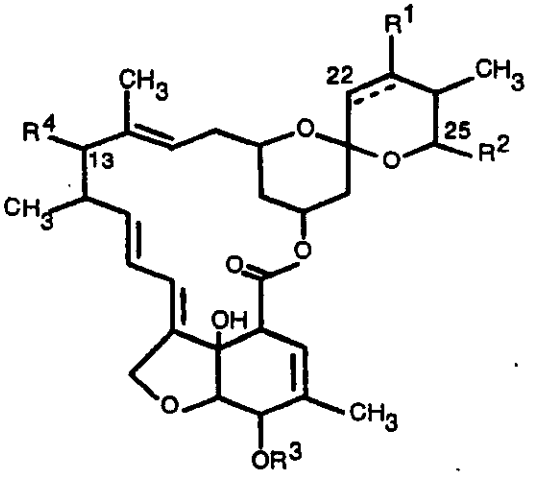
17. A compound of the formula I as claimed in any one of claims 1 to 10 or a composition thereof as claimed in claims 14 to 16 for use in the treatment or prevention of parasitic infections in humans and animals.

18. A method of combating insect or parasite infections or infestations, including parasitic conditions in non-human animals and agricultural or horticultural pest infestations, which comprises applying an effective amount of a compound of the formula (I) as claimed in any one of claims 1 to 10 to the organism responsible for said infection or infestation or to the location thereof.

Claims for the Contracting State: AT

1. A process for producing a novel avermectin derivative having an unnatural substituent group at the 25-position which comprises adding a carboxylic acid, or a salt, ester or amide thereof or oxidative precursor therefor, to a fermentation of an avermectin producing strain of the organism *Streptomyces avermitilis,* and isolating the novel avermectin derivative.

2. A process for producing a compound of the formula:

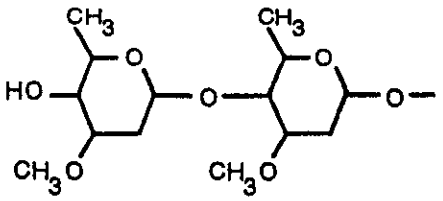


(I)

wherein the broken line at the 22—23 position represents an optional double bond and wherein R^1 is H or OH and the double bond is absent, or, the double bond is present and R^1 is absent;

R^2 is an alpha-branched C_3—C_8 alkyl, alkenyl, alkoxyalkyl or alkylthioalkyl group; an alpha-branched C_4—C_8 alkynyl group; a (C_5—C_8 cycloalkyl)alkyl group wherein the alkyl group is an alpha-branched C_2—C_5 alkyl group; a C_3—C_8 cycloalkyl or C_5—C_8 cycloalkenyl group, either of which may optionally be substituted by methylene or one or more C_1—C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C_1—C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;
R^4 is H or a 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group of the formula:



with the proviso that when R^2 is alkyl it is not isopropyl or sec-butyl, and when R^4 is H, R^2 is not 2-buten-2-yl, 2-penten-2-yl or 4-methyl-2-penten-2-yl; which comprises fermenting an avermectin producing strain of the organism *Streptomyces avermitilis* in the presence of a carboxylic acid of the formula R^2CO_2H wherein R^2 is as previously defined, or a salt, ester or amide thereof or oxidative precursor therefor, and isolating the compound of formula (I) wherein R^1 is OH and the double bond is absent or wherein the double bond is present and R^1 is absent and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy and, if desired, reducing the compound wherein the double bond is present and R^1 is absent to obtain the compound of formula (I) wherein R^1 is H and the double bond is absent or, if desired, removing the 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group by hydrolysis followed by halogenation and reduction to yield the compound of formula (I) wherein R^4 is H.

3. A process as claimed in claim 2 wherein the organism is *Streptomyces avermitilis* NCIB 12121.

4. A process as claimed in claim 2 wherein the acid is added as a salt.

5. A process as claimed in claim 2 wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy and the optional removal step is not performed.

6. A process as claimed in claim 5 wherein R^2 is a C_5 or C_6 cycloalkyl or cycloalkenyl group which may optionally be substituted by one or more C_1—C_4 alkyl groups.

7. A process as claimed in claim 6 wherein R^2 is cyclopentyl.

8. A process as claimed in claim 5 wherein R^2 is cyclohexyl.

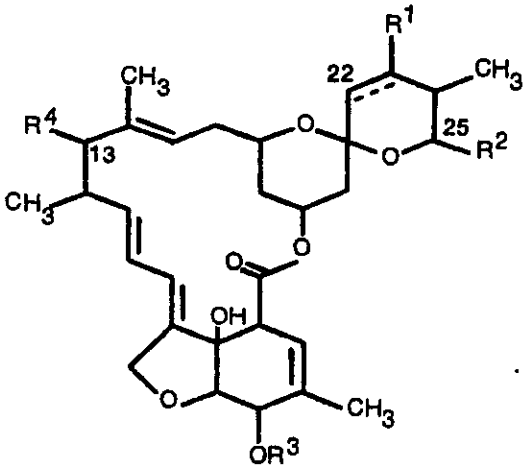
9. A process as claimed in claim 5 wherein R^2 is cyclobutyl.

10. A process as claimed in claim 5 wherein R^2 is 3-thienyl.

11. A process as claimed in claim 5 wherein R^2 is 1-methylthioethyl.

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

1. Verbindung der Formel



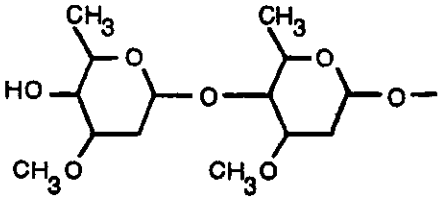
(I)

worin

die gestrichelte Linie an der 22-23-Position eine fakultative Doppelbindung repräsentiert und worin R^1 gleich H oder OH ist und die Doppelbindung fehlt oder worin die Doppelbindung vorliegt und R^1 fehlt,

R^2 eine alpha-verzweigte C_3—C_8-Alkyl-, —Alkenyl-, -Alkoxyalkyl- oder -Alkylthioalkylgruppe, eine alpha-verzweigte C_4—C_8-Alkinylgruppe, eine (C_5—C_8-Cycloalkyl)alkylgruppe, worin die Alkylgruppe eine alpha-verzweigte C_2—C_5-Alkylgruppe ist, eine C_3—C_8-Cycloalkyl- oder C_5—C_8-Cycloalkenylgruppe, von denen jede fakultativ mit Methylen oder einer oder mehreren C_1—C_4-Alkylgruppen oder Halogenatomen substituiert sein kann, oder ein 3- bis 6-gliedriger, Sauerstoff oder Schwefel enthaltender, heterocyclischer Ring ist, der gesättigt oder vollständig oder teilweise ungesättigt sein kann und der fakultativ durch eine oder mehrere C_1—C_4-Alkylgruppen oder Halogenatome substituiert sein kann,

R^3 Wasserstoff oder Methyl ist,
R^4 gleich H oder eine 4'-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy-Gruppe mit der Formel:



ist, unter der Voraussetzung, daß, wenn R^2 Alkyl ist, es nicht Isopropyl oder sec.-Butyl ist, und wenn R^4 gleich H ist, R^2 nicht 2-Buten-2-yl, 2-Penten-2-yl oder 4-Methyl-2-penten-2-yl ist.

2. Verbindung der Formel I gemäß Anspruch 1, worin R^4 4'-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy ist.

3. Verbindung gemäß Anspruch 2, worin R^2 eine C_5- oder C_6-Cycloalkyl- oder -Cycloalkylengruppe ist, die fakultativ mit einer oder mehreren C_1—C_4-Alkylgruppen substituiert sein kann.

4. Verbindung gemäß Anspruch 3, worin R^2 Cyclopentyl ist.

5. Verbindung gemäß Anspruch 3, worin R^2 Cyclohexyl ist.

6. Verbindung gemäß Anspruch 2, worin R^2 Cyclobutyl ist.

7. Verbindung gemäß Anspruch 2, worin R^2 ein 5- oder 6-gliedriger, Sauerstoff oder Schwefel enthaltender, heterocylischer Ring ist, der fakultativ durch eine oder mehrere C_1—C_4-Alkylgruppen oder Halogenatome substituiert sein kann.

8. Verbindung gemäß Anspruch 7, worin R^2 3-Thienyl ist.

9. Verbindung gemäß Anspruch 2, worin R^2 eine C_3—C_8-Alkylthioalkylgruppe ist.

10. Verbindung gemäß Anspruch 9, worin R^2 1-Methylthioethyl ist.

11. Verfahren zur Herstellung eines neuen Avermectin-Derivates mit einer ungewöhnlichen Substituentengruppe in der 25-Position, das das Zugeben einer Carbonsäure oder eines Salzes, Esters oder Amids hiervon oder eines oxidativen Vorläufers hierfür zu einer Fermentation eines Avermectin produzierenden Stammes des Organismus *Streptomyces avermitilis* und Isolieren des neuen Avermectin-Derivates umfaßt.

12. Verfahren zur Herstellung einer Verbindung der Formel I gemäß Anspruch 1, das das Fermentieren eines Avermectin produzierenden Stammes des Organismus *Streptomyces avermitilis* in Gegenwart einer Carbonsäure der Formel R^2CO_2H, worin R^2 wie in Anspruch 1 definiert ist, oder eines Salzes, Esters oder Amids hiervon oder eines oxidativen Vorläufers hierfür und das Isolieren der Verbindung der Formel (I), worin R^1 OH ist und die Doppelbindung fehlt oder worin die Doppelbindung vorliegt und R^1 fehlt und R^4 4'-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy ist, und falls gewünscht, das Reduzieren der Verbindung, worin die Doppelbindung vorliegt und R^1 fehlt, zur Bildung einer Verbindung der Formel (I), worin R^1 gleich H ist und die Doppelbindung fehlt, oder, falls gewünscht, das Entfernen der 4-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy-Gruppe durch Hydrolyse, gefolgt vom Halogenieren und Reduzieren zur Bildung der Verbindung der Formel (I), worin R^4 gleich H ist, umfaßt.

13. Verfahren gemäß Anspruch 12, worin der Organismus *Streptomyces avermitilis* NCIB 12121 ist.

14. Zusammensetzung zur Behandlung und Verhütung parasitärer Infektionen bei Mensch und Tier, einschließlich ectoparasitärer, insektizider, acarizider und anthelminthischer Zusammensetzungen, die eine Verbindung der Formel I gemäß irgendeinem der Ansprüche 1 bis 10 zusammen mit einem inerten Verdünnungsmittel oder Träger umfaßt.

15. Zusammensetzung nach Anspruch 14 in Form eines Arzneitrankes oder einer oralen oder injizierbaren Formulierung.

16. Zusammensetzung gemäß Anspruch 14 in Form einer Tiernahrung oder in Form einer Vormischung oder eines Zusatzes für die Zugabe zur Tiernahrung.

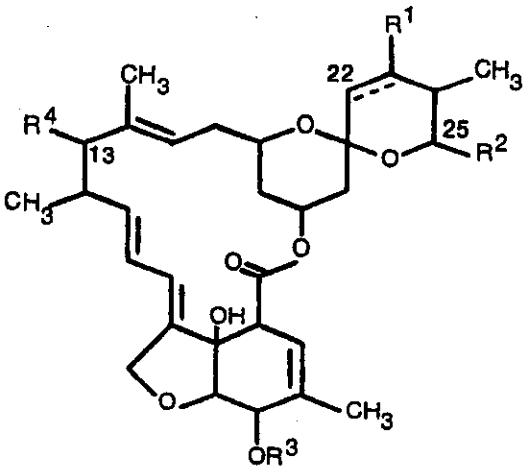
17. Verbindung der Formel I gemäß irgendeinem der Ansprüche 1 bis 10 oder eine Zusammensetzung derselben gemäß den Ansprüchen 14 bis 16 zur Verwendung bei der Behandlung oder Verhütung parasitärer Infektionen bei Mensch und Tier.

18. Verfahren zur Bekämpfung von Infektionen oder einem Befall durch Insekten oder Parasiten einschließlich parasitärer Erkrankungen bei Tieren, den Menschen ausgeschlossen, und von Schädlingsbefall in der Landwirtschaft oder im Gartenbau, das das Aufbringen einer wirksamen Menge einer Verbindung der Formel I gemäß irgendeinem der Ansprüche 1 bis 10 auf den Organismus, der für die Infektion oder den Befall verantwortlich ist, oder auf den Ort des Befalles umfaßt.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung eines neuen Avermectin-Derivates, das eine ungewöhnliche Substituentengruppe in 25-Position besitzt, welches die Zugabe einer Carbonsäure oder eines Salzes, Esters oder Amids hiervon oder eines oxidativen Vorläufers hierfür zu einer Fermentation eines Avermectin produzierenden Stammes des Organismus *Streptomyces avermitilis* und Isolieren des neuen Avermectin-Derivates.

2. Verfahren zur Herstellung einer Verbindung mit der Formel:



(I)

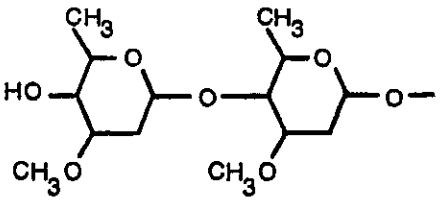
worin

die gestrichelte Linie an der 22-23-Position eine fakultative Doppelbindung repräsentiert und worin R^1 gleich H oder OH ist und die Doppelbindung fehlt oder worin die Doppelbindung vorliegt und R^1 fehlt,

R^2 eine alpha-verzweigte C_3—C_8-Alkyl-, —Alkenyl-, -Alkoxyalkyl- oder -Alkylthioalkylgruppe, eine alpha-verzweigte C_4—C_8-Alkinylgruppe, eine (C_5—C_8-Cycloalkyl)alkylgruppe, worin die Alkylgruppe eine alpha-verzweigte C_2—C_5-Alkylgruppe ist, eine C_3—C_8-Cycloalkyl- oder C_5—C_8-Cycloalkenylgruppe, von denen jede fakultativ mit Methylen oder einer oder mehreren C_1—C_4-Alkylgruppen oder Halogenatomen substituiert sein kann, oder ein 3- bis 6-gliedriger, Sauerstoff oder Schwefel enthaltender, heterocyclischer Ring ist, der gesättigt oder vollständig oder teilweise ungesättigt sein kann und der fakultativ durch eine oder mehrere C_1—C_4-Alkylgruppen oder Halogenatome substituiert sein kann,

R^3 Wasserstoff oder Methyl ist,

R^4 gleich H oder eine 4'-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy-Gruppe mit der Formel:



ist, unter der Voraussetzung, daß, wenn R^2 Alkyl ist, es nicht Isopropyl oder sec.-Butyl ist, und wenn R^4 gleich H ist, R^2 nicht 2-Buten-2-yl, 2-Penten-2-yl oder 4-Methyl-2-penten-2-yl ist, welches das Fermentieren eines Avermectin produzierenden Stammes des Organismus *Streptomyces avermitilis* in Gegenwart einer Carbonsäure mit der Formel R^2CO_2H, worin R^2 wie vorstehend definiert ist, oder eines Salzes, Esters oder Amids hiervon oder eines oxidativen Vorläufers hierfür und das Isolieren der Verbindung mit der Formel (I), worin R^1 OH ist und die Doppelbindung fehlt, oder worin die Doppelbindung vorhanden ist und R^1 fehlt, und R^4 4'-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy ist, und auf Wünsch das Reduzieren der Verbindung, in der die Doppelbindung vorhanden ist und R^1 fehlt, um die Verbindung mit der Formel (I) zu gewinnen, worin R^1 gleich H ist und die Doppelbindung fehlt, oder auf Wünsch das Entfernen der 4-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy-Gruppe durch Hydrolyse und anschließendes Halogenieren und Reduzieren, um die Verbindungen mit der Formel (I), in der R^4 gleich H ist, zu gewinnen, umfaßt.

3. Verfahren gemäß Anspruch 2, worin der Organismus *Streptomyces avermitilis* NCIB 12121 ist.

4. Verfahren gemäß Anspruch 2, worin die Säure als Salz zugesetzt wird.

5. Verfahren gemäß Anspruch 2, worin R^4 4'-(alpha-L-Oleandrosyl)-alpha-L-oleandrosyloxy ist und der Fakultative Entfernungsschritt nicht durchgeführt wird.

6. Verfahren gemäß Anspruch 5, worin R^2 eine C_5- oder C_6-Cycloalkyl- oder -Cycloalkenylgruppe ist, die fakultativ durch eine oder mehrere C_1—C_4-Alkylgruppen substituiert sein kann.

7. Verfahren gemäß Anspruch 6, worin R^2 Cyclopentyl ist.

8. Verfahren gemäß Anspruch 5, worin R^2 Cyclohexyl ist.

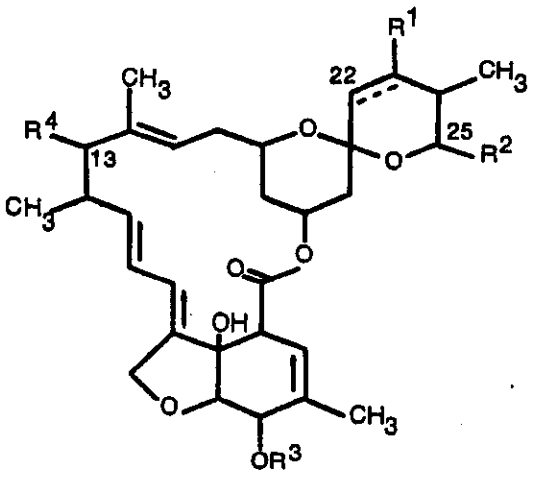
9. Verfahren gemäß Anspruch 5, worin R^2 Cyclobutyl ist.

10. Verfahren gemäß Anspruch 5, worin R^2 3-Thienyl ist.

11. Verfahren gemäß Anspruch 5, worin R^2 1-Methylthioethyl ist.

Revendications pour les Etats contractants: BE CH DE FR GB IT LI LU NL SE

1. Composé de formule:



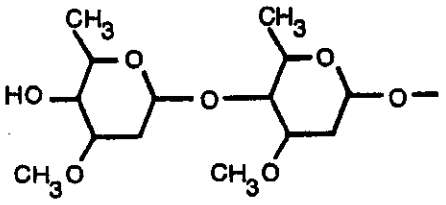
(I)

dans laquelle la ligne en pointillés en position 22—23 représente une double liaison facultative et dans laquelle soit R^1 représente H ou OH, auquel cas la double liaison n'existe pas, soit la double liaison existe et R^1 n'existe pas;

R^2 est un groupe alkyle, alkényle, alcoxyalkyle ou alkylthioalkyle en C_3—C_8 ramifié en alpha; un groupe alkynyle en C_4—C_8 ramifié en alpha; un groupe alkyl(cycloalkyl en C_5—C_8) dans lequel le groupe alkyle est un groupe alkyle en C_2—C_5 ramifié en alpha; un groupe cycloalkyle en C_3—C_8 ou cycloalkényle en C_5—C_8, l'un ou l'autre pouvant être éventuellement substitués par un groupe méthylène ou un ou plusieurs groupes alkyle en C_1—C_4 ou atomes d'halogène; ou un noyau hétérocyclique ayant de 3 à 6 chaînons et contenant de l'oxygène ou du soufre, lequel noyau peut être saturé ou totalement ou partiellement insaturé et qui peut être éventuellement substitué par un ou plusieurs groupes alkyle en C_1—C_4 ou atomes d'halogène;

R^3 représente l'hydrogène ou un groupe méthyle;

R^4 représente H ou un groupe 4'-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy de formule:



étant entendu que lorsque R^2 représente un groupe alkyle, il ne s'agit pas d'un groupe isopropyle ou sec-butyle, et lorsque R^4 représente H, R^2 ne représente pas un groupe 2-butèn-2-yle, 2-pentèn-2-yle ou 4-méthyl-2-pentèn-2-yle.

2. Composé de formule (I) selon la revendication 1, dans laquelle R^4 est un groupe 4'-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy.

3. Composé selon la revendication 2, dans lequel R^2 est un groupe cycloalkyle ou cycloalkényle en C_5 ou C_6 qui peut être éventuellement substitué par un ou plusieurs groupes alkyle en C_1—C_4.

4. Composé selon la revendication 3, dans lequel R^2 est un groupe cyclopentyle.

5. Composé selon la revendication 3, dans lequel R^2 est un groupe cyclohexyle.

6. Composé selon la revendication 2, dans lequel R^2 est un groupe cyclobutyle.

7. Composé selon la revendication 2, dans lequel R^2 est un noyau hétérocyclique à 5 ou 6 chaînons contenant de l'oxygène ou du soufre qui peut être éventuellement substitué par un ou plusieurs groupes alkyle en C_1—C_4 ou atomes d'halogène.

8. Composé selon la revendication 7, dans lequel R^2 est un groupe 3-thiényle.

9. Composé selon la revendication 2, dans lequel R^2 est un groupe alkylthioalkyle en C_3—C_8.

10. Composé selon la revendication 9, dans lequel R^2 est un groupe 1-méthylthioéthyle.

11. Procédé de production d'un nouveau dérivé de l'avermectine ayant un groupe substituant artificiel en position-25 qui consiste à ajouter un acide carboxylique, ou un sel, ester ou amide ou précurseur oxydatif de celui-ci, à un milieu de fermentation d'une souche de l'organisme *Streptomyces avermitilis* produisant l'avermectine et à isoler le nouveau dérivé de l'avermectine.

12. Procédé de production d'un composé de formule (I) selon la revendication 1, qui consiste à faire fermenter une souche productrice d'avermectine de l'organisme *Streptomyces avermitilis* en présence d'un acide carboxylique de formule R^2CO_2H dans laquelle R^2 est tel que défini dans la revendication 1, ou un sel, ester ou amide ou précurseur oxydatif d'un tel composé, et à isoler le composé de formule (I) dans lequel soit R^1 représente OH auquel cas la double liaison n'existe pas, soit la double liaison existe et R^1 n'existe pas, et R^4 est un groupe 4'-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy et, si désiré, à réduire le composé lorsqu'il présente une double liaison et que R^1 n'existe pas pour obtenir le composé de formule (I) dans lequel R^1 représente H et la double liaison est inexistante ou, si on le souhaite, à éliminer le groupe 4-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy par hydrolyse suivie d'une halogénation et d'une réduction pour donner le composé de formule (I) dans lequel R^4 représente H.

13. Procédé selon la revendication 12, dans lequel l'organisme est *Streptomyces avermitilis* NCIB 12121.

14. Composition pour le traitement et la prévention d'infections à parasites chez l'homme et l'animal, comprenant les compositions ectoparasiticides, insecticides, acaricides et anthelmintiques qui renferment un composé de formule (I) selon l'une quelconque des revendications 1 à 10 avec un diluant ou un véhicule inerte.

15. Composition selon la revendication 14, sous la forme d'un breuvage liquide ou d'une formulation pour l'administration par voie orale ou injectable.

16. Composition selon la revendication 14 sous la forme d'un aliment pour animaux ou sous la forme d'un prémélange ou d'un supplément pour l'adjonction à l'alimentation animale.

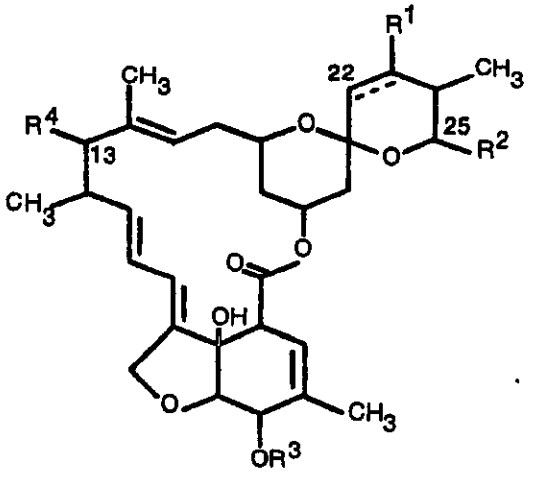
17. Composé de formule (I) selon l'une quelconque des revendications 1 à 10, ou composition d'un tel composé selon les revendications 14 à 16, destinés au traitement ou à la prévention d'infections à parasites chez l'homme et l'animal.

18. Procédé de lutte contre les infections et les infestations par les insectes ou les parasites, y compris les infections par les parasites chez les animaux, l'homme excepté, et les infections par les parasites agricoles ou horticoles, qui consiste à appliquer une quantité efficace d'un composé de formule (I) selon l'une quelconque des revendications 1 à 10 sur l'organisme responsable de ladite infection ou infestation ou de son lieu de manifestation.

Revendications pour l'Etat contractant: AT

1. Procédé de production d'un nouveau dérivé d'avermectine ayant un groupe substituant artificiel en position-25 qui consiste à ajouter un acide carboxylique un sel, un ester, un amide ou un précurseur oxydatif du celui-ci à un milieu de fermentation d'une souche productrice d'avermectine de l'organisme *Streptomyces avermitilis* et à isoler le nouveau dérivé d'avermectine.

2. Procédé de production d'un composé de formule:



(I)

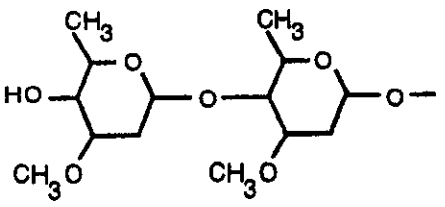
dans laquelle la ligne en pointillés en position 22—23 représente une double liaison facultative et dans laquelle soit R^1 représente H ou OH, auquel cas la double liaison n'existe pas, soit la double liaison existe et R^1 n'existe pas;

R^2 est un groupe alkyle, alkényle, alcoxyalkyle ou alkylthioalkyle en C_3—C_8 ramifié en alpha; un groupe alkynyle en C_4—C_8 ramifié en alpha; un groupe alkyl(cycloalkyl en C_5—C_8) dans lequel le groupe alkyle est un groupe alkyle en C_2—C_5 ramifié en alpha; un groupe cycloalkyle en C_3—C_8 ou cycloalkényle en C_5—C_8, l'un ou l'autre pouvant être éventuellement substitués par un groupe méthylène ou un ou plusieurs groupes alkyle en C_1—C_4 ou atomes d'halogène; ou un noyau hétérocyclique ayant de 3 à 6 chaînons et contenant de l'oxygène ou du soufre, lequel noyau peut être saturé ou totalement ou partiellement insaturé

et qui peut être éventuellement substitué par un ou plusieurs groupes alkyle en C_1—C_4 ou atomes d'halogène;

R^3 représente l'hydrogène ou un groupe méthyle;

R^4 représente H ou un groupe 4'-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy de formule:



étant entendu que lorsque R^2 représente un groupe alkyle, il ne s'agit pas d'un groupe isopropyle ou sec-butyle, et lorsque R^4 représente H, R^2 ne représente pas un groupe 2-butèn-2-yle, 2-pentèn-2-yle ou 4-méthyl-2-pentèn-2-yle, qui consiste à faire fermenter une souche productrice d'avermectine de l'organisme *Streptomyces avermitilis* en présence d'un acide carboxylique de formule R^2CO_2H dans laquelle R^2 est tel que précédemment défini, ou un sel, ester, amide ou précurseur oxydatif d'un tel composé, et à isoler le composé de formule (I) dans lequel soit R^1 représente OH auquel cas la double liaison n'existe pas, soit la double liaison existe et R^1 n'existe pas, et R^4 est un groupe 4'-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy et, si désiré, à réduire le composé lorsqu'il présente une double liaison et que R^1 n'existe pas, pour obtenir le composé de formule (I) dans lequel R^1 représente H et la double liaison est inexistante ou, si on le souhaite, à éliminer le groupe 4-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy suivie par hydrolyse d'une halogénation et d'une réduction pour donner le composé de formule (I) dans lequel R^4 représente H.

3. Procédé selon la revendication 2, dans lequel l'organisme est *Streptomyces avermitilis* NCIB 12121.

4. Procédé selon la revendication 2, dans lequel l'acide est ajouté sous la forme d'un sel.

5. Procédé selon la revendication 2, dans lequel R^4 est un radical 4'-(alpha-L-oléandrosyl)-alpha-L-oléandrosyloxy et en ce que l'étape facultative d'élimination n'est pas mise en oeuvre.

6. Procédé selon la revendication 5, dans lequel R^2 est un groupe cycloalkyle ou cycloalkényle en C_5 ou C_6 qui peut être éventuellement substitué par un ou plusieurs groupes alkyle en C_1—C_4.

7. Procédé selon la revendication 6, dans lequel R^2 est un groupe cyclopentyle.

8. Procédé selon la revendication 5, dans lequel R^2 est un groupe cyclohexyle.

9. Procédé selon la revendication 5, dans lequel R^2 est un groupe cyclobutyle.

10. Procédé selon la revendication 5, dans lequel R^2 est un groupe 3-thiényle.

11. Procédé selon la revendication 5, dans lequel R^2 est un groupe 1-méthylthioéthyle.

REGISTER ENTRY FOR EP0214731

European Application No EP86305604.0 filing date 22.07.1986

Priorities claimed:
27.07.1985 in United Kingdom - doc: 8518999
09.08.1985 in United Kingdom - doc: 8520069
24.04.1986 in United Kingdom - doc: 8610063
02.05.1986 in United Kingdom - doc: 8610862

Designated States BE CH DE FR GB IT LI LU NL SE AT

Title ANTIPARASITIC AVERMECTIN AND MILBEMYCIN DERIVATIVES AND PROCESS FOR THEIR PREPARATION

Applicant/Proprietor
PFIZER LIMITED, Ramsgate Road, Sandwich Kent CT13 9NJ, United Kingdom [ADP No. 50601020001]

Inventors
STEPHEN PAUL GIBSON, 25, George V Avenue, Westbrook Margate Kent, United Kingdom [ADP No. 54673843001]

KELVIN SCOTT HOLDOM, 9, Tothill Street, Minster Ramsgate Kent, United Kingdom [ADP No. 54673850001]

ALEXANDER CROSSAN GOUDIE, 1, Hildersham Close, St. Peters Broadstairs Kent, United Kingdom [ADP No. 54673868001]

JOHN DESMOND BU'LOCK, 205, Stockport Road, Marple Manchester, United Kingdom [ADP No. 54673876001]

Classified to
C2C C6Y U1S
C07D A01N A23K A61K C07H C12P

Address for Service
DR. JAMES WILLIAM MOORE, Pfizer Limited Ramsgate Road, Sandwich Kent CT13 9NJ, United Kingdom [ADP No. 50178987001]

EPO Representative
DR. JAMES WILLIAM MOORE, Pfizer Limited Ramsgate Road, Sandwich Kent CT13 9NJ, United Kingdom [ADP No. 50178987001]

Publication No EP0214731 dated 18.03.1987
Publication in English

Examination requested 22.07.1986

Patent Granted with effect from 02.01.1991 (Section 25(1)) with title ANTIPARASITIC AVERMECTIN AND MILBEMYCIN DERIVATIVES AND PROCESS FOR THEIR PREPARATION.

23.07.1987 EPO: Search report published on 22.07.1987
Entry Type 25.11 Staff ID. Auth ID. EPT

03.12.1990 Notification from EPO of change of Applicant/Proprietor details
from
PFIZER LIMITED, Ramsgate Road, Sandwich Kent CT13 9NJ, United Kingdom [ADP No. 50601020001]
to
PFIZER LIMITED, Ramsgate Road, Sandwich Kent CT13 9NJ, United Kingdom [ADP No. 50601020001]
Entry Type 25.14 Staff ID. RD06 Auth ID. EPT

21.10.1991 EPO: Opposition filed on 01.10.1991
Entry Type 25.1 Staff ID. RD06 Auth ID. EPT

**** END OF REGISTER ENTRY ****

RENEWAL DETAILS

PUBLICATION NUMBER EP0214731

PROPRIETOR(S)

Pfizer Limited, Ramsgate Road, Sandwich Kent CT13 9NJ, United Kingdom

DATE FILED 22.07.1986

DATE GRANTED 02.01.1991

DATE NEXT RENEWAL DUE 22.07.1993

DATE NOT IN FORCE

DATE OF LAST RENEWAL 19.06.1992

YEAR OF LAST RENEWAL 07

STATUS PATENT IN FORCE