Derivatives of C-076 compounds.

Priority: 11.05.81 US 262082

Date of publication of application: 24.11.82 Bulletin 82/47

Publication of the grant of the patent: 14.08.85 Bulletin 85/33

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

References cited:
EP-A-0 001 689
EP-A-0 008 184

Proprietor: MERCK & CO. INC.
126, East Lincoln Avenue P.O. Box 2000
Rahway New Jersey 07065 (US)

Inventor: Buhs, Rudolf P.
2 Sylvan Way
Short Hills New Jersey 07078 (US)
Inventor: Jacob, Theodore A.
828 St. Marks Avenue
Westfield New Jersey 07090 (US)
Inventor: Miwa, Gerald
562 Ridgewood Road
Maplewood New Jersey 07040 (US)
Inventor: Sestokas, Elena
1494 Valley Road
Rahway New Jersey 07065 (US)
Inventor: Taub, Rae
54 Wistar Avenue
Metuchen New Jersey 08841 (US)
Inventor: Walsh, John S.
2202 Village Drive
Avenel New Jersey 07001 (US)

Representative: Crampton, Keith John Allen et al
D YOUNG & CO 10 Staple Inn
London WC1V 7RD (GB)

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Description

The C-076 family of compounds are a series of macrolides isolated from the fermentation broth of a strain of *Streptomyces avermitilis*. The C-076 compounds are characterized by having a 16-membered cyclic backbone substituted with a disaccharide and having a bicyclic spiroketal fused thereon. The compounds have the structure:

![Chemical Structure](image)

wherein the broken line indicates a single or a double bond; \( R_1 \) is hydroxy and is present only when said broken line indicates a single bond; \( R_2 \) is iso-propyl or sec-butyl; and \( R_3 \) is methoxy or hydroxy.

The C-076 compounds are named using a system of designations which corresponds to the structural variations as is set forth in the following table.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1a</td>
<td>Double bond</td>
<td>sec-butyl</td>
<td>(-\text{OCH}_3)</td>
</tr>
<tr>
<td>A1b</td>
<td>Double bond</td>
<td>iso-propyl</td>
<td>(-\text{OCH}_3)</td>
</tr>
<tr>
<td>A2a</td>
<td>(-\text{OH})</td>
<td>sec-butyl</td>
<td>(-\text{OCH}_3)</td>
</tr>
<tr>
<td>A2b</td>
<td>(-\text{OH})</td>
<td>iso-propyl</td>
<td>(-\text{OCH}_3)</td>
</tr>
<tr>
<td>B1a</td>
<td>Double bond</td>
<td>sec-butyl</td>
<td>(-\text{OH})</td>
</tr>
<tr>
<td>B1b</td>
<td>Double bond</td>
<td>iso-propyl</td>
<td>(-\text{OH})</td>
</tr>
<tr>
<td>B2a</td>
<td>(-\text{OH})</td>
<td>sec-butyl</td>
<td>(-\text{OH})</td>
</tr>
<tr>
<td>B2b</td>
<td>(-\text{OH})</td>
<td>iso-propyl</td>
<td>(-\text{OH})</td>
</tr>
</tbody>
</table>

The above compounds are isolated from the fermentation broth of *Streptomyces avermitilis* using normal extraction and isolation procedures. The C-076 producing culture and the morphological characteristics thereof along with the procedures used for separating and isolating the C-076 compounds, are fully described in Great Britain Patent 1573955.

The fermentation is carried out in an aqueous medium and includes an assimilable source of carbon, an assimilable source of nitrogen and inorganic salts and the fermentation is generally carried out under aerobic conditions. The specific nutrients and parameters for the fermentation are described completely in the above cited Great Britain Patent.

The C-076 producing culture and a mutant thereof have been deposited in the permanent culture collection of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. The cultures are accessible under the accession numbers ATCC 31287 for the basic culture and ATCC 31272 (lyophilized tube) and ATCC 31271 (frozen vial) for the mutant. The C-076 compounds are potent antiparasitic agents with very broad spectrum anthelmintic, acaricidal, nematocidal and insecticidal activity.

Derivatives of the C-076 compounds have been prepared which also have considerable antiparasitic
activity. In particular a mixture of at least 80% of 22,23-dihydro C-076 Bla and no more than 20% of 22,23-dihydro C-076 Blb is particularly effective and such a mixture has been given the generic name of ivermectin. Such compounds are disclosed in U.S. Patent 4,199,569. European Patent Specification EP-0-001-689-A1 discloses compounds having the formula

![Chemical Structure](image)

in which $R_1$ is iso-propyl or sec-butyl; $R_2$ is methoxy, hydroxy or loweralkanoyloxy; and $R_3$ is hydrogen, lower alkanoil; or certain oleandrosyl-comprising groups. European Patent Specification EP-0-008-184-A1 discloses compounds having the formula

![Chemical Structure](image)

where the broken line indicates that a single or a double bond is present in the 22(23) position; $R_1$ is hydroxy, loweralkanoyloxy or hydrocarbonoxy and is present only when the broken line indicates a single bond; $R_2$ is iso-propyl or sec-butyl; $R_3$ is hydrogen, methyl, loweralkanoyl or a hydrocarbon. $R$ is hydrogen, loweralkanoyl, a hydrocarbon, or certain oleandrosyl-comprising groups; and $R_3$ is hydrogen or methyl. There are certain provisos on carbon content in both these disclosures.

The compounds in these two European Patent Specifications are produced by chemical treatment (e.g. hydrogenation, hydrolysis and substitution) of the basic compounds, themselves obtained by microbiological fermentation as stated above. It will in particular be noted that these compounds have a 24-methyl substituent, and have no hydroxy substituents that are not directly attached to a carbon atom forming part of a ring.

This invention provides compounds of the following formula:
wherein \( R_1 \), \( R_2 \) and the 22, 23 broken line have the following meanings:

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>22, 23-bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>( O )</td>
<td>(-CH_2CH_3)</td>
<td>double bond</td>
</tr>
<tr>
<td>II</td>
<td>(-H)</td>
<td>(-CH_2CH_3)</td>
<td>single bond</td>
</tr>
<tr>
<td>III</td>
<td>( O )</td>
<td>(-CH_2CH_3)</td>
<td>single bond</td>
</tr>
<tr>
<td>IV</td>
<td>(-H)</td>
<td>(-CH_3)</td>
<td>single bond</td>
</tr>
<tr>
<td>V</td>
<td>( O )</td>
<td>(-CH_3)</td>
<td>single bond</td>
</tr>
</tbody>
</table>

It will be noted that the compounds of the present invention have a 24-hydroxymethyl substituent, the hydroxy portion of which is not attached to a ring-forming carbon atom. Moreover, as will appear below, their method of preparation involves the incubation of ivermectin or the like in liver, rather than a chemical modification.

The compounds of this invention are prepared by either of two methods. The first is the \textit{in vitro} incubation of ivermectin in rat or steer liver microsomes. The second is the \textit{in vivo} method of extraction of the liver of animals, primarily rats, sheep or cattle, that had been administered a C-076 compound (substrate).
In Vitro Method.

The rat or steer liver microsomes are prepared using standard techniques for doing so wherein livers from such animals are homogenized in a buffer solution and subjected to fractional centrifugation. The initial centrifugation at about 25,000 x g or less removes the bulk of the solid material leaving the microsomes suspended in the buffer solution. Recentrifugation of the supernatant at higher rotations of about 100,000 x g or higher results in a pellet of liver microsomes.

The substrate is then incubated with the liver microsomes at a rate of about one mg of 1-mercaptotetrazol with from 10 to 100 mg of liver microsomal protein. Preferably for each mg of substrate about 20 mg of liver microsomal protein is employed. The incubation is carried out in a mixture of solvents, preferably employing a lower alcohol such as methanol or ethanol, and an aqueous buffer solution. The buffering salts used in the microsomal incubation system are generally standard buffers known to those skilled in the art. Examples of such are alkali metal phosphates, preferably potassium phosphate.

Also present in the microsomal incubation system is an NADPH-generating system such as NADP, glucose-6-phosphate dehydrogenase, and glucose-6-phosphate. These agents provide cofactors for the liver microsomes and provide an enzyme system such as would be found in vivo. (“NADP” is nicotinamide-adenine dinucleotide phosphate). While all three of the foregoing are needed to properly generate NADPH, those skilled in the art will appreciate that other similar agents may be substituted for one or more of the listed agents while still retaining NADPH-generating properties. NADP is generally used at a rate of from about 0.05 to 5 micromoles (µM) for each mg of liver microsomal protein. Preferably about 0.25 µM of NADP is used for each mg of liver microsomal protein. From 0.5 to 10 units, preferably 1 to 2 units, of glucose-6-phosphate dehydrogenase is generally used for each 10 mg of liver microsomal protein. Additionally from 0.5 to 50 µM, preferably about 2.5 µM, of glucose-6-phosphate is added to the microsomal incubation mixture for each mg of liver microsomal protein in order to provide an adequate source of NADPH.

The incubation is carried out generally in two stages over a period of from 3 to 4 hours. Approximately one half of the liver microsomal protein is added to the remainder of the materials and incubated for about 15 minutes to 2 hours. Then the remainder of the liver microsomal protein is added and incubated for an equal period of time. The incubation is carried out aerobically and with vigorous stirring or shaking. The incubation is carried out at from about 25 to 40°C. The preferred incubation temperature is about 37°C.

At the end of the incubation period the reaction is stopped by the addition of a water miscible organic solvent, such as acetone to precipitate the protein with may be removed by filtration if desired. The novel compounds thus produced are isolated and purified using solvent extraction and chromatography techniques. In particular, high performance liquid chromatography (HPLC) has been found to be especially useful for the isolation of the instant compounds.

The compounds of this invention are also isolated in an in vivo preparation by extracting the livers of animals, preferably sheep or cattle, that had been administered 1-mercaptotetrazol. The animals are generally administered from 10 to 500 µg/kg of animal body weight. The liver is excised and homogenized in a mixture of water and an organic solvent, preferably acetone, and filtered. Using extraction techniques followed by sequential HPLC treatments, purified metabolite fractions containing the compounds of this invention are obtained.

The novel compounds of this invention have significant parasiticidal 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 other species, such as Haemonchus and Ostertagia, are more prevalent in the stomach while 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, subcutaneous and lymphatic tissue. The parasitic infections known as helminthiasis lead to anemia, malnutrition, weakness, weight loss, 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, Aspiculuris in rodents, arthropod ectoparasites of animals and birds such as ticks, mites, lice, flies, 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 are also useful against parasites which infect humans. The most common genera of parasites of the gastro-intestinal tract of parasites of man are Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, and Enterobius. Other medicinally 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, Blatella 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, (Acrystosiphon 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.

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 about 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 dicalcium phosphate.

When 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 compound usually are employed. 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 our invention may be administered to animals parenterally, for example, by intramuscular, intravenous, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the animal material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil and cotton seed oil. Other parenteral vehicles such as organic preparation using solvents, glycerol, formal 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 agent 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 to be employed for best results will, of course, depend upon the particular compound employed, the species of animal to be treated and the type and severity of parasitic infection or infestation. Generally, good results are obtained with our novel compounds by the oral administration of from about 0.001 to 10 mg. per kg. of animal body weight, such 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 about 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 being employed. 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 oral 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 about 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 about 0.0002 to 0.3% by weight of the active compound.

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
0 065 403

desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular C-076 compound employed, the compounds of this invention are usually fed at concentrations of between 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 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 microsomal incubation or liver extraction.

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 as sprays, dusts and emulsions, to the growing or stored crops to effect protection from such agricultural pests.

The following examples describe the isolation and purification procedures for the preparation of the instant compounds. The examples are provided in order that the invention might be more fully understood. They should not be construed as limiting of the invention.

Example 1

METHODS:

A. Preparation of Liver Microsomes

Microsomes from either steer or rat livers are prepared by homogenizing freshly obtained liver samples (10 g) with 0.05 M Tris, pH 7.5, containing 1.16% KCl (30 ml). The samples are centrifuged for 20 min. at 10,000 × g. The supernatant is centrifuged for 60 min. at 105,000 × g. The resulting microsomal pellet is washed by resuspending it in 1.15% KCl containing 10 mM EDTA. Centrifugation for 60 min. at 105,000 × g gives a washed microsomal pellet which was resuspended in 0.25 m sucrose and stored at −80°C at a protein concentration of approximately 30—40 mg/ml.

B. Substrate

In a typical preparative incubation, an alcohol solution of 10 ml containing 10 mg of 22,23-dihydro C-076 Bis placed in a 500 ml Erlenmeyer flask and the alcohol solvent removed under a stream of nitrogen. The substrate residue is then redissolved in 0.5 ml methanol and the following added (a) microsomes from either steer or rat livers equivalent to 200 mg protein, (b) 10 ml of 1M potassium phosphate buffer, pH 7.4, (c) 10 ml of 10 mM NADP, (d) 20 ml of 0.05 M glucose-6-phosphate, (e) 5 ml of 14 units/ml glucose-6-phosphate dehydrogenase and (f) water to bring the total volume to 90 ml.

C. Incubation

The sample is aerobically incubated with shaking at 37°C for 30 min, after which another addition of microsomes (200 mg in 40 ml 0.1 M potassium phosphate buffer, pH 7.4) is made and the sample incubated an additional 30 min. The reaction is stopped and the protein precipitated by the addition of 100 ml of acetone.

D. Sample Cleanup by Solvent Extraction

The samples are partially purified by solvent extraction. The quenched samples are extracted with methylene chloride (3 × 200 ml) and the organic extract evaporated to dryness on a rotary evaporator. The residue is dissolved in three successive 33 ml volumes of ethanol/0.1 M K2PO4, pH 7.0 (40/60). This aqueous solution is extracted with cyclohexane (3 × 60 ml) to remove the unmetabolized substrate. The aqueous solution is then extracted with methylene chloride (2 × 100 ml and 1 × 50 ml) to recover the metabolites. The methylene chloride is removed by rotary evaporation and the metabolite residue redisolved in 5 ml methylene chloride. The polar metabolites in this solution are adsorbed onto a Silica Gel Sep Pak (Waters Assoc., Milford, Massachusetts) and eluted with ethyl acetate. The ethyl acetate eluate is evaporated to dryness and the residue dissolved in a small volume (200 μl) of methanol. Any insoluble material is removed by centrifugation.

E. HPLC Purification of Metabolites

The entire sample (200 μl) is injected onto a 4.6 mm inside diameter × 25 cm Zorbax ODS column (DuPont Co., Wilmington, Delaware) and isocratically eluted with acetonitrile/methanol/water (39/26/35) for 25—30 min at a flow rate of 1.6 ml/min. A 15 min linear gradient to 100% acetonitrile/methanol (80/40) is then used to remove any lipids or residual substrate. The UV profile at 248 nm was continuously monitored while fractions equivalent to 1 min (1.6 ml) were collected and 100 μl aliquots taken for counting. Under these conditions, two fractions containing polar metabolites (identified as peaks I and II) eluted in fractions 5—8 and 10—12 corresponding to retention times of approximately 7 and 11 mins, respectively.

The fractions containing the more polar metabolite (peak I) are pooled, 3 ml of water added and the metabolite extracted into methylene chloride (2 × 3 ml). The organic extract is evaporated to dryness under a stream of nitrogen and the residue dissolved in about 100 μl of methanol. The sample is then injected onto the same Zorbax column and eluted isocratically for 30 min with acetonitrile/methanol/water (36/24/40) before initiating the acetonitrile/methanol gradient (60/40). This HPLC purification procedure is
repeated, as necessary until a single, symmetrical peak is eluted from the column as assessed by UV absorbance. This is Compound II (11.8 μg).

Samples of the later eluting metabolite corresponding to peak II are also purified by HPLC. The fractions rich in peak II from the first HPLC are pooled, extracted into methylene chloride and purified by HPLC in a manner identical to that used for peak II affording Compound III (80—80 μg).

Example 2
The procedure of Example 1 is followed using 10 mg of 22,23-dihydro C-076 Blb in place of 22,23-dihydro C-076 Bla and then in recovered Compound IV (11.8 μg) and Compound V (63.7 μg).

Example 3
The procedure of Example 1 is followed using 10 mg of C-076 Bla and Compound I (10.4 μg) is obtained.

Example 4

I. Isolation Procedure
Two 500 g liver samples from 14-day post-dose steers have been administered 0.3 mg/kg of ivermectin, were extracted by the following procedure:
1. Five portions of 100 g liver, 200 ml of water, and 300 ml of acetone are homogenized in a blender.
2. The homogenate is filtered through a sintered glass funnel packed with a layer of supercel. The residue is washed with 1260 ml of acetone/water (1/1).
3. The filtrate and washes are then extracted with about 1500 ml of methylene chloride. The methylene chloride extract is evaporated, and the residue dissolved in 102 ml of absolute alcohol.
4. To the alcohol solution is added 150 ml of 0.1 M pH 7 phosphate buffer and extracted with 150 ml of cyclohexane. The cyclohexane layer is removed.
5. The ethanol-buffer layer is re-extracted with 300 ml of methylene chloride. The methylene chloride layer is removed and evaporated. The residue is dissolved in 10 ml of methanol and transferred to a 15 ml centrifuge tube.
6. The methanol solution is evaporated to dryness, the residue dissolved in 1 ml of isooctane/ethanol (75/25), and centrifuged. The supernatant is subjected to HPLC purification.

II. Purification by HPLC
The final extract from each 500 g tissue sample is chromatographed four times before the two partially purified metabolites are combined and rechromatographed as described in II.4.
1. The first chromatography on the final liver extract is an adsorption chromatography through a silica gel column with isooctane/ethanol (75/25) as the solvent system.
2. Thereafter, five HPLC chromatographies (No. 2—6) are completed using the reversed-phase conditions with a Zorbax ODS column and acetonitrile/methanol/water (36/24/40) as the solvent system.
3. Peak fractions from each chromatography are sequentially pooled and extracted with methylene chloride. The extract is evaporated, and the residue dissolved in 0.1 ml of methanol and centrifuged in preparation for HPLC injection.
4. The peak fractions from the 4th HPLC of each sample are combined and the extract is evaporated, and the residue dissolved in Methanol for the 5th and 6th chromatographies using similar conditions as in No. 2.
5. The final peak fractions from the 6th HPLC are combined and evaporated to dryness.

This sample weighing 8.5 μg is identified using nuclear magnetic resonance and mass spectrometry, as Compound III.
Claims

1. A compound having the formula:

![Chemical Structure]

wherein \( R_1 \), \( R_2 \) and the 22,23 broken line have the following meanings:

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>22,23-bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>HO</td>
<td>(-\text{CH}_2\text{CH}_3)</td>
<td>double bond</td>
</tr>
<tr>
<td>II</td>
<td>(-\text{H})</td>
<td>(-\text{CH}_2\text{CH}_3)</td>
<td>single bond</td>
</tr>
<tr>
<td>III</td>
<td>HO</td>
<td>(-\text{CH}_2\text{CH}_3)</td>
<td>single bond</td>
</tr>
<tr>
<td>IV</td>
<td>(-\text{H})</td>
<td>(-\text{CH}_3)</td>
<td>single bond</td>
</tr>
<tr>
<td>V</td>
<td>HO</td>
<td>(-\text{CH}_3)</td>
<td>single bond</td>
</tr>
</tbody>
</table>

2. A process for preparing a compound as claimed in Claim 1, comprising incubating with liver microsomal protein a substrate of the corresponding compound having a 24-methyl group instead of a 24-hydroxymethyl group.

3. A process as claimed in Claim 2 carried out in a mixture of solvents, buffering agents and fortification agents.

4. A process as claimed in Claim 3 carried out in a mixture of aqueous and lower alcohol solvents, aqueous alkali metal phosphate solution as buffering agents and nicotinamideadenine dinucleotide phosphate (NADP) glucose-6-phosphate dehydrogenase and glucose-6-phosphate as a source of NADPH.
5. A process as claimed in any one of Claims 2 to 4, carried out using from 10 to 100 mg of liver microsomal protein for each mg of substrate.
6. A process for the preparation of Compound III of Claim 1 comprising extracting the livers of animals to which ivermectin had been administered.
7. A composition useful as an antiparasitic agent comprising an inert carrier and an effective amount of a compound as claimed in Claim 1.
8. A compound as claimed in Claim 1, for use in the treatment of parasitic infections.

Patentansprüche

1. Eine Verbindung mit der Formel:

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
R_1 & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{OH} \\
\text{CH}_3 & \quad \text{OH} \\
\text{CH}_3 & \quad \text{CH}_2\text{OH} \\
\end{align*}
\]

worin \( R_1 \), \( R_2 \) und die strichlierte Linie in Stellung 22, 23 die folgenden Bedeutungen haben:
2. Ein Verfahren zur Herstellung einer Verbindung wie im Anspruch 1 beansprucht, umfassend das Bebrühen eines Substrats der entsprechenden Verbindung, die eine 24-Methylgruppe anstelle einer 24-Hydroxymethylgruppe aufweist, mit Lebermikrosomenprotein.


4. Ein Verfahren wie im Anspruch 3 beansprucht, ausgeführt in einem Gemisch aus wässerigen und Niedrigalkohol-Lösungsmitteln, wässriger Alkalimetallphosphatlösung als Puffermittel und Nicotinamidadenindinucleotidphosphat (NADP)glucose-6-phosphatdehydrogenase und Glucose-6-phosphat als eine Quelle für NADPH.

5. Ein Verfahren wie in einem der Ansprüche 2 bis 4 beansprucht, ausgeführt unter Verwendung von 10 bis 100 mg Lebermikrosomenprotein je mg Substrat.


7. Eine als ein antiparasitäres Mittel nützliche Zusammensetzung, umfassend einen inerten Träger und eine wirksame Menge einer Verbindung, wie im Anspruch 1 beansprucht.

8. Eine Verbindung wie im Anspruch 1 beansprucht, zur Verwendung bei der Behandlung von parasitären Infektionen.
Revendications

1. Un composé ayant pour formule:

   ![Chemical Structure](image)

   dans laquelle \( R_1 \), \( R_2 \) et le trait discontinu 22, 23 ont les significations suivantes:

<table>
<thead>
<tr>
<th>Composé</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>liaison 22, 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>H</td>
<td>-CH(_2)CH(_3)</td>
<td>double liaison</td>
</tr>
<tr>
<td>II</td>
<td>H</td>
<td>-CH(_2)CH(_3)</td>
<td>simple liaison</td>
</tr>
<tr>
<td>III</td>
<td>H</td>
<td>-CH(_2)CH(_3)</td>
<td>simple liaison</td>
</tr>
<tr>
<td>IV</td>
<td>H</td>
<td>-CH(_3)</td>
<td>simple liaison</td>
</tr>
<tr>
<td>V</td>
<td>H</td>
<td>-CH(_3)</td>
<td>simple liaison</td>
</tr>
</tbody>
</table>

2. Un procédé pour la préparation d’un composé comme revendiqué dans la revendication 1, comprenant l’incubation avec une protéine de microsomes hépatiques d’un substrat du composé correspondant ayant un groupe 24-méthyle au lieu d’un groupe 24-hydroxyméthyle.

3. Un procédé comme revendiqué dans la revendication 2 effectué dans un mélange de solvants, d’agents tampons et d’agents adjuvants.

4. Un procédé comme revendiqué dans la revendication 3 effectué dans un mélange d’eau et de solvants de type alcools inférieurs, une solution aqueuse de phosphate de métal alcalin comme agent tampon et du nicotinamide-adénine dinucléotide phosphate (NADP), de la glucose-6-phosphate-déshydrogénase et du glucose-6-phosphate comme source de NADPH.
5. Un procédé comme revendiqué dans l'une quelconque des revendications 2 à 4 effectué par emploi de 10 à 100 mg de protéines de microsomes hépatiques pour chaque mg de substrat.

6. Un procédé pour la préparation du composé III de la revendication 1, comprenant l'extraction des foies d'animaux auxquels on a administré de l'ivermectin.

7. Une composition utile comme agent antiparasitaire comprenant un support inerte et une quantité efficace d'un composé comme revendiqué dans la revendication 1.

8. Un composé comme revendiqué dans la revendication 1 pour l'emploi dans le traitement d'infections parasitaires.