Hereby apply for the grant of a Patent for an invention entitled

"CHEMICAL PROPERTIES AND PRODUCTS"

"Long-acting Antihistamines and Phenothiazines"

which is described in the accompanying complete specification.

This application is a Convention Application and is based on the application numbers 786,157 and 852,403

for a patent or similar protection made in

the United States of America

on 11 April 1977 and 17 November 1977, respectively.

Our address for service is:

Care: SPRUSON & FERGUSON
PATENT ATTORNEYS
ESSO HOUSE, 127 KENT STREET
SYDNEY, NEW SOUTH WALES.
AUSTRALIA.

Dated January 24, 1978

MERCK & CO., INC.

The Commissioner of Patents

The Common Seal of

MERCK & CO., INC.

was hereto affixed

in the presence of:

James F. Naughton
Manager-Administration
Off. of V.P. & Gen. Counsel

To:
The Commissioner of Patents
Declaraion in Support of a Convention Application for a Patent or Patent of Addition

In support of the Convention Application made for a patent for an invention entitled "CHEMICAL PROCESSES AND PRODUCTS"

I, JAMES F. NAUGHTON

of MERCK & CO., Inc., 126 East Lincoln Avenue,
Rahway, New Jersey, United States of America

do solemnly and sincerely declare as follows:

1. I am authorised by MERCK & CO., Inc., the applicant for the patent to make this declaration on its behalf.

2. The basic applications as defined by Section 141 of the Act were made in the United States of America on 11 April 1977 by MICHAEL HERBERT FISHER and RICHARD LEE TOLMAN and on 17 November 1977 by MICHAEL HERBERT FISHER and RICHARD LEE TOLMAN

3. MICHAEL HERBERT FISHER and RICHARD LEE TOLMAN 1140 Concord Drive, Bridgewater, New Jersey; and 140 Briarwood Drive, East, Berkeley Heights, New Jersey; United States of America, respectively

are the actual inventor/s of the invention and the facts upon which the said Company is entitled to make the application are as follows:

The said Company is the assignee of the inventor/s.

4. The basic applications referred to in paragraph 2 of this Declaration were the first applications made in a Convention country in respect of the invention the subject of the application.

Declared at Rahway, New Jersey, U.S.A.

this 23 day of January 1978

MERCK & CO., Inc.

James F. Naughton

Manager-Administration

Off. of V.P. & Gen. Counsel

To:
The Commissioner of Patents,
Commonwealth of Australia.
A compound having the formula:

\[
\begin{array}{c}
\text{III} \\
\text{OR'}
\end{array}
\]

wherein R is hydrogen or a glycosyloxy moiety and \( R_1, R_2, R_3, R_4 \) and \( R' \) are defined as follows:

\[
\begin{array}{cccccc}
R_1 & R_2 & R_3 & R_4 & R' \\
\text{H} & \text{H} & \text{CH}_3 & \text{CH}_3 & \text{H or glycosyl} \\
\text{H} & \text{H} & \text{CH}_3 & \text{CH}_3 & \text{CH}_3 \\
\text{H} & \text{H} & \text{C}_2\text{H}_5 & \text{CH}_3 & \text{H or glycosyl} \\
\text{H} & \text{H} & \text{C}_2\text{H}_5 & \text{CH}_3 & \text{CH}_3
\end{array}
\]

1. The product is isolated using techniques known to those skilled in the art.
2. The orthoester process prepares sugar.
\( R_1 \quad R_2 \quad R_3 \quad R_4 \quad R' \)

- OH or glycosyloxy  \(-O-C-\text{CH}_4\text{H}_9\) \CH_3\ CH_3 \quad H or glycosyl

- OH or glycosyloxy  \(-O-C-\text{CH}_4\text{H}_9\) \CH_3\ CH_3 \quad CH_3

- OH or glycosyloxy  \(-O-C-\text{CH}_4\text{H}_9\) \text{C}_2\text{H}_5\ CH_3 \quad H or glycosyl

- OH or glycosyloxy  \(-O-C-\text{CH}_4\text{H}_9\) \text{C}_2\text{H}_5\ CH_3 \quad CH_3

H \quad H \quad \text{CH}_3 \quad \text{-CH}_2\text{OCN}\quad H or glycosyl

H \quad H \quad \text{CH}_3 \quad \text{-CH}_2\text{OCN}\quad H or glycosyl

.../3
wherein \( R \) is hydrogen or a glycosyloxy moiety and \( R_2, R' \) and \( R'' \) are defined as follows:

<table>
<thead>
<tr>
<th>( R_2 )</th>
<th>( R' )</th>
<th>( R'' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
<td>(-\text{OH or glycosyloxy})</td>
</tr>
<tr>
<td>( \text{C}_2\text{H}_5 )</td>
<td>( \text{CH}_3 )</td>
<td>(-\text{OH or glycosyloxy})</td>
</tr>
<tr>
<td>( \text{CH}_3 )</td>
<td>( \text{H or glycosyl} )</td>
<td>( \text{H} )</td>
</tr>
</tbody>
</table>

provided that in formula III at least one of \( R, R_1 \) and \( R' \) is a glycosyl or glycosyloxy moiety and in formula IV at least one of \( R, R' \), and \( R'' \) is a glycosyl or glycosyloxy moiety.
COMPLETE SPECIFICATION

FOR OFFICE USE:

Application Number: 34760/78

Class: 34760

Int. Class: 34760

Complete Specification Lodged:

Priority: 31 JUL 1981

Related Art:

Name of Applicant: MERCK & CO., INC.

Address of Applicant: 126 East Lincoln Avenue, Rahway, New Jersey, United States of America

Actual Inventors: MICHAEL HERBERT FISHER and RICHARD LEE TOLMAN


Complete Specification for the invention entitled:

"CHEMICAL PROCESSES AND PRODUCTS"

"CARBAMOYL DERIVATIVES OF MUNDAMINE AND DERIVATIVES THEREOF"

The following statement is a full description of this invention, including the best method of performing it known to me/us:

AUSTRALIAN
- 4 BR 197B

PATENT OFFICE

16012 IA

EXAMPLE 2
ABSTRACT OF THE DISCLOSURE

Carbohydrate derivatives of the antibiotic substance milbemycin, also identified as B-41, and of 13-hydroxy milbemycin are prepared. The carbohydrate groups are attached to the available hydroxy groups of milbemycin and to the hydroxy group synthesized at the 13-position. The reactions may be made selectively such that more than one carbohydrate group may be attached to a single position, or that multiple carbohydrate groups may be attached at different positions on the molecule. The described carbohydrate derivatives have antiparasitic activity.

BACKGROUND OF THE INVENTION

Milbemycin, or B-41, is a substance which is isolated from the fermentation broth of a milbemycin producing strain of Streptomyces. The microorganism, the fermentation conditions, and the isolation procedures are more fully described in U.S. Patent 3,950,360 and U.S. Patent 3,984,564. The structures of seven of the thirteen milbemycin compounds are described in said patents and the structures of all thirteen compounds are described in the Journal of Antibiotics 29 (6) June 1976 pages 76-35 to 76-42 and pages 76-14 to 76-16. The milbemycin compounds described in said patents do not have any carbohydrate groups substituted thereon.
SUMMARY OF THE INVENTION

The carbohydrate derivatives of milbemycin and 13-hydroxy milbemycin are prepared by various procedures, and such compounds have been found to be active antiparasitic agents. Such carbohydrate derivatives are prepared by using one of several reactions. The classical Koenigs-Knorr reaction is successfully employed as are the silver triflate (silver trifluoromethylsulfonate) modification and the Helferich modification, and the reaction utilizing orthoester intermediates. Thus, it is an object of this invention to describe the carbohydrate derivatives of the milbemycin compounds and of the 13-hydroxy milbemycin compounds. A further object is to describe the processes employed to prepare such carbohydrate derivatives. A still further object is to describe the antiparasitic uses of such compounds. Further objects will be apparent from reading the following description.

The milbemycin compounds were originally named as B-41 compounds and given the nomenclature A₁, A₂, A₃, A₄, B₁, B₂, B₃, C₁ and C₂. Later, however, four additional milbemycin compounds were isolated from the fermentation broth and the structures of all thirteen compounds determined. The series was then named as milbemycin and the nomenclature was changed to α₁ to α₁₀ and β₁ to β₃, recognizing the two basic structural differences between the two series of compounds. The following structural formulae and tables fully describes the milbemycin compounds and the relationship between the old and new nomenclature.
1 Milbemycin

2 \( R_1 \) H H CH\(_3\) CH\(_3\) -OH A3
3 \( R_2 \) H H CH\(_3\) CH\(_3\) -OCH\(_3\) B2
4 \( R_3 \) H H C\(_2\)H\(_5\) CH\(_3\) -OH A4
5 \( R_4 \) H H C\(_2\)H\(_5\) CH\(_3\) -OCH\(_3\) B3
6 \( R_5 \) -OH -OC-CH-C\(_4\)H\(_9\) \( R_3 \) CH\(_3\) CH\(_3\) -OH A2
7 \( R_6 \) -OH -OC-CH-C\(_4\)H\(_9\) CH\(_3\) CH\(_3\) -OCH\(_3\) B1
8 \( R_7 \) -OH -OC-CH-C\(_4\)H\(_9\) C\(_2\)H\(_5\) CH\(_3\) -OH
9 \( R_8 \) -OH -OC-CH-C\(_4\)H\(_9\) C\(_2\)H\(_5\) CH\(_3\) -OCH\(_3\)
10 \( R_9 \) H H CH\(_3\) -CH\(_2\)-O-\( R_4 \) -OH C1
11 \( R_{10} \) H H C\(_2\)H\(_5\) -CH\(_2\)O-N\( R_4 \) -OH C2

EXAMPLE 9
The 13-hydroxy milbemycin compounds may be prepared from the milbemycin compounds which are unsubstituted at the 13-position by allylic bromination with N-bromosuccinimide followed by treatment with an alkali metal alkanoate such as sodium acetate, and finally by removing the alkanoyl group by hydrolysis.

This process affords the 13-hydroxy group which is then available for substitution with the below described carbohydrate groups.

In the above formulae, at various times, there are found hydroxy groups at the 5, 13 and 22 positions and on the methyl group at the 8 position of formula II. Any one or more of these hydroxy groups may be substituted with a carbohydrate or sugar moiety (also known as a glycosyl group) to form the compounds of this invention. Such compounds are more precisely defined in the following formulae:
wherein R is hydrogen or a glycosyloxy moiety and R₁, R₂, R₃, R₄ and R' are defined as follows:

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R'</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-O-C-CH₄H₉</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-O-C-CH-C₄H₉</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-OC-CH-C₄H₉</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-OC-CH-C₄H₉</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
</tbody>
</table>
wherein \( R \) is hydrogen or a glycosyloxyl moiety and \( R_2, R', \) and \( R'' \) are defined as follows:

<table>
<thead>
<tr>
<th>( R_2 )</th>
<th>( R' )</th>
<th>( R'' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
<td>(-\text{OH} \text{ or glycosyloxy})</td>
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<td>(-\text{OH} \text{ or glycosyloxy})</td>
</tr>
<tr>
<td>( \text{CH}_3 )</td>
<td>( \text{H} \text{ or glycosyl} )</td>
<td>( \text{H} )</td>
</tr>
</tbody>
</table>

provided that in formula III at least one of \( R, R_1 \) and \( R' \) is a glycosyl or glycosyloxyl moiety and in formula IV at least one of \( R, R', \) and \( R'' \) is a glycosyl or glycosyloxyl moiety.
The nature of the glycosyl or glycosyloxy groups is not critical and any sugar may be substituted onto the milbemycin substrate using the procedures described below. The preferred glycosyl or glycosyloxy groups are derived from glucopyranose, galactopyranose, mannopyranose, maltose, arabinoxyranose, lyxopyranose, xylopyranose, ribopyranose, oleandrose, rhamnopyranose, fucopyranose, lactose, ribofuranose, mannofuranose, glucofuranose, arabinofuranose, mycarose, cladinose, desosaminose, daunosaminose, mycaminose and the like.

The foregoing sugars are available generally in the D or L configuration. The instant invention includes both of the possible configurations for attachment to the milbemycin substrate.

The above glycosyl or glycosyloxy groups may be substituted on the milbemycin or 13-hydroxy milbemycin compounds as mono, di or trisaccharides wherein one of the above sugars is further substituted with another of the same or different sugar. In addition, where there is more than one hydroxy group available for substitution, the sugar groups may be present on only one or on more than one of such hydroxy groups, and the substitution may be with identical or different sugars.

The preferred sugars are with glucopyranose, rhamnopyranose, oleandrose or daunosaminose. The most preferred sugars are glucopyranose and oleandrose.
The processes for the substitution of the carbohydrate groups are substituted onto the hydroxy groups of the substrate molecule using the Koenigs-Knorr process, the silver triflate process or the orthoester process.

The carbohydrate starting materials employed for the Koenigs-Knorr, the Helferich modification thereof and the silver triflate processes are protected by acylating all of the free hydroxy groups. The preferred protecting group is the acetyl group, however, other groups such as the benzoate may be employed. The processes for the blocking of the hydroxy groups are well known to those skilled in the art. The acetyl blocking groups are also easily removed at the completion of the reaction by catalytic hydrolysis, preferably base-catalyzed hydrolysis such as with an alcoholic ammonia solution.

The Koenigs-Knorr and silver triflate processes use as starting materials the acetohalo-sugars such as the appropriate acetobromohexoses and acetobromopentoses of the sugar groups listed above. The bromine atom is substituted on a carbon atom adjacent to an acetyl group and the sugar moiety becomes bonded to the substrate at the carbon atom to which the halogen was attached.

In the Koenigs-Knorr reaction the milbemycin compound is dissolved under anhydrous conditions in an aprotic solvent. Ether is the preferred solvent, however, methylene chloride, acetonitrile, nitromethane, dimethoxy ethane and the like may also be employed. To the substrate solution is added the acetohalosugar.
and silver oxide. A single molar equivalent of the
sugar is required, however, an addition 1 to 3 moles
occasionally aids the reactions, however, molar excesses
beyond 3 tend to make the isolation of the product more
difficult. It has been found preferable to employ freshly
prepared silver oxide for the reaction, since the material
tends to lose its catalytic efficiency upon standing
for prolonged periods. The silver oxide is prepared
from silver nitrate using known procedures. The
reaction may be carried out at from 10-50°C, however,
reaction at room temperature is preferred. The
reaction generally requires from 2 to 10 days for
completion. Reaction progress is monitored by taking
aliquots from the reaction mixture and examining
them with thin layer chromatographic techniques.
Possible side reactions are avoided by carrying out the
reaction in the dark, and this method is preferred.
The product is isolated using techniques known to
those skilled in the art.

In one modification of the Koenigs-Knorr
reaction, known as the Helferich modification thereof,
a mercuric halide, such as mercuric chloride or bromide,
alone or in combination with mercuric oxide or mercuric
cyanide is substituted for the silver oxide. The above
described reaction conditions may be employed except that
nitromethane and benzene are the preferred solvents and reflux
temperature is the preferred reaction temperature.
The silver triflate reaction uses the reagent silver triflate (silver trifluoromethyl sulfonate) and the acetohalosugar in the same solvents listed above, with ether being preferred. The silver triflate is best if highly purified and prepared fresh just prior to its use. Methods for the preparation of silver triflate are well known to those skilled in the art. All of the reactants are combined in the solvent and the reaction conducted at from 10 to 50°C for from 2 to 48 hours. Generally, however, the reaction is complete in about 24 hours at room temperature. The progress of the reaction may be followed by thin layer chromatography techniques. Again the reaction is preferably carried out in the dark, and with absolutely dry reactants and equipment.

A single mole of the sugar is required, however, a single molar excess is often used to aid in the course of the reaction.

During the course of the reaction a mole of triflic acid (trifluoromethanesulfonic acid) is liberated. This is a very strong acid and a molar equivalent of a base is required to neutralize the acid. Preferred bases are non-nucleophilic bases such as tertiary amines, preferably triethylamine, diisopropylethylamine, diazabicycloundecane, diazabiclyclononane and the like. Since triflic acid is such a strong acid, if the base used is not a strong enough base to neutralize all of the acid, the residual acid will adversely affect the course of the reaction and of the isolation of the product. The
4 derivatives of the milbemycin compounds from ortho-
5 esters of a lower alkanol and of the above sugars at
6 the hydroxy function of said milbemycins. The ortho
7 esters are prepared from the acetohalosugars using
8 a lower alkanol and procedures which are well known to
9 those skilled in the art. The reaction is carried out
10 in an aprotic solvent such as dichloroethane,
11 nitromethane, methylene chloride, dimethoxy ether,
12 acetonitrile, tetrahydrofuran and the like. Dichloro-
13 ethane, nitromethane, dimethoxy ethane and tetrahydrofuran
14 are preferred. The reaction is preferably carried out
15 at the reflux temperature of the reaction mixture and
16 is generally complete in from about 4 to 24 hours.
17 Catalytic amounts of mercuric bromide or mercuric chloride
18 are added to aid in the reaction. During the course of
19 the reaction one mole of the alcohol used to make the
20 orthoester is liberated. Thus, the preferred method
21 is to azeotropically distill off the solvent to remove
22 the alcohol and to force the reaction to completion. To
23 prevent any volume reduction, fresh solvent is
24 added as the distillation proceeds to maintain a constant
25 volume. To isolate the product, the solvent is generally
26 removed and the residue washed with a reagent to remove
27 the mercury salts, such as aqueous potassium iodide. The
28 product is then isolated using known techniques.
29
30 Where there is more than one position with
31 a hydroxy group which is susceptible to reaction (the

-11-
7-position tertiary hydroxy has been found to be less reactive toward substitution with a sugar moiety than the others), selective substitution may be obtained by careful ordering of the reaction steps. For example, if the 13-hydroxy-5-glycosyloxy milbemycin \(a_1\) is desired, the sugar reactions may be carried out on the 13-unsubstituted milbemycin \(a_1\) and then the reactions required to prepare the 13-hydroxy may be carried out. Further glycosylation reactions may then be carried out on the 13-hydroxy-5-glycosyloxy milbemycin \(a_2\) to prepare a compound with different sugar groups at the 5- and 13-positions. Alternatively, selective acylation of one of the hydroxy groups may be used to direct the glycosylation to another hydroxy group. If the 13-glycosyloxy milbemycin \(a_1\) is desired, the 5-hydroxy would be protected by acylation thereof, using known techniques and standard acylation reagents such as acid halides, anhydrides and the like. Then the 13-hydroxy group would be prepared and the glycosylation reactions carried out. The desired product would then be prepared by simple hydrolysis of the 5-acyl group.

Milbemycine \(a_6\) and \(a_8\) have hydroxy groups at the 22 and 13 positions and processes for selectively glycosylating these compounds would follow a procedure similar to that employed for glycosylating compounds at the 5 and 13 positions. The milbemycin \(a_5\) and \(a_7\) compounds have hydroxy groups at the 5, 13, and 22 positions. The selectivity of the 5 and 22 positions has been found to be very similar, thus reactions under the foregoing conditions will produce a mixture of compounds with sugar moieties.

2. The compound of Claim 1 wherein the glycosyl or glycosyloxy moieties are derived from a mono-, di- or trisac-
moieties at the 5, the 22 and at both positions.

Chromatographic techniques have been found to be very useful in separating mixtures of these compounds. In this manner, any combination of compounds with more than one available hydroxy group may be selectively substituted with the above sugar moieties.

The following examples are provided in order that the reaction might be more fully understood. They should not be construed as limitative of the invention.

EXAMPLE 1

13-(2,3,4,6-Tetra-O-acetyl-O-glucopyranosyloxy) milbemycin α2

To a solution of 13-hydroxy milbemycin α2 (280 mg.) in anhydrous ether (precautions are taken to insure anhydrous conditions of solvent and glassware) is added freshly prepared silver oxide (230 mg.) and then acetobromoglucose (410 mg.). The mixture is magnetically stirred at ambient temperature for 4 days in the dark. The solids are filtered and washed with ether and the volume of the filtrate is reduced in vacuo. The resulting solution is purified by column chromatography on silica gel using dichloromethane-methanol mixtures as eluant, affording 13-(2,3,4,6-tetra-O-acetyl-O-glucopyranosyloxy) milbemycin α2.
EXAMPLE 2

13-(D-glucopyranosyloxy) milbemycin \( \alpha_2 \)

The acetylated glucopyranosyl derivative (50 mg.) of Example 1 is treated with sufficient methanolic ammonia (2 ml., presaturated at 0\(^\circ\)) to cover the starting material, and the reaction is monitored by thin layer chromatography at one hour intervals. The reaction is complete in 6 hours and the solvent is removed in vacuo. The product purified by column chromatography using a dichloromethane-methanol mixture as eluant.

EXAMPLE 3

5-0-(2,3,4,6-Tetra-0-acetyl-D-0-glucopyranosyl)
milbemycin \( \alpha_1 \)

To a mixture of milbemycin \( \alpha_1 \) (560 mg.), acetobromoglucose (820 mg.) and diisopropylethylamine (260 mg.) in anhydrous ether (precautions are taken to insure anhydrous conditions of solvent and glassware) is added silver triflate (280 mg.). The mixture is stirred (magnetically) at ambient temperature in the dark until further reaction stops (as monitored by thin layer chromatography). 24 Hours reaction time is required. The solids are filtered, washed with ether and the filtrate is partitioned with aqueous dilute sodium bicarbonate, separated, washed with water, and dried over sodium sulfate. The solvent is removed in vacuo and the product is purified by column chromatography on silica gel using dichloromethane-methanol mixtures as eluant.

DATED this TWENTY-SEVENTH day of FEBRUARY, 1978

MERCK & CO., INC.
EXAMPLE 4

5-O-(β-D-Glucopyranosyl) milbemycin \(_{\alpha_1}\)

Following the procedure of Example 2, the peracetylated glucopyranosyl milbemycin \(_{\alpha_1}\) of Example 3 is treated with methanolic ammonia and the product 5-O-(β-D-glucopyranosyl) milbemycin \(_{\alpha_1}\) isolated.

EXAMPLE 5

13-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyloxy) milbemycin \(_{\alpha_1}\)

To a solution of 13-hydroxy milbemycin \(_{\alpha_1}\) (250 mg.) in vigorously anhydrous dichloroethane is added 3,4-di-O-acetyl-1,2-methyloehaaceteyl-β-L-rhamnopyranose (450 mg.) and mercuric bromide (360 mg.) The mixture is heated at reflux under nitrogen with slow removal of solvent (and formed methanol) by distillation. Solvent removed by distillation is replaced with fresh solvent from a dropping funnel. The reaction is monitored by thin layer chromatography and when it ceases to make further progress is cooled, washed with 30% aqueous potassium iodide, water and dried over sodium sulfate. Column chromatography using chloroform-methanol mixtures as eluants resolves the glycosidic products. After lyophilization from benzene the product 13-(2,3,4-tri-O-α-L-rhamnopyranosyloxy) milbemycin \(_{\alpha_1}\) is isolated as an amorphous solid.

EXAMPLE 6

13-(α-L-rhamnopyranosyloxy) milbemycin \(_{\alpha_1}\)

Following the procedure of Example 2 using the product of Example 5 as starting material, there is obtained 13-(α-L-rhamnopyranosyloxy) milbemycin \(_{\alpha_1}\).
EXAMPLE 7

13-(L-oleandrosyl-a-L-oleandrosyl)-milbemycin α₁

The procedure of Example 1 is followed employing

100 mg. of 5-O-acetyl-13-hydroxy milbemycin α₁ in place
of 13-hydroxy milbemycin α₂ and 250 mg. of 4-O-acetyl-
a-L-oleandrosyl-L-oleandrosyl-L-oleandrosyl chloride in
place of acetobromoglucone. (The halogenose is also
known as 4-O(4-O-acetyl-2,6-dideoxy-3-O-methyl-a-L-
lyxo-hexopyranosyl)-2,6-dideoxy-3-O-methyl-L-lyxo-
hexopyranosyl chloride). The product is purified using
preparative layer chromatography affording 13-(4-O-acetyl-
a-L-oleandrosyl-L-oleandrosyl)-5-O-acetyl-milbemycin α₁.

The above compound is hydrolyzed according to
the procedures of Example 2, affording 13-(L-oleandrosyl-
a-L-oleandrosyl) milbemycin α₁.

EXAMPLE 8

5-O-Acetyl-22-(4-O-Acetyl-a-L-Oleandrosyl-a-L-Oleandrosyl-
oxo) Milbemycin α₆

A solution of 100 mg. of milbemycin α₆ is

treated with 250 mg. of 4-O-acetyl-a-L-oleandrosyl-L-
oleandrosyl chloride (also named as 4-O-(4-O-acetyl-2,6-
dideoxy-3-O-methyl-a-L-lyxo hexopyranosyl)-2,6-dideoxy-
3-O-methyl-L-lyxo hexopyranosyl chloride) following the
procedure of Example 3. The product 22-(4-O-acetyl-a-L-
oleandrosyl-a-L-oleandrosylxy) milbemycin α₆ is isolated
and purified on preparative layer chromatography plates
using methylene chloride methanol mixtures as solvent.
EXAMPLE 9

22-(4-O-Acetyl-o-L-Oleandrosyl-o-L-oleandrosyloxy) Milbemycin a₆

The product of Example 8 is hydrolized following the procedure of Example 2 to produce 22-
(ο-L-oleandrosyl-ο-L-oleandrosyloxy) milbemycin a₆.

PREPARATIONS

A. 13-Bromo milbemycin a₂

A solution of 542 mg. of milbemycin a₂ and 178 mg. of N-bromosuccinimide in 10 ml. of carbon
tetrachloride is stirred under irradiation with ultraviolet light for 1 hour at room temperature. The
mixture is cooled to 0°C, the succinimide is filtered off and the solvent is removed by evaporation under
reduced pressure. Chromatography of a solution of the residue in a mixture of chloroform and tetrahydrofuran
(95:5) over a column of silica yields 13-bromo milbemycin a₂.

B. 13-Acetoxy milbemycin a₂

A solution of 621 mg. of 13-bromo milbemycin a₂ and 82 mg. of anhydrous sodium acetate in 10 ml. of
acetic acid is stirred for 24 hours at 20°C-30°C. The acetic acid is evaporated under reduced pressure and
the product is separated from the sodium bromide by extraction with ether and evaporation. Chromatography
of the product extracted into the ether in a mixture of chloroform and tetrahydrofuran (95:5) over a
column of silica yields 13-acetoxy milbemycin a₂.
1 C. 13-Hydroxy milbemycin $a_2$

A solution of 600 mg. of 13-acetoxy milbemycin $a_2$ and 44 mg. of sodium hydroxide in a mixture of 8 ml. of methanol and 2 ml. of water is stirred for 10 hours at 0°-10°C. The solvent is evaporated under reduced pressure and the residue is dissolved in chloroform. Chromatography of the chloroform solution over a column of silica yields 13-hydroxy milbemycin $a_2$.

D. Other milbemycin compounds such as $a_3$, $a_4$, $a_5$, $a_6$, $a_7$, $a_8$, $a_9$, $a_{10}$, $\beta_1$, $\beta_2$ and $\beta_3$ may be similarly converted into the 13-hydroxy derivatives either before or after the sugar reactions described above.

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 others, such as Haemonchus and Ostertagia, are
more prevalent in the stomach, while still others such
as Dicyoecauluas 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, sub-
cutaneous and lymphatic tissue and the like. The
parasitic infections known as helminthiases 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 milbemycin derivatives of
this invention have unexpectedly high activity against
these parasites, and in addition are also active
against Dirofilaria in dogs, Nematospirides, Syphacia,
Aspiculuris in rodents, arthropod octoparasites of
animals and birds such as ticks, mites, lice, fleas,
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 instant compounds are also useful against
parasites which infect humans. The most common genera
of parasites of the gastro-intestinal tract of man are
Ancylostoma, Necator, Ascaris, Strongyloides,
Trichinella, Capillaria, Trichuris, and Enterobius.
Other medically important genera of parasites which are
found in the blood or other tissues and organs outside
the gastro-intestinal tract are the filiarial 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, (Acyrthosiphon sp.);
against migratory orthopterans such as locusts and
immature stages of insects living on plant tissue.
The compounds are useful as a nematocide for the control
of soil nematodes and plant parasites such as Meloidogyne
spp. which may be of importance in agriculture.

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 contains 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 di-calcium phosphate.

Where it is desired to administer the milbemycin derivatives 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, vegetable gums and the like. 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 intraruminal, intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably
of the vegetable oil variety such as peanut oil, cotton
seed oil and the like. Other parenteral vehicles such
as organic preparation using solketal, glycerol, formal
and aqueous parenteral formulations are also used. The
active milbemycin 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.
enough base to neutralize all of the acid, the residual acid will adversely affect the course of the reaction and of the isolation of the product. The

The claims, defining the invention are as follows:

1. A compound having the formula:

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wherein R is hydrogen or a glycosyloxy moiety and R₁, R₂, R₃, R₄ and R' are defined as follows:
```

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R'</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-O-C-CH₂C₄H₉</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-O-C-CH₂C₄H₉</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-OC-CH₂C₄H₉</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H or glycosyl</td>
</tr>
<tr>
<td>-OH or glycosyloxy</td>
<td>-OC-CH₂C₄H₉</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
</tbody>
</table>
wherein R is hydrogen or a glycosyloxy moiety and $R_2$, $R'$ and $R''$ are defined as follows:

<table>
<thead>
<tr>
<th>$R_2$</th>
<th>$R'$</th>
<th>$R''$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_3$</td>
<td>$\text{CH}_3$</td>
<td>$-\text{OH}$ or glycosyloxy</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_5$</td>
<td>$\text{CH}_3$</td>
<td>$-\text{OH}$ or glycosyloxy</td>
</tr>
<tr>
<td>$\text{CH}_3$</td>
<td>$\text{H}$ or glycosyl</td>
<td>$\text{H}$</td>
</tr>
</tbody>
</table>

provided that in formula III at least one of $R$, $R_1$ and $R'$ is a glycosyl or glycosyloxy moiety and in formula IV at least one of $R$, $R'$, and $R''$ is a glycosyl or glycosyloxy moiety.
2. The compound of Claim 1 wherein the glycosyl or glycosyloxy moieties are derived from a mono-, di- or trisaccharide selected from glucopyranose, galactopyranose, manno-pyranose, maltose, arabinopyranose, lyxopyranose, xylopyranose, ribopyranose, oleandrose, rhamnopyranose, fucopyranose, lactose, ribofuranose, mannofuranose, glucofuranose, arabinofuranose, mycarose, cladinose, desosaminose, daunosaminose or mycaminose.

3. The compounds of Claim 2 wherein the glycosyl or glycosyloxy moiety is derived from mono-or disaccharide selected from glucopyranose, rhamnopyranose, oleandrose or daunosaminose.

4. The compound of Claim 3 wherein the glycosyl or glycosyloxy moiety is derived from a mono- or disaccharide selected from glucopyranose and oleandrose.

5. 13-( β-D-glucopyranosyloxy) milbemycin α₂.
6. 5-O-( β-D-glucopyranosyl) milbemycin α₁.
7. The compound of Claim 4 wherein the glycosyl or glycosyloxy moiety is derived from L-oleandrosyl-α-L-oleandrose.

8. 13-(L-oleandrosyl-α-L-oleandrosyloxy) milbemycin α₁.
9. 5-O-(L-oleandrosyl-α-L-oleandrosyloxy) milbemycin α₁.
10. A compound of Claim 7 which is 13-(L-oleandrosyl-α-L-oleandrosyl) milbemycin α₂.

11. A process for the preparation of a compound of Claim 1 which comprises treating a milbemycin compound or a 13-hydroxy milbemycin compound with an aceto halo sugar in the presence of silver oxide, and removing the acetyl protecting groups by hydrolysis.

12. The process of Claim 11 wherein a mercuric halide alone or in combination with mercuric oxide or mercuric cyanide is employed in place of silver oxide.

13. A process for the preparation of a compound of Claim 1 which comprises treating a milbemycin compound or a 13-hydroxy milbemycin compound with an aceto halo sugar in the presence of silver trifluoromethylsulfonate and a non-nucleophylic base.

14. A process for the preparation of a compound of Claim 1 which comprises treating a milbemycin compound or a 13-hydroxy milbemycin compound with an ortho ester of a sugar in the presence of catalytic amounts of mercuric bromide or mercuric chloride.

15. A composition for the treatment of parasitic infections, which comprises administering to an animal infected with parasites an effective amount of a compound of Claim 1.
DATED this TWENTY-SEVENTH day of FEBRUARY, 1978

MERICAN & CO., INC.

Patent Attorneys for the Applicant
SPRUSON & FERGUSON
Following the procedure of Example 2 using the product of Example 5 as starting material, there is obtained 13-(α-L-rhamnopyranosyloxy) milbemycin α₁.