MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1961-A

1.0
1.1
1.25
1.4
1.6
2.0
2.2
2.5
COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952
APPLICATION FOR A STANDARD PATENT

K

We BEECHAM GROUP LIMITED,
of Beecham House,
Great West Road,
Brentford, Middlesex, England

hereby apply for the grant of a Standard Patent for an invention entitled:

"TREATING PLANT INFECTIONS"

which is described in the accompanying complete specification.

Details of basic application(s):

<table>
<thead>
<tr>
<th>Number</th>
<th>Convention Country</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>8020674</td>
<td>Great Britain</td>
<td>24 June, 1980</td>
</tr>
</tbody>
</table>

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

Dated this 25th day of June, 1981.

To: THE COMMISSIONER OF PATENTS

(a member of the firm of DAVIES & COLLISON for and on behalf of the Applicant).

Davies & Collison, Melbourne and Canberra.
DECLARATION IN SUPPORT OF CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT

In support of the Application made for a patent for an invention entitled: Treating Plant Infections

Ronald Smither 72175/81
of Beecham House, Great West Road, Brentford, Middlesex, England.

I do solemnly and sincerely declare as follows:

1. (a) I am authorized by Beecham Group Limited the applicant for the patent to make this declaration on its behalf.
2. (a) Peter Charles Thomas Hannon, 35 Vogan Close, Reigate, Surrey, England.

I am the actual inventor of the invention and the facts upon which the applicant is entitled to make the application are as follows:

by virtue to his employment by Beecham Group Limited, whereby the applicant would, if a patent were granted upon an application made by the said actual inventor be entitled to have the patent assigned to it.

3. The basic application as defined by Section 141 of the Act was made in United Kingdom on the 24th June 1980 by Beecham Group Limited.

4. The basic application referred to in paragraph 3 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.
A method for the treatment of plant infections, which
comprises administering to a plant or plant growth
site an effective amount of pseudomonic acid A, of
formula (I), or a non-toxic salt or ester thereof.

\[
\begin{align*}
\text{O} & \quad \text{OH} \\
\text{OH} & \quad \text{O} \\
\text{OH} & \quad \text{CO}_2(\text{CH}_2)_8\text{CO}_2\text{H}
\end{align*}
\]
Name of Applicant: BEECHAM GROUP LIMITED,

Address of Applicant: of Beecham House,
Great West Road,
Brentford, Middlesex, England

Actual Inventor(s): PETER CHARLES THOMAS HANNON

Address for Service: DAVIES & COLLISON, Patent Attorneys,
1 Little Collins Street, Melbourne, 3000.

Complete specification for the invention entitled:
"TREATING PLANT INFECTIONS"

The following statement is a full description of this invention,
including the best method of performing it known to us :-
This invention relates to a method for the treatment of plant infections, particularly, but not exclusively, mycoplasmal infections.

The term 'plant' is used herein to mean any member of the vegetable kingdom, including shrubs, trees and fungi.

Mycoplasma or mycoplasma-like organisms, such as spiroplasmas, cause numerous 'yellowing' or stunting diseases in plants, particularly in crops of economic importance. Some examples of particular diseases are as follows:

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>HOST PLANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn stunt</td>
<td>Corn</td>
</tr>
<tr>
<td>Citrus stubborn</td>
<td>Citrus trees</td>
</tr>
<tr>
<td>Coconut lethal yellowing</td>
<td>Coconut palms</td>
</tr>
<tr>
<td>Peach x disease</td>
<td>Peach trees</td>
</tr>
<tr>
<td>Aster yellows</td>
<td>Asters, &amp; Chrysanthemums</td>
</tr>
<tr>
<td>Potato witches' broom</td>
<td>Tomato</td>
</tr>
<tr>
<td>Rice yellow dwarf</td>
<td>Rice</td>
</tr>
<tr>
<td>Grapevine 'flavescence doree'</td>
<td>Grapevines</td>
</tr>
<tr>
<td>Tobacco Yellow dwarf</td>
<td>Tobacco</td>
</tr>
</tbody>
</table>

There are thought to be well over 50 plant diseases attributable to such micro-organisms. The diseases are spread by insect vectors, such as leaf hoppers, and can also be transmitted by certain parasitic plants.
Hitherto, the only really successful chemotherapy of these infections has been with the tetracycline group of compounds, particularly oxytetracycline.

It has now been found that pseudomonic acid A or its salts or esters can provide treatment of these infections.

According to the present invention there is provided a method for the treatment of plant infections, which comprises administering to the plant or plant growth site an effective amount of pseudomonic acid A or a non-toxic salt or ester thereof.

Pseudomonic acid A has the formula (I):

```
\[
\text{OH} \quad \text{OH} \quad \text{CO}_2(CH_2)_8\text{CO}_2\text{H}
\]
```

and is disclosed in British Patent Specification No. 1395907.

Suitable non-toxic salts of pseudomonic acid which may be administered include metal salts, e.g. aluminium, alkali metal salts such as sodium or potassium, alkaline earth metal salts such as calcium or magnesium, and ammonium or substituted ammonium salts for example those with lower alkylamino such as triethylamine, hydroxy-(C_1-6)-alkylamino such as 2-hydroxyethylamino, bis-(2-hydroxyethyl)-amino or tri-(2-hydroxyethyl)-amine, cycloalkylamines such as bicyclohexylamine, or with procaine, dibenzylamine, N,N-dibenzyl-ethylene-diamine, l-ephanamine, N-ethylpiperidine, N-benzyl-β-phenethyl-amine,
dehydroabietylamine, N,N'-bis-dehydroabietylethylenediamine, or bases of the pyridine type such as pyridine, collidine or quinoline.

Preferred salts are alkali metal salts.

Suitable esters include alkyl, aryl and aralkyl groups, any of which may be substituted with a hydroxy, amino or halogen group. For example the ester group may be a C\textsubscript{1-6} alkyl group in particular, methyl, ethyl, n- or iso-propyl, n, sec-, iso or tert-butyl; a halo-(C\textsubscript{1-6})-alkyl group such as trifluoromethyl, 2,2,2-trichloroethyl; an aminoalkyl group such as aminomethyl, 2-aminoethyl; hydroxymethyl, hydroxyethyl; phenyl; substituted phenyl; or a benzyl group.

Preferred esters are C\textsubscript{1-6} alkyl esters.

For administration, the pseudomonic acid, salt or ester thereof will be formulated into a suitable composition with an inert carrier or diluent adapted for administration to the plants or the plant growth site.

Preferably the carrier includes a surface active agent, such as Triton.

The carrier may comprise an aqueous medium which may be buffered to a slightly acidic or neutral pH.
The method of application will depend on the nature of the plant to be treated, but the following methods are suitably employed:

1. Foliar spray
2. Root, shoot or leaf immersion
3. Trunk injection
4. Hydroponic culture
5. Direct soil application

The concentration of the active compound in the treatment composition will vary according to the method of application, higher concentrations being necessary for direct soil application and large trees.

In general, a concentration of from 10 to 1000 µg/ml of active compound is suitable for sprays; root, shoot or leaf immersion or hydroponic culture; from 1 to 3 grams of active ingredient per tree for tree protection; and from 5 to 20 grams per tree for treatment against existing infection.

Examples of specific protection treatments are:

(i) Protection of coconut palms against natural lethal yellowing disease by injection of 1 to 3 grams of active ingredient at 4 monthly intervals.

(ii) Inducing remission of peach x-disease symptoms for one year by a single injection of 1.25 to 3.75 grams of active ingredient per tree.

(iii) Curing white leaf disease of sugarcane by immersion of the seed cuttings from infected stalks in solutions containing 100 µg/ml of active ingredient for 72 hours before planting.
The following are typical of the type of compositions which may be employed.

**Foliar Spray and Trunk Injection**

The active ingredient is dissolved in a 0.01 M phosphate buffer at pH 6.0 to 7.0. Triton is employed as a surface active agent.

**Hydroponic culture**

The active ingredient is incorporated into a hydroponic culture solution, for example 0.003 M Ca(NO$_3$)$_2$, 0.002 M KNO$_3$, 0.002 M MgSO$_4$ or 0.002 M KH$_2$PO$_4$. 
BIOLOGICAL DATA

The activity of sodium pseudomonate A in vitro against various plant and insect-born mycoplasmas was found using the following tests:

*Spiroplasma citri* and *Bee Spiroplasma* were each separately incubated at 32°C in a liquid medium consisting of:

- PPLO broth (Difco) 21 grams/litre
- Sorbitol 70 grams/litre
- Glucose 1 gram/litre
- Fructose 1 gram/litre
- Horse Serum 100 mls/litre
- Phenol red added to a final concentration of 0.002% to 0.005%.

In addition, corn stunt spiroplasma was grown in a similar manner as above except that the horse serum was replaced with foetal calf serum (final concentration 10%) which had been dialysed against 1.1% aqueous KCl, and α keto glutarate was added to a concentration of 100 μg/ml. The incubation temperature was 28 to 29°C.

The IC concentrations of sodium pseudomonate A were found when the controls had grown, judged by phenol red colour change and dark field microscopy.

The results are given in the table below:
<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>MIC (µg/ml) of sodium pseudomonate A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiroplasma citri Strain</td>
<td></td>
</tr>
<tr>
<td>R8A2  (Maroc)</td>
<td>0.8</td>
</tr>
<tr>
<td>ATCC 27556</td>
<td></td>
</tr>
<tr>
<td>Spiroplasma citri</td>
<td></td>
</tr>
<tr>
<td>Israeli strain</td>
<td>0.4</td>
</tr>
<tr>
<td>NCPPB 2566</td>
<td></td>
</tr>
<tr>
<td>Corn Stunt</td>
<td></td>
</tr>
<tr>
<td>Spiroplasma (Rio Grande E 275)</td>
<td>6.25</td>
</tr>
<tr>
<td>Bee Spiroplasma (BC 3)</td>
<td>6.25</td>
</tr>
</tbody>
</table>

The levels of activity are similar to those of the tetracyclines.
CLAIMS
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for the treatment of plant infections, which comprises administering to a plant or plant growth site an effective amount of pseudomonic acid A, of formula (I), or a non-toxic salt or ester thereof:

   ![Chemical Structure](attachment:image.png)

   (I)

2. A method according to claim 1, in which the salt is a metal salt or an ammonium or substituted ammonium salt.

3. A method according to claim 1, in which the ester is a C\textsubscript{1-6} alkyl ester.

4. A method according to any one of claims 1 to 3, in which the active compound is administered in the form of a composition containing an inert carrier or diluent adapted for administration to the plant or plant growth site.

5. A method according to claim 4, in which the composition contains from 10 to 1000 µg/ml of active compound.
6. A method according to any one of claims 1 to 4, in which from 1 to 3 grams of active compound is administered to a tree in order to protect the tree against infection.

7. A method according to any one of claims 1 to 4, in which from 5 to 20 grams of active compound is administered to a tree in order to treat the tree for an existing infection.

8. A plant whenever treated by a method according to any one of claims 1 to 7.

9. The steps or features disclosed herein or any combination thereof.

Dated this 25th day of June, 1981
BEECHAM GROUP LIMITED
by its Patent Attorneys
DAVIES & COLLISON.