FIG. 1A

'\text{A}' Strain

\begin{align*}
\text{ABSORBANCE} & : 1.2, 0.6, 0.2, 0.1 \\
\text{HOURS} & : 10, 20, 30 \\
\text{NO. 1} & \\
\text{NO. 2} & \\
\text{NOS. 3, 4, 5} & 
\end{align*}

FIG. 1B

'\text{B}' Strain

\begin{align*}
\text{ABSORBANCE} & : 0.4, 0.3, 0.2, 0.1 \\
\text{HOURS} & : 10, 20, 30 \\
\text{NO. 1} & \\
\text{NO. 2} & \\
\text{NO. 3} & \\
\text{NO. 4} & \\
\text{NO. 5} & 
\end{align*}

FIG. 1C

'\text{C}' Strain

\begin{align*}
\text{ABSORBANCE} & : 0.25, 0.2, 0.1 \\
\text{HOURS} & : 10, 20, 30 \\
\text{NO. 1} & \\
\text{NO. 2} & \\
\text{NO. 3} & \\
\text{NO. 4} & \\
\text{NO. 5} & 
\end{align*}

FIG. 1D

'\text{D}' Strain

\begin{align*}
\text{ABSORBANCE} & : 1.2, 0.6, 0.2, 0.1 \\
\text{HOURS} & : 10, 20, 30 \\
\text{NO. 1} & \\
\text{NO. 2} & \\
\text{NO. 3} & \\
\text{NO. 4} & \\
\text{NO. 5} & 
\end{align*}

FIG. 1E

'\text{E}' Strain

\begin{align*}
\text{ABSORBANCE} & : 0.25, 0.2, 0.1 \\
\text{HOURS} & : 10, 20, 30 \\
\text{NOS. 1, 2} & \\
\text{NO. 4} & \\
\text{NO. 5} & \\
\text{NO. 3} & 
\end{align*}

FIG. 1F

'\text{F}' Strain

\begin{align*}
\text{ABSORBANCE} & : 0.25, 0.2, 0.1 \\
\text{HOURS} & : 10, 20, 30 \\
\text{NOS. 1, 2} & \\
\text{NO. 3} & \\
\text{NOS. 4, 5} & 
\end{align*}
FIG. 3A

'\textit{A}' Strain

FIG. 3B

'\textit{C}' Strain

FIG. 3C

'\textit{G}' Strain
N-MONOACYL DERIVATIVES OF ARGinine
Tadasami Saito, 890 Fukuya-cho, Totsuka-ku, Yokohama-
shi; Masahiro Takehara, 1060 Ogura, Kawasaki-shi;
Kazuhiko Yamada, 22-22, 3-chome, Fujigakou, Fuji,
Sawagawa-shi; and Ryosynoke Yoshida, 1265-11 Kajiwa-
ra, Kamakura-shi, all of Kanagawa-ken, Japan; and Yukiko
Sasaki, 28-6, 3-chome, Minami-ku, Yokohumishi, Yok-
ohama-ken, Japan.

Continuation-in-part of abandoned application Ser. No.
99,768, Dec. 21, 1970. This application June 28, 1971,
Ser. No. 157,584

Claims priority, application Japan, Dec. 30, 1969,
45/1,750

U.S. Cl. 260—326.45

6 Claims

ABSTRACT OF THE DISCLOSURE

The lower alkyl or benzyl esters and amides of the N-
monoaoyl derivatives of the basic amino acids (α,γ-di-
aminobutyric acid, oroticine, citrulline, lysine, arginine,
and histidine) and their N-methyl and N-benzyl deriv-
atives in which acyl is the acyl radical of an aliphatic mono-
acrylyc acid having 6 to 20 carbon atoms have bact-
eriostatic effects of the order of magnitude of that of
hexachlorophene. They may be used as antibacterial agents in
the form of their water-soluble salts with hydrochloric acid,
pyrrolidine carbonate acid, and other inorganic and
organic acids.

This application is a continuation-in-part of the copending
application Ser. No. 99,768, filed on Dec. 21, 1970
and now abandoned.

This invention relates to antiseptic, disinfectant, med-
ical, preservative, bactericidal, bacteriostatic, germicidal
and fungicidal materials and compositions.

Corrosive sublimate, cresol, alcohol, invert soap and
etc. have been used as disinfectants or antimicrobial deter-
gents in food hygiene field, environment sanitary field
and medical field. However, these disinfectants have the
following unsatisfactory properties. Corrosive sublimate
has powerful disinfectant action, but it has a strong irrita-
tion against skin and causes a serious problem in the dis-
posal of waste matters owing to mercury containing mate-
rals. And cresol has high toxicity to man and beasts, while
alcohol has weak disinfectant action. Although invert soap
such as "Hyamine" (Product of Rohm & Haas Co., Inc.)
has a powerful disinfectant action, the effect is markedly
decreased by co-existence of protein, fat, sodium chloride,
or soap, and it has high toxicity to man and beasts (For
example, the acute oral toxicity (LD₅₀) value of "Hy-
amine" is 420±25 mg. per kg. of body weight of mice.)

Recently, a disinfectant incorporating soap or synthetic
detergent, the so-called deodorant detergent is recom-
ended. As disinfectants or deodorizers in these deter-
gents, for example hexachlorophene, dichloroacetamide,
tribromosalicylanide, tetramethylthiuram disulfide and
the like have been practically used. However, each of these
desorizers has irritation of the skin and is hardly soluble
or insoluble in water, and hence it is necessary that these
desorizers are incorporated in a relatively large amount
into detergent for purpose to retain their antimicrobial ac-
tivity. And the use of such a large amount causes further
strong irritation on the skin. Particularly, hexachlorophene
or tetramethylthiuram disulfide incorporating detergent has
been reported to cause eczematoid, sensitization to the
skin when exposed to sunlight after its use. Also, usual
cationic surfactants which exhibit in general strong anti-
microbial action are water soluble, but they cannot be
used as deodorizers of detergents, since an insoluble pre-
cipitation is formed when they are incorporated in deter-
gent such as soap and thereby antibacterial activity is re-
duced or disappeared as well as the detergency is markedly
reduced.

On the other hand, cosmetics, leather goods, rubber
goods, paints, foods and feed are easily attacked and de-
teriorated by microorganism and hence almost all of them
does not stand long storage or use.
Various antiseptic agents and fungicidal agents have been
hitherto developed and employed in this field. However,
there has not yet been proposed antiseptic agent which
possesses low or non-toxicity, non-irritation in addition to
the complete inhibition of the growth of microorganisms.
For example, since cosmetics are composed mainly of
water and oil and since they also contain glycerin, protein
and other materials which are easily attacked by micro-
organisms, they are able to be changed in quality and ap-
pearance as well as foods. Hence, benzoic acid, its deriva-
tives hexachlorophene and the like have been employed as
antiseptic agent for cosmetics, but all of them have the
following disadvantages: (1) The concentration in water
phase which is easily attacked by microorganisms is low
because of their oil-soluble properties. (2) Their antiseptic
action is greatly reduced by the action of surface active
agents which are present in cosmetics. (3) They have skin
irritation and high toxicity.

In addition, sorbic acid, sodium propionate, dehydro
acetic acid, nitrofuran series compounds and etc. have been
used as antiseptic agents for food but the amount added of
them is restricted from the standpoint of toxicity and so,
they can not exert sufficient antiseptic action within
their allowance of addition. Recently, higher alkyl deriva-
tives of peptide containing glutamic acid or basic amino
acid have been used as antiseptic agents, but the former
is hard to handle because of its very low water solubility,
whereas the latter injures the taste of food because of
very bitter taste in addition to high cost.

An object of the present invention is to provide deodor-
zers which are suitable to be incorporated into a deter-
gent and which have the desired characteristic properties
in that they exert excellent antimicrobial action, cause no
irritation to the skin and are effective to enhance cleaning
effect.

It is another object of the present invention to provide
an antiseptic material composition in which the disadvan-
tages of the known antiseptic materials are reduced or
obviated.

It has been found that mono-N-higher aliphatic acyl
basic amino acid deritives of α,γ-diaminobutyric acid,
arginine, ornithine, citrulline, lysine and histidine, and salts
thereof, hereinafter described in detail, have the desirable
and satisfactory characteristic property as deodorizers of
detergents, owing to their water soluble property, their
excellent antimicrobial activity against microorganisms
such as bacteria and fungi, their good surface active ac-
tion and their mildness to the skin. Moreover, by the use
of them, skin troubles such as irritation of the skin, ex-
ematoid and sensitization are not entirely recognized, and
even when incorporated in soap and other synthetic deter-
gents, they greatly exert their excellent antimicrobial ac-
tion without reducing cleaning effect. Also, in case where
they are incorporated in synthetic detergents which usually
roughen the skin, they have skin protecting action prevent-
ing the skin from chapping.

It has further been found that said mono-N-higher al-
iphatic basic amino acid derivatives antiseptic, medicina-
185   l, preservative, bactericidal, bacteriostatic, germicidal
and fungicidal properties.

According to the present invention, there are provided
antiseptic, disinfectant, medicinal, preservative, bacterici-
dal, bacteriostatic, germicidal and fungicidal agents which
are much secure to men and beasts, comprising at least one
mono-N-higher aliphatic acyl basic amino acid derivatives
Compounds which are incorporated as deodorizers in detergent and which are also useful as antiseptic material or germicide according to the present invention may be easily prepared and are cheaply available. That is, among the compounds of the general formulas (I) and (II), ones wherein \( n \) is 2 are \( \alpha,\gamma \)-diaminobutyric acid derivatives, ones wherein \( n \) is 3 are ornithine derivatives and ones wherein \( n \) is 4 are lysine derivatives. And, the compounds of general formula (III) are arginine, citrulline and histidine derivatives. Each of these mono-N-higher aliphatic basic amino acid derivatives may be easily prepared by the reaction of corresponding basic amino acid with higher fatty acid chloride in alkaline aqueous medium.

Either of optically active L- or D-form or racemic form of basic amino acid components are effective. As salts of these N-higher aliphatic acyl basic amino acid derivatives, for example mineral acid salts such as hydrochloride and sulfate, salts with organic acid such as an optically active or inactive \( \alpha \)-pyrrolidonecarboxylic acid, an optically active or inactive acidic amino acids (e.g. glutamic acid and aspartic acid), lactic acid, citric acid and acetic acid are taken. Especially, the use of hydrochloride and salt with DL- or L-\( \alpha \)-pyrrolidonecarboxylic acid are suitable from a point of view of crystalline nature.

Representative examples of mono-N-higher aliphatic acyl basic amino acid derivatives are the following N-cocoyl-N,N,N,N-diethyl-\( \gamma \)-aminobutyric acid methyl ester hydrochloride, N-lauroyl-L-ornithine methyl ester hydrochloride, N-lauroyl-N,N,N,N-dimethylornithine benzyl ester hydrochloride, N-palmitoyl-N,N,N,N-trimethylornithine methyl ester hydrochloride, N-lauroyl-N,N,N,N-methyl-\( \gamma \)-aminobutyric acid methyl ester hydrochloride, N-lauroyl-N,N,N,N-benzyl-N,N,N,N-methyl-\( \gamma \)-aminobutyric acid methyl ester hydrochloride, and Staphylococcus aureus is 170, 85 and 250.

<table>
<thead>
<tr>
<th>TABLE 1. Microorganism Compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>The present invention: N-cocoyl-L-arginine ethyl ester pyrrolidone carboxylic acid</td>
<td>75-100</td>
<td>150-200</td>
<td>50-100</td>
<td>50-100</td>
<td>400-500</td>
<td>100-150</td>
<td>2,000-2,500</td>
</tr>
<tr>
<td>N-lauroyl-L-arginine ethyl ester pyrrolidone carboxylic acid</td>
<td>75-100</td>
<td>150-200</td>
<td>50-100</td>
<td>50-100</td>
<td>400-500</td>
<td>100-150</td>
<td>2,000-2,500</td>
</tr>
<tr>
<td>N-lauroyl-L-lysine methyl ester hydrochloride</td>
<td>75-100</td>
<td>150-200</td>
<td>50-100</td>
<td>50-100</td>
<td>400-500</td>
<td>100-150</td>
<td>2,000-2,500</td>
</tr>
<tr>
<td>N-lauroyl-L-lysine methyl ester hydrochloride</td>
<td>75-100</td>
<td>150-200</td>
<td>50-100</td>
<td>50-100</td>
<td>400-500</td>
<td>100-150</td>
<td>2,000-2,500</td>
</tr>
<tr>
<td>N-lauroyl-L-lysine methyl ester hydrochloride</td>
<td>75-100</td>
<td>150-200</td>
<td>50-100</td>
<td>50-100</td>
<td>400-500</td>
<td>100-150</td>
<td>2,000-2,500</td>
</tr>
<tr>
<td>The reference example: &quot;Hyamine&quot; (hexaethanolamine chloride)</td>
<td>75-100</td>
<td>150-200</td>
<td>50-100</td>
<td>50-100</td>
<td>400-500</td>
<td>100-150</td>
<td>2,000-2,500</td>
</tr>
</tbody>
</table>

Note.—The numerical value in table 1 indicates inhibiting concentration of compound against the growth of microorganism in per 7,000 ml, under contacting of each microorganism in these water solution for ten minutes.
The microorganisms, culture media and preparation of cultured cells employed in the test, and the assay method of bactericidal activity are as follows:

(a) Microorganisms employed:

1. Escherichia coli (ATCC 3655)
2. Pseudomonas aeruginosa (IAM 1002)
3. Proteus vulgaris (IAM 1025)
4. Staphylococcus aureus (ATCC 6538)
5. Bacillus subtilis (ATCC 6633)
6. Candida albicans (ATCC 10259)
7. Aspergillus niger (ATCC 9642)

(b) Culture media employed:

1. Meat extract 1.0%, polypeptone 1.0%, NaCl 0.25% pH 7.0 (used for microorganisms 1-5).
2. Yeast extract 0.3%, malt extract 0.3%, polypeptone 0.5%, glucose, 1.0%, pH 6.2 (used for microorganisms 6 and 7).

(c) Preparation of cultured cells: Microorganisms 1-6 were cultured statically at 31°C for 20 to 24 hr. in test tubes in which are above-mentioned media were poured, and microorganism 7 was cultured at 31°C for 4 days on the yeast-malt agar slant.

(d) Assay method of bactericidal activity: 0.5 ml. of the above-mentioned cultured cells was inoculated respectively to each test tube in which 10 ml. of sterilized water solution containing each concentration of various compounds was poured. After cells were contacted with the compound, one loopful of cell suspension of microorganisms 1 to 5 was spread on a nitririn-bouillon agar plate, while the yeast-malt agar plate was used in case of microorganisms 6 and 7. After cultivation at 31°C for 48 hr., living or died of cells was judged.

The third characteristic feature in this invention is the fact that they possess, only a small, or nontoxicity and do not cause any significant skin irritation. For example, in an acute oral toxicity test (LD₅₀) carried out on mice, the respective LD₅₀ values of N-lauroyl-L-arginine methyl ester DL-a-pyroline carbonate and N-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate are 2-3.0 g/kg, 10.75 g/kg, body weight. Accordingly, injuring to men and beasts are scarcely considered. Moreover, after this amino acid derivative was well mixed with polyethylene glycol, it was spread on the cloth of a sticking plaster for a patch test used in the wet state, and then applied to the skin for 24 hours. As the result of this patch test, almost no irritation against the skin was observed. And since this effective materials are water soluble cationic surface active agents, they have a remarkable detergent effect owing to strong foaming action.

TABLE 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Microorganism</th>
<th>Time for pre-cultivation, hours</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>N-lauroyl-L-lysine methyl ester hydrochloride</td>
<td>24</td>
<td>Nutrient bouillon, pH 7.</td>
</tr>
<tr>
<td>2</td>
<td>N-lauroyl-L-arginine methyl ester hydrochloride</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>N-palmitoyl-N,N-dimethyl-L-lysine methyl ester hydrochloride</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>N-myristoyl-N,N-dimethyl-L-lysine methyl ester hydrochloride</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>N-lauroyl-L-arginine methyl ester hydrochloride</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>N-lauroyl-L-lysine methyl ester pyrrolidone carboxylate</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>N-lauroyl-L-lysine methyl ester hydrochloride</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&quot;A-2&quot;</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>&quot;B-2&quot;</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Stearic acid</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Lauryl sarcosine</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Penicillin</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Sorbic acid</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Figures in Tables represent the concentration (mg/ml.) of compounds which causes the growth inhibition of microorganisms.

Microorganisms tested, culture conditions and culture medium are as follows:

The second characteristic feature in this invention is the fact that these non-N-higher aliphatic acyl basic amino acid derivatives possess a powerful antibacterial activity against microorganisms which possess relatively strong resistance to various known antiviral compounds, such as Bacillus subtilis, Candida albicans and Aspergillus niger. For example, the bacteriostatic or inhibitory effect of the compounds of the present invention in comparison with that of nitrofurans series compound, palmitoyl-L-lysyl-L-lysine methyl ester dihydrochloride, streptomycin, penicillin and sorbic acid lauroyl sarcosine are illustrated in the Table 2.

Moreover, as they have strong emulsifying power to cosmetic and are water-soluble, the solubility in water phase, which is easily attacked by microorganisms, is higher than that in oil phase and they show appreciable antiseptic effect even when added in a small amount to cosmetics. And they possess strong penetrating power to a fiber. Accordingly, they may be applied to wide use.
The following shows the water solubilities of various mono-N-higher aliphatic acyl basic amino acid derivatives:

<table>
<thead>
<tr>
<th>Compound:</th>
<th>Solubility (g./dl. 40°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N*-Lauroyl-L-arginine methyl ester hydrochloride</td>
<td>5</td>
</tr>
<tr>
<td>N*-Lauroyl-L-arginine methyl ester DL-a-pyrrolidonecarboxylate</td>
<td>20</td>
</tr>
<tr>
<td>N*-Lauroyl - N*,N* - dimethyl - DL - ornithine methyl ester hydrochloride</td>
<td>2.5-3.0</td>
</tr>
<tr>
<td>N*-Palmitoyl-L-lysine methyl ester hydrochloride</td>
<td>25-30</td>
</tr>
<tr>
<td>N*-Cocoyl-L-arginine methyl ester-L-a-pyrrolidonecarboxylate</td>
<td>15</td>
</tr>
<tr>
<td>N*-Lauroyl - N*,N* - dimethyl - DL - ornithine methyl ester hydrochloride</td>
<td>5-8</td>
</tr>
</tbody>
</table>
| Mono-N-higher acyl basic amino acid derivatives or salts thereof can be compounded, applied or sprayed according to the shape of soap, synthetic detergent, food, feed, cosmetics, fiber good, leather goods and paint in any form of liquid, powder, or emulsion. In addition, we want to refer to a few following concrete cases of the above-mentioned amino acid derivatives. Firstly, it was found that antiseptics comprising one or more than two kinds of these esters or salts as an effective component prevent the water-soluble “biochi putrefaction” rather completely.

The phenomenon of “biochi putrefaction” means that the “sake” (Japanese wine), synthetic sake which contains partially brewing alcohol, or the products of brewing alcohol such as “mirin” (a sweet kind of “sake”) becomes impossible to eat and drink because of white muddy and rancidity during the storage, or after the bottling. Generally, “biochi putrefaction” is caused by Lactobacillus heterochoi, Lactobacillus japonicus or Lactobacillus homohioch, which comes from brewage.

The example 9 and 10 indicate the antibacterial activity of N*-Lauroyl-L-arginine-methyl ester pyrrolidone carboxylate in comparison with salicylic acid which has been used habitually for prevention of “biochi putrefaction.” Moreover it is confirmed that these compounds adhered to a mucosa of oral cavity and were disinfectant in it for a long time. From these findings these compounds have marked antibacterial activity against both a bacteria belonging to genus Lactobacillus, a main pathogen of dental caries, and a bacteria belonging to genus Staphylococcus, a main pathogen of alveolar pyorrhoea.

Table 3 shows the result of antibacterial test of these compounds against genus Lactobacillus, i.e., Lactobacillus fermenti-36 ATCC 9938, and Streptococcus faecalis ATCC 8083, and genus Staphylococcus, Staphylococcus aureus ATCC 65389.

As shown in Table 3, the antibacterial activity of generally used sodium N-lauroyl-sarcosinate was a control.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lactobacillus fermenti</th>
<th>Streptococcus faecalis</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>N*-cocooyl-L-arginine methyl ester hydrochloride</td>
<td>14</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>N*-lauroyl-L-arginine methyl ester</td>
<td>18</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>N*-cocooyl-L-arginine ethyl ester PCA</td>
<td>21</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>N*-palmitoyl-L-lysine methyl ester acetate</td>
<td>17</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>Sodium-N-lauroyl-sarcosinate</td>
<td>79</td>
<td>49</td>
<td>80</td>
</tr>
</tbody>
</table>

1 The growth of Lactobacillus and Streptococcus was measured after 24 hr. standing culture at 37°C. and Staphylococcus was after 48 hr. at 37°C.
2 Fifty acid residue of cocoyl oil.
3 DL-Pyrrolidone carboxylic acid.

Figures in Table 3 represent the concentration (µ/mL) of compounds which causes the fifty percents inhibition of bacterial growth.

It is evident from the Table 3 that any of them is more effective than a control compound.

They possess nearly the same foaming power, which is a desirable surface active action as dentifrice, is the same as a control, sodium-N-lauroyl sarcosinate as shown in Table 4. The foaming power of 0 minute value are illustrated in the method of JISK 3362. As the results, above mentioned each compound possesses many extremely satisfactory properties as combining component of dentifrice.

| TABLE 4 |
|-----------------------------|------------------------|
| Name of compound: | Foaming power (mm.) |
| N*-cocooyl-L-arginine methyl ester PCA salt | 190 |
| N*-cocooyl-L-arginine ethyl ester PCA salt | 192 |
| N*-lauroyl-L-arginine methyl ester hydrochloride | 185 |
| Sodium-N-lauroyl sarcosinate | 200 |

Also, these active compounds are incorporated in soap or other synthetic detergents in 0.1-50% by weight, preferably 0.1-5% by weight based thereon. As synthetic detergents, for example, detergents containing predominantly at least one of N*-higher acyl glutamate, N*-higher acyl aspartate, alkylbenzenesulfonate, higher alkylsulfonate, α-olefin sulfonate and alkylethersulfate are taken. The shape of detergent may be bar, tablet, powder, paste or liquid according to the appearance of a product required.

In the appended drawing, FIGS. 1A to 1H diagrammatically illustrate the bacteriostatic effects of N*-lauroyl-arginine methyl ester a-pyrrolidonecarboxylate of this invention against respective strains of microorganisms when added to culture media for the eight strains together with soap.

FIGS. 2A to 2D illustrate the bacteriostatic effects of the compound of FIGS. 1A to 1H in cooperation with a synthetic detergent when added to culture media of the strains illustrated in FIGS. 1A, 1C, 1E, and 1G respectively.

FIGS. 3A to 3C diagrammatically illustrate the bacteriostatic effects of a milled soap containing the same compound of the invention when added to culture media of the strains illustrated in FIGS. 1A, 1C, and 1G.

The drawing is explained in more detail in Examples 14, 15, and 16 hereinbelow.

EXAMPLE 1

Effect of 0.1% N*-cocooyl-L-arginine ethyl ester-pyrrolidone carboxylate (CAE-P) on finger's disinfection and washing was examined.

Various kinds and numbers of bacteria were found to exist on the fingers of fifteen persons selected as a panel. Each member immersed his hands and forearms by 35 cm. length from the tip of the middle finger in a washbowl containing 2 l. of tap water, and then repeated the same thing for a minute in 0.1% N*-cocooyl-L-arginine ethyl ester pyrrolidone carboxylate solution. Finally he washed them in 2 l. of sterilized water.

The number of viable cells in these washings was counted after the performance of all members. On the other hand, in another group consisting of fifteen members the same experiment was carried out as control by using tap water instead of 0.1% N*-cocooyl-L-arginine ethyl ester pyrrolidone carboxylate solution.

| TABLE 8 |
|-----------------------------|------------------------|
| Compound | Lactobacillus fermenti | Streptococcus faecalis | Staphylococcus aureus |
| N*-cocooyl-L-arginine methyl ester hydrochloride | 14 | 26 | 5 |
| N*-lauroyl-L-arginine methyl ester | 18 | 28 | 12 |
| N*-cocooyl-L-arginine ethyl ester PCA | 21 | 45 | 8 |
| N*-palmitoyl-L-lysine methyl ester acetate | 17 | 28 | 5 |
| Sodium-N-lauroyl-sarcosinate | 79 | 49 | 80 |

Before washing | After washing
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of viable cells (in 0.1 ml.)</td>
<td>341</td>
</tr>
</tbody>
</table>

With 0.1% CAE-P

Without 0.1% CAE-P

348 | 289
EXAMPLE 2

The Bath Preparations were prepared by mixing such components as written in the following.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-cocoyl-L-arginine ethyl ester DL-α-pyrrolidone carboxylate</td>
<td>500 mg</td>
</tr>
<tr>
<td>Sodium iodide</td>
<td>1 mg</td>
</tr>
<tr>
<td>Sodium bromide</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Lithium carbonate</td>
<td>0.01 mg</td>
</tr>
<tr>
<td>Manganese sulfate</td>
<td>0.01 mg</td>
</tr>
<tr>
<td>Iron sulfate</td>
<td>0.01 mg</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>15 mg</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>40 mg</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>66.4 mg</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>96.0 mg</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>280.5 mg</td>
</tr>
</tbody>
</table>

EXAMPLE 3

A Preventive Cleansing agent was prepared by mixing such components as shown in the following table.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-lauroyl-L-arginine ethyl ester DL-α-pyrrolidone carboxylate</td>
<td>3.0 %</td>
</tr>
<tr>
<td>Triethanol amine</td>
<td>2.0 %</td>
</tr>
<tr>
<td>Perfume</td>
<td>0.3 %</td>
</tr>
<tr>
<td>Water</td>
<td>94.7 %</td>
</tr>
</tbody>
</table>

EXAMPLE 4

The O/W type cold cream was prepared as follows.

Fifty grams of mineral oil, 7 g. of beeswax, 2 g. of “tween 40” (a registered trademark), 8 g. of “Atlas G-1726” (a registered trademark), were emulsified with 33 ml. of aqueous 1% N-myristoyl-N,N'-dimethyl-L-lysine hydrochloride at 70°C.

On the other hand, the control cold cream containing 33 ml. of water was also prepared in the same way.

The newly prepared Cream Containing Lysine derivative caused a good emulsion and any change of the quality could not be found on storage at 30°C and at moisture 90% during two months.

The control cream showed the growth of mold and resulted in coloration.

EXAMPLE 5

The toilet water was prepared as follows.

Ten part of Ethanol, 0.05 part of gum tragacanth, 5 part of propylene glycol, 1 part of N-lauroyl-L-arginine methyl ester-DL-α-pyrrolidone carboxylate were mixed with 85 part of water.

On the other hand, the control toilet water containing polyoxyethylene sorbitan monolaureate instead of arginine derivative was prepared in the same way.

In the newly prepared toilet water containing arginine derivative any change of the quality could not be found on storage at 30°C and at moisture 90% during one month in a room.

The control toilet water showed the growth of mold.

EXAMPLE 6

0.5 g. of N-parmiroyl-N,N'-dimethyl-L-ornithine amide was mixed with 1 kg. of assorted feed.

In the newly prepared assorted feed containing ornithine derivative any change of the quality could not be found on storage at room temperature and during two weeks.

The control assorted feed showed the growth of mold and a putrid smell.

EXAMPLE 7

0.5 ml. of aqueous 3% N-lauroyl-L-arginine ethyl ester DL-α-pyrrolidone carboxylate was sprayed per 100 cm² of surface of dressed oxhide.

In the sprayed dressed oxhide any change of the quality could not be found on storage at 30°C and at moisture 90% during a month.

EXAMPLE 8

The control dressed oxhide showed the growth of mold.

EXAMPLE 9

An aqueous culture medium comprising 100 ml of “sake” (Japanese wine) (16% alcohol concentration) and 0.8 g. of beef liver extract was adjusted to pH 5.0.

After sterilization, sample (N-lauroyl-L-arginine methyl ester pyrrolidone carboxylate) shown in Table 5 was added to the medium in the concentration of 1%/ml., 10%/ml. or 50%/ml. Three microorganisms of hiocchi-bacteria listed in Table 5 were inculated, and cultured at 30°C. Table 5 indicates the visible growth after 3 weeks culture in case of 10 and 50%/ml. Salicylic acid (LD₅₀ orally in rat: 0.5 g./kg.) was used as a control in the example.

<table>
<thead>
<tr>
<th>Lactobacillus acidoinciliis</th>
<th>Lactobacillus acidophilus</th>
<th>Lactobacillus helveticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>10 x 10⁶</td>
<td>10 x 10⁷</td>
</tr>
</tbody>
</table>

1 Cell growth was not detected.
2 Cell growth was clearly observed.

EXAMPLE 10

“Sake” A (17% as spirits) added with 0.001% of N-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate and “Sake” B (17% as spirits) added with 0.001% of Na-salicylate were stored at 30°C for 2 months, and the flavor and taste were compared each other.

“Sake” A did not turn sour at all even after six months, while “Sake” B began to turn sour virtually after a month.

EXAMPLE 11

Kamaboko was prepared according to a known method by employing the following components; 70.0% of frozen fish meat, 2.6% of sodium chloride, 0.3% of “HI-2ME” (Product of Ajinomoto Co., Inc.), Mixture of sodium inosinate and sodium glutamate), 5.5% of egg albumin, 2.1% of sucrose, 13.8% of starch and 7.0% of cold water.

“Kamaboko” A added with 0.012% of L-citrulline methyl ester hydrochloride together with 0.012% of “AF-2” (Veno Pharmaceutical Co., Nitrofuram compounds) and “Kamaboko” B added with only 0.012% of “AF-2” were stored at 30°C for 3 days in a petri dish with a cover. The result was that colonies of fungi conceived as Penicillium occurred on the “Kamaboko” B. However, any spoilage with bacterial slime or infection with fungi was not detected on the “Kamaboko” A in addition to no change in taste.
The tooth paste were prepared by mixing such components as written in the following. Percent by weight

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicalcium phosphate 2H₂O</td>
<td>4.5</td>
</tr>
<tr>
<td>Gum tragacanth</td>
<td>2.0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.8</td>
</tr>
<tr>
<td>N⁺-cocoyl-L-arginine ethyl ester DL-α-pyrolidone carboxylate</td>
<td>3.0</td>
</tr>
<tr>
<td>Saccharin</td>
<td>0.4</td>
</tr>
<tr>
<td>Flavoring material</td>
<td>1.0</td>
</tr>
<tr>
<td>Water</td>
<td>30.6</td>
</tr>
</tbody>
</table>

The wet dentifrice was prepared by mixing such components as shown in the following table.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium carbonate precipitate</td>
<td>70.2</td>
</tr>
<tr>
<td>Sodium carboxymethyl cellulose</td>
<td>22.0</td>
</tr>
<tr>
<td>N⁺-lauroyl-L-arginine methyl ester DL-α-pyrolidone carboxylate</td>
<td>3.0</td>
</tr>
<tr>
<td>Saccharin</td>
<td>0.5</td>
</tr>
<tr>
<td>Flavoring material</td>
<td>1.3</td>
</tr>
<tr>
<td>Water</td>
<td>3.0</td>
</tr>
</tbody>
</table>

N⁺-Lauroyl - L - arginine methyl ester DL-α-pyrolidone-carboxylate (referred to as DL-I) was added to 2.0 g. of fatty acid soap material in an amount given in Table 6 and the mixture was dissolved in 100 ml. of the culture medium shown in Table 7, then was adjusted to a pH of between 6.2 and 7.3. 4 ml. of the respective solutions was inoculated with each of the eight kinds of microorganisms shown in Table 2 and cultivated under the condition shown in Table 2. The growth of microorganisms was estimated by increased turbidity of the culture solution and was verified by measuring at times absorbance (optical density) at 560 mλ with a spectrophotometer. The results were as shown in FIG. 1 from which the culture mediums (Nos. 4 and 5) added with DL-I were recognized to exhibit marked inhibitory effect to the growth of microorganisms as compared with the culture medium alone (No. 1) and with the culture medium (No. 2) added with fatty acid soap material alone, and also their inhibitory effect was recognized to be approximately equal with that of hexachlorophene.

<table>
<thead>
<tr>
<th>TABLE 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Control:</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>1 G-11: hexachlorophene</td>
</tr>
</tbody>
</table>

N⁺-Lauroyl - L - (or DL) - arginine methyl ester L-α-pyrolidone-carboxylate (referred to as L-I or DL-I respectively) was added to 2.0 g. of monosodium N⁺-cocoyl-L-(or DL)-glutamate (referred to as L-II or DL-II respectively) in an amount given in Table 8. Subsequently, inhibitory tests against various microorganisms were carried out according to the similar manner in Example 14.

<table>
<thead>
<tr>
<th>TABLE 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
</tr>
<tr>
<td>Control:</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>The present invention:</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
</tbody>
</table>

Each of the culture media was recognized to exhibit a marked inhibitory effect against A-H microorganisms. The results of inhibitory tests against A, C, E and G microorganisms were shown in FIG. 1. It can be seen from FIG. 2 that even the culture medium (No. 2) added with DL-II alone exhibits some extent of antibacterial activity compared with the culture medium alone (No. 1) but the culture media (Nos. 4 and 5) added with further L-I or DL-I exhibit more excellent antibacterial activity, that is comparable to hexachlorophene.

EXAMPLE 16

2 Parts of N⁺-lauroyl - DL - arginine methyl ester L-α-pyrolidone-carboxylate (referred to as DL-I) was sufficiently compounded with 100 parts of soap material with a small roller. The mixture was warmed to 50-60°C. and extruded into a bar of soap having 25 mm. in diameter with a prodder for making soap and then moulded in a metal mould installed to an impact type moulding machine. The appearance of the bar soap thus prepared was equal with that of the bar soap prepared similarly from soap material alone. Also, there is recognized no difference between both the products in solubility by rubbing which was measured according to the Japanese Industrial Standards M-3304, and in disintegration in water.

Next, 2.0 g. of each of samples was scraped from the respective products, i.e., the bar soap added with DL-I and one added with no DL-I, and dissolved respectively in 100 ml. of culture solution as in the Example 14. 4 ml. of the solution was inoculated with the microorganisms shown in Table 7 and cultivated under the same conditions. The growth of microorganisms was estimated from the turbidity of the culture solution, which was measured using a spectrophotometer according to the similar manner as in Example 14.

As the result, the culture medium added with DL-I was recognized to exhibit an inhibitory effect against all of the A-H strains. Only the results of inhibitory test to the
A, C and G strains were shown in FIG. 3 wherein a dotted line means the case where DL-I was added and a line means the case where DL-I was not added. It may be seen that the soap containing DL-I is effective in inhibiting the growth of microorganisms as compared with the soap not containing DL-I and especially it exhibits a marked inhibitory effect against A microorganism.

EXAMPLE 17

10 Parts of sodium sulfate was mixed with 90 parts of sodium dodecyl sulfate (referred to as SDS) and Nlauroyl-N\textsuperscript{2}, N\textsuperscript{3}-dimethyl-DL-ornithine methyl ester lactate (referred to as DL-III) was added thereto in an amount given in Table 9 to prepare shampoo composition. Each sample was dissolved in 100 ml of the culture medium shown in Table 6 and 4 ml of the solution was inoculated with each of A-H microorganisms and cultivated. Subsequently, the growth of microorganisms was measured according to the similar manner as in Example 1. As the results, in case of the culture medium (No. 1) not added with DL-III, symptom of the growth of microorganisms was recognized in one hour after cultivation, while the culture medium (Nos. 4 and 5) added with DL-III was recognized to inhibit the growth of microorganisms for 10-25 hours after cultivation. This was approximately equal to the inhibitory effect of the culture medium (No. 2) with hexachlorophene.

**TABLE 9**

<table>
<thead>
<tr>
<th>Number</th>
<th>Amount of SDS, g.</th>
<th>Amount of deodorizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.8</td>
<td>0.2 None</td>
</tr>
<tr>
<td>2.</td>
<td>1.8</td>
<td>0.2 O-Y 0.40 g.</td>
</tr>
<tr>
<td>3.</td>
<td>1.8</td>
<td>0.2 DL-III 0.040 g.</td>
</tr>
<tr>
<td>4.</td>
<td>1.8</td>
<td>0.2 DL-III 0.225 g.</td>
</tr>
</tbody>
</table>

1 G-11: hexachlorophene.

**EXAMPLE 18**

5 Parts of each of the eleven kinds of mono N-higher aliphatic acid basic amino acid derivatives shown in Table 10 was sufficiently compounded together with 100 parts of soap material and with 1-3 parts of water using a rotor. The mixture was warmed to 50-55° C and extended into a bar soap having 25 mm in diameter using a prodder, and the bar soap was stamped.

The result of having measured the solubility by rubbing according to the Japanese Industrial Standards K-3304 on the bar soap prepared in the above and the bar soap similarly prepared from soap material alone showed that any of the bar soaps containing the compounds of the present invention has a value within 20% from the measured value of the bar soap of control. Also, there was no appreciable difference between both the products in disintegration in water.

**TABLE 10**

1. N\textsuperscript{2}-Myristoyl-L-lysine methyl ester hydrochloride
2. N\textsuperscript{2}-Myristoyl-L-lysine amide hydrochloride
3. N\textsuperscript{3}-palmitoyl - N\textsuperscript{2}, N\textsuperscript{3}-dimethyl-L-lysine methyl ester hydrochloride
4. N\textsuperscript{3} - Lauroyl-N\textsuperscript{2}, N\textsuperscript{3}, N\textsuperscript{3}-trimethyl-DL-ornithine methyl ester hydrochloride
5. N\textsuperscript{3}-cocoyl-N\textsuperscript{3}-methyl-DL-ornithine methyl ester hydrochloride
6. N\textsuperscript{3}-palmitoyl-N\textsuperscript{3}, N\textsuperscript{3}-dimethyl - DL - ornithine benzyl- ester hydrochloride
7. N\textsuperscript{3}-Lauroyl-N\textsuperscript{3}-benzyl-N\textsuperscript{3}-methyl-DL-ornithine methyl ester hydrochloride
8. N\textsuperscript{3}-cocoyl-N\textsuperscript{3}, N\textsuperscript{3}-dimethyl - DL - \(\gamma\)-diaminobutyric acid methyl ester hydrochloride
9. N\textsuperscript{3}-palmitoyl-N\textsuperscript{3}-hydrogenated tallowyl - DL - ornithine ethyl ester hydrochloride
10. N\textsuperscript{3}-hydrogenated tallowyl arginine methyl ester DL-\(\varepsilon\) pyrrolidone carboxylate
11. N\textsuperscript{3}-semihydrogenated tallowyl arginine butyl ester hydrochloride

2 g. of the each sample was scraped from the respective bar soaps containing the above compounds and the bar soap for control, and dissolved in 100 ml of culture medium shown in Table 6. 4 ml of the each solution was inoculated with each microorganism of A and E microorganisms and cultivated. The growth of the microorganisms was estimated from the turbidity of the culture solution according to Example 14. As the result of carrying out the standing cultivation for 22 hours with respect to A microorganism, the absorbance of the culture solution added with the bar soap for control in 10 hours after cultivation was 0.80, while the absorbance of any culture solutions added with the bar soaps containing the additives in the that time was below 0.10. Also, as the result of carrying out the shaking cultivation for 12 hours with respect to E microorganism, the absorbance of the culture solution added with the bar soap for control in 8-10 hours after cultivation was 0.20, while that of any culture solutions added with the bar soaps containing the additives in that time was 0.05. As apparent from these results, Mono-N-higher aliphatic acid basic amino acid derivatives were recognized to exhibit deodorant effect.

**SUPPLEMENTAL EXAMPLE**

(i) Preparation of N\textsuperscript{3}-lauroyl-L-lysine methyl ester hydrochloride

66.2 g. (0.2 m.) of N\textsuperscript{3}-carboxbenzyloxy-L-lysine methyl ester hydrochloride were dissolved in 200 ml of tetrahydrofuran and then added with 38.5 ml. (0.42 m.) of triethylamine and 43.7 g. (0.2 m.) of lauroyl chloride under stirring and cooling. The resulting mixture was allowed to stand overnight at room temperature. The resulting reaction mixture was neutralized with 800 ml of 1N HCl.

The precipitate was filtered and it was dissolved in 400 ml of ethanol. The ethanol solution was added slowly into 800 ml of aqueous ammonia under cooled to 0° C. The precipitate of separates out was filtered and dried.

The thus obtained 39.8 g. of N\textsuperscript{3}-lauryl-N\textsuperscript{3}-carboxbenzyloxy-L-lysine methyl ester were dissolved in 600 ml of ethanol and then with 12.7 ml. of conc. hydrochloric acid and 23 g. of 10% palladium-carbon. Thereafter, the reaction solution was reduced by hydrogen under stirring and atmospheric pressure. The catalyst was filtered off. The reaction mixture concentrated under reduced pressure to remove solvent and water completely. The residue was dissolved in methanol and then added with ethyl acetate. Whereby white crystals were precipitated and filtered out. The crude crystals were recrystallized from ethanol and ethyl acetate to obtain 13.3 g. of the purified product of N\textsuperscript{3}-lauryl-L-lysine methyl ester hydrochloride. Yield: 88.0%, m.p. 96-98° C.

(ii) Preparation of N\textsuperscript{3}-cocoyl-L-arginine ethyl ester DL-\(\varepsilon\)-pyrrolidone carboxylate

35.0 g. (0.2 m.) of L-arginine were dissolved in 200 ml of acetone and 150 ml of water and then added dropwise under cooled to 10-20° C, stirring and adjusting to pH 11.5-12.0 with 8N sodium hydroxide and 40 g. (0.18 m.) of cocoyl chloride (coconut oil fatty acid chloride). The reaction mixture was neutralized with 6 N HCl to pH 5.0 and it was added into 300 ml of cold water. The precipitate of separates out, was filtered and dried, 0.5 g. of crude crystalline N\textsuperscript{3}-cocoyl-L-arginine was obtained. Yield: 77.9%, m.p. 230-235° C.

35.6 g. (0.1 m.) of above compound was saturated with 200 ml of ethanol solution containing hydrogen chloride and allowed to stand overnight at room temperature. The insoluble material of resulting reaction mixture in filtered off. The filtrate was concentrated under reduced pressure. The residue was dissolved in 200 ml of ethyl acetate and then added with triethyl amine under cooling. Organic solvent layer was washed with water and then added with
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12.9 g. of DL-α-pyrrolidone carboxylic acid under heating at 30° C. Thereafter, the reaction mixture was filtered to remove a small amount of insoluble material. The filtrate was concentrated under reduced pressure. The residue was recrystallized from ethanol. 12.4 g. of white crystal of Na-cocoyl-L-arginine ethyl ester DL-α-pyrrolidone carboxylate was obtained. m.p. 181–184° C. (dec.).

What we claim is:
1. A compound which is the lower alkyl or benzyl ester of an N-monooacyl derivative of arginine or of the N*-methyl or N*-benzyl derivative of said arginine, or a water soluble salt of said ester with hydrochloric acid, sulfuric acid, α-pyrrolidonecarboxylic acid, glutamic acid, aspartic acid, lactic acid, citric acid, or acetic acid, said ester having the formula

\[
\text{NH}_2 \quad R_1-\text{N}^{-}\text{CO}-R
\]

\[
\text{NH}=\text{C}-\text{NH}-\text{CH}_2-\text{CH}-\text{CH}-\text{CO}-X
\]

wherein R–CO is the acyl radical of a fatty acid having 6 to 20 carbon atoms, R_1 is hydrogen, methyl or benzyl, and X is lower alkoxy having up to four carbon atoms or benzyl oxy.

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2. A compound as set forth in claim 1 which is an ester of N-monooacyl arginine or a salt of said ester.
3. A compound as set forth in claim 2, wherein acyl is lauroyl.
4. A compound as set forth in claim 3 wherein said ester is a lower alkyl ester of N-lauroyl arginine.
5. A compound as set forth in claim 4, wherein said compound is a salt of a lower alkyl ester of N-lauroyl arginine with hydrochloric acid or α-pyrrolidonecarboxylic acid.
6. N*-Lauroylarginine ethyl ester α-pyrrolidonecarboxylate.

References Cited

JOSEPH A. NARCAGAVE, Primary Examiner

U.S. Cl. X.R.

260—471 A, 482 R, 404.5, 404, 561 A, 309; 424—273, 274, 311