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(72) Inventors EGBERT CHARLET, UWE KEUP, WERNER FROMMER and WINFRIED WINGENDER



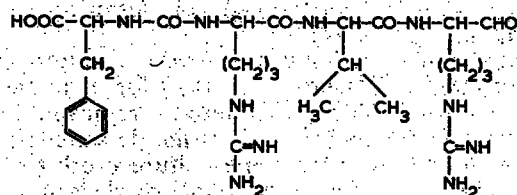
(54) DEODORANT METHOD AND COMPOSITIONS EMPLOYING A PROTEINASE INHIBITOR

(71) We, BAYER AKTIENGESELLSCHAFT, a body corporate organised under the laws of Germany, of Leverkusen, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to the use of a known proteinase inhibitor of microbial origin as an active compound in deodorant compositions.

It is known that certain proteinase inhibitors of animal or vegetable origin can be used as deodorants (German Offenlegungsschrift (German Published Specification) 1,939,419, British Patent Specification No. 1,286,011). However, these proteinase inhibitors can only be used in aqueous solutions since, owing to their low solubility in organic solvents (for example, methanol, ethanol, isopropanol and butanol), they cannot be considered for use in aerosol sprays. Furthermore, their preparation entails substantially greater effort than the microbial processes of preparation.

This invention now provides a deodorizing composition containing as a deodorizing ingredient the proteinase inhibitor of the formula (I):—



mixed with a semi-solid or solid diluent or carrier.

The invention also provides a deodorizing composition containing the proteinase inhibitor of the formula (I) mixed with a liquid diluent or carrier and a solubilizing agent or emulsifier.

Deodorizing compositions containing 0.05—10 wt% of the inhibitor mixed with a liquid diluent or carrier also come within the scope of the invention.

Surprisingly, the inhibitor of the formula I used according to the invention is a more effective deodorant than the previously known inhibitors of animal or vegetable origin. The deodorant action of the inhibitor of formula I can be due to both an inhibition of the proteolytic enzymes and an inhibition of the esterolytic enzymes. The use according to the invention of the proteinase inhibitor of the formula I thus represents an enrichment of the art.

The proteinase inhibitor to be used according to the invention is already known (compare "The Journal of Antibiotics", Volume XXV, No. 4, pages

263—270 (1972) and "Enzyme Inhibitors of Microbial Origin" by H. Umezawa, University Park Press, Baltimore (1972), pages 29—32).

It can be prepared, as hitherto, using strains of the species *Streptomyces michiganensis* (Corbaz et al), especially the strain *Streptomyces michiganensis* CBS 538.68. The species *Streptomyces michiganensis* has been described by Corbaz et al., Arch. Microbiol. 26 (1957), page 192. Strain CBS 538.68, which is particularly suitable, was deposited in the Centraalbureau voor Schimmelcultures in Baarn, Netherlands, as long ago as 1968. The strains Ami 634 and Halde 1,160, which are described in Arch. Microbiol. 32 (1959), page 187 and are deposited in the Centraalbureau voor Schimmelcultures in Baarn, Netherlands, under numbers CBS 194.73 and CBS 193.73 are also suitable for the preparation of the inhibitor according to the invention. However, the origin of the inhibitor is not material to the present invention.

To prepare the active ingredient of the formula I, the nutrient solutions customary for streptomycetes can be used, at the temperatures customary for streptomycetes. A nutrient solution of maize starch, glucose, casein hydrolysate and yeast extract is particularly suitable for the preparation of the inhibitor. Instead of maize starch and glucose it is also possible to use glycerine, sorbitol, mannitol and inositol or mixtures of these compounds as the source of carbon. Equally, soya flour or peptone or meat extract can also be used instead of casein hydrolysate and yeast extract. Under certain nutritional conditions the addition of salts which stabilise the pH, such as calcium carbonate or potassium phosphates or sodium phosphates, can assist the production of inhibitor. The concentration of the constituents of the nutrient solution can vary within wide limits. Preferably, a nutrient solution containing 2% of maleic acid, 1% of glucose, 0.5% of casein hydrolysate, 1% of yeast extract and 0.4% of calcium carbonate is used to prepare the inhibitor of the formula I; sterilisation is for 1 hour at 121°C.

In carrying out the process, the nutrient solutions are steam-sterilised in the usual manner. The culture is inoculated with 0.3—10%, preferably 1—5%, of a pre-culture and is incubated with intensive aeration at 20—40°C, preferably 25—30°C, until a maximum inhibitor concentration is reached, preferably 2—5 days.

The crude inhibitor can be isolated from the culture broth, after separating off the mycelia by centrifuging or filtration; by various methods:

(a) Concentration of the culture broth under reduced pressure (10—50 mm Hg), at bath temperatures of 20—100°C, preferably 40—80°C, to about 1/5 to 1/10 of the initial volume. The concentrated extract is filtered or centrifuged and the clear filtrate (or the clear supernatant liquid) is lyophilised.

(b) Precipitation of the inactive impurities from the culture broth (or from the culture broth which has been concentrated as described in (a)) by adding a water-soluble organic solvent; such as an alcohol or ketone (preferably methanol, ethanol or acetone) up to a content of 60—80% by volume. The precipitate which separates out is removed by centrifuging or filtration and the filtrate is concentrated *in vacuo* and lyophilised.

(c) Salting out of the inactive impurities from the culture broth (or from the culture broth concentrated as described in (a)), for example with ammonium sulphate or sodium chloride. The precipitate is removed by centrifuging or filtration and the filtrate is concentrated *in vacuo*, de-salted and lyophilised.

(d) Extraction of the inhibitor from the culture broth (or the culture broth which has been concentrated as described in (a)) by shaking with *n*-butanol. The butanol extract is concentrated *in vacuo* and lyophilised.

(e) Adsorption of the inactive impurities from the culture broth (or the culture broth concentrated as described in (a)) on ion exchangers, preferably on the weakly basic ion exchanger Amberlite (Registered Trade Mark) 45 in the OH⁻ form. The eluate is concentrated *in vacuo* and lyophilised.

(f) Treatment of the culture broth (optionally concentrated as described in (a)) with adsorbents, preferably active charcoal and/or macro-porous polystyrene resins, whereby the inhibitor is bound practically completely. The desorption is effected with water-soluble organic solvents such as, for example, methanol, ethanol, isopropanol or acetone (preferably 80% strength ethanol) in the acid or neutral range, preferably at pH 2, and at 30—80°C, preferably 60°C. The desorbate is concentrated *in vacuo* and lyophilised.

The inhibitor of the formula I is in each of the above cases obtained in the solid form as a crude inhibitor which can be subjected to fine purification according to the generally customary methods (for example ion exchange chromatography, adsorption chromatography and/or electrophoresis).

The term "inhibitor in the crude form" as used in this specification therefore means the inhibitor of general formula I which has been subjected to one of the above methods of purifications (a)–(f), or its equivalent, without further purification.

The inhibitor of the formula I shows excellent toleration (no side-effects even in the case of sensitive persons) and a strong deodorant action in human subjects.

The inhibitor of the formula I can be employed in the pure form but also, particularly advantageously, in a crude form (as defined above) obtained from the microbial process of preparation and containing impurities, since the impurities arising from the microbial preparation do not interfere. Fine purification, which is very involved and expensive, can thus be dispensed with.

This invention provides three broad classes of deodorant composition:—

- (1) liquid compositions;
- (2) semi-solid compositions; and
- (3) solid compositions

The liquid compositions according to the invention can be solutions or suspensions, preferably emulsions. Suitable diluents and carriers for these compositions include water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, animal and vegetable oils (especially cottonseed oil, groundnut oil, maize germ oil, olive oil, castor oil and sesame oil), glycerine, glycerineformal, tetrahydrofurfuryl alcohol, polyethylene glycol, and fatty acid esters of sorbitane, and mixtures of two or more of these substances.

The liquid compositions may be adapted for use in an aerosol dispenser by the use of a diluent or carrier comprising an aerosol propellant; the usual propellants may be used, especially fluorochlorohydrocarbons. A preferred composition for use in an aerosol dispenser comprises:—

- (i) as the propellant, a fluorochlorohydrocarbon; and
- (ii) ethanol or isopropanol; and
- (iii) a viscous or tacky ester, such as isopropyl myristate or glycerine monoacetic acid ester.

Compositions for use in aerosol dispensers are preferably anhydrous.

The liquid compositions according to the invention may contain the conventional auxiliaries, such as solvents, solubilizing agents and emulsifiers.

The semi-solid compositions according to the invention may for example take the form of ointments, pastes, creams or gels. Generally they will comprise a hydrophilic semi-solid vehicle.

Preferred diluents and carriers for use in the semi-solid compositions are animal and vegetable fats (for example, lanolin), waxes, paraffins, starch, tragacanth, cellulose derivatives (such as hydroxymethyl cellulose), polyethylene glycol, silicones, bentonites, silica, talc, zinc oxide, stearic acid, and stearates and mixtures of two or more thereof.

A preferred semi-solid deodorant composition according to the invention is a semi-solid composition adapted to be moulded into a deodorant stick. Such a composition is generally characterized by the presence of a stearate and water. It may be moulded into a stick and mounted in a holder.

The solid compositions of the invention include both coherent solids and powders. Preferred diluents and carriers for such compositions are solid soaps, lactose, talc, silica, alumina, aluminium hydroxide, calcium silicate, zinc oxide and polyamide powder, and mixtures of two or more thereof. In particular moulded soap tablets may be made of compositions of the invention in which the carrier is a solid soap.

All the deodorant compositions of the invention may include the adjuvants and auxiliaries conventional in cosmetics, for example perfumes and preservatives, and may also include other organic, inorganic or biochemical deodorants, such as aluminium chlorhydroxide. All the ingredients of the compositions must of course be non-toxic and tolerated by human skin. Examples of deodorant formulations such as may be used in the present invention are given in Hermann Römpf's "Chemie Lexicon" (Chemical Encyclopedia), 6th Edition, Vol. I, columns 1,402–1,403.

Because of the powerful activity and good toleration of the inhibitor of the formula I, the content of inhibitor in the abovementioned compositions can be varied within exceptionally wide limits. Preparations which contain the inhibitor in amounts of 0.05 to 10 wt%, preferably 0.1 to 1 wt%, and especially 0.2 to 0.5 wt% are particularly suitable. The particular optimum content of active compound in a

particular formulation can easily be ascertained by those skilled in the art from their expert knowledge, in the same way as with conventional deodorants.

The abovementioned compositions can be prepared according to generally customary methods, for example by mixing the inhibitor of the formula I with the appropriate diluent or carrier and, in the case of shaped articles, by subsequently casting or pressing the mixtures in the desired moulds.

The invention also provides a method of combating body-odour in human and non-human animals (including prevention, reduction and elimination of body odour), comprising applying the proteinase inhibitor of formula I, either alone or as a composition of the invention as defined above, locally to a human or non-human animal. The active compound or a composition containing the active compound can, for this purpose, be brought into contact with the skin by any of very diverse number of customary methods, for example by spreading and spraying. Because of the extremely good activity and toleration of the inhibitor of the formula I, the amount of inhibitor applied to the skin can be varied over a very wide range. In the case of liquid preparations, for example, about 0.1 ml per cm² of skin can be applied. The other preparations can be employed in corresponding amounts. The optimum amount can easily be ascertained for each preparation by anyone skilled in the art, on the basis of his expert knowledge of conventional deodorant compositions.

Examples of deodorant compositions according to the invention which contain the inhibitor of general formula I (in the pure or crude form).

1. Aerosol spray

0.2 g of inhibitor of the formula I, 0.7 g of perfume, 0.7 g of glycerine, 0.7 g of isopropyl myristate, 3.5 g of dipropylene glycol and 64.2 g of ethanol are mixed and 12 g of CCl₂F₂ and 18 g of C₂Cl₂F₄ are added thereto in a pressure can.

2. Aerosol spray

1.0 g of inhibitor of the formula I, 0.5 g of perfume, 90.0 g of isopropanol and 10.0 g of glycerine monoacetic acid ester are mixed. 80 g of a mixture of CCl₂F₂ and C₂Cl₂F₄ are added to 20.0 g of this mixture in a pressure can.

3. Stick

8.0 g of sodium stearate, 5.0 g of sorbitol, 75.0 g of propylene glycol, 10.0 g of water, 1.75 g of perfume and 0.25 g of inhibitor of the formula I are mixed and converted into sticks in a suitable mould.

4. Cream

19 g of aluminium phenol-sulphonate, 25.0 g of cetyl alcohol, 25.0 g of petroleum wax, 12.0 g of sodium laurylsulphate and 3.0 g of water are mixed. 9.9 g of this mixture are mixed with 0.1 g of inhibitor of the formula I.

5. Cream

20.0 g of inhibitor of the formula I, 35.0 g of ethoxylated fatty acid ester of sorbitane (Tween 60), 30.0 g of a fatty acid ester of sorbitane, 150.0 g of wool fat, 30.0 g of groundnut oil, 5.0 g of silicone oil, 5.0 g of cetyl alcohol, 20.0 g of tylose, 75.0 g of sorbitol and 620.0 g of water are mixed.

6. Powder

Sufficient perfume and talc are added to 5.0 g of zinc oxide, 0.9 g of inhibitor of the formula I, 5.0 g of titanium dioxide, 10.0 g of CaCO₃, 0.9 g of wool fat, 1.0 g of cetyl alcohol, 2.0 g of sodium aluminium sulphate, 5.0 g of salicylic acid and 2.0 g of boric acid to give 100 g of mixture. The constituents are mixed carefully.

7. Powder

1.5 g of glycerine dilaurate, 0.8 g of inhibitor of the formula I, 0.4 g of aluminium stearate, 0.3 g of zinc oxide, 52.0 g of talc and 45.0 g of maize starch are mixed.

The excellent deodorant action of the inhibitor according to the invention can be demonstrated by the following experiments.

20 women and 14 men, who had to perform medium to heavy physical work during the day, were available as subjects for the experiments.

Two assistants trained in cosmetics and perfumery carried out the odour test

independently of one another, as a duplicate blindfolded test. The results can be seen in Tables 1 to 4.

Experiment 1

The test subjects washed their armpits on the morning of the first day of the experiment with water only and used neither deodorant preparation nor soap. The body odour in the armpits was tested at the start of their work. Thereafter, one armpit was rubbed with 1 ml of a solution of 0.2 g of the inhibitor of the formula I to be used according to the invention, in 99 g of an ethyl alcohol/water mixture (1:1 by volume). The test subjects then followed their normal work. At the end of the working time of the test subjects, that is to say after about 9 hours, a comparative assessment of the treated and untreated armpits was made by smelling. The test subjects were not allowed to wash with deodorants or soap either on the evening of that day or on the following morning. Only dabbing with a dry cloth was permitted. On the morning of the following (second) day, an assessment was again made by a smelling test when starting work.

Experiment 2

(Comparison with a proteinase inhibitor of animal origin)

The experiment was carried out as Experiment 1.

The comparison substance used was a proteinase inhibitor from cattle organs which is a basic polypeptide of molecular weight 6512 and contains 16 different aminoacids in a chain totalling 58 aminoacid radicals (Kallikrein—Trypsin inhibitor).

The inhibitor of the formula I was employed in a concentration of 0.2% (by weight) and the comparison substance was employed in a concentration of 1% (by weight). Water-ethanol (1:1 by volume) was used as the solvent.

Experiment 3.

(Comparison with a proteinase inhibitor of vegetable origin)

The experiment was carried out like Experiment 1.

The active compound was used in the form of an 0.2% (by weight) solution in water. A proteinase inhibitor from potatoes, as a 4% (by weight) solution in water, was used as the comparison substance. It was found that the deodorant action of the 0.2% solution of the inhibitor of the formula I corresponds to that of an about 4% solution of the comparison substance.

The intensity of the odour was assessed in accordance with the following scheme (compare Tables 1 to 4);

0 = no body odour

+ = moderate body odour

++ = distinct body odour

+++ = unpleasantly strong body odour.

TABLE 1.

A. Female test subjects

Test person	Initial value on morning of 1st day untreated	Evening of 1st day		Morning of 2nd day	
		untreated	treated	untreated	treated
1	+++	+++	++	+++	++
2	+++	+++	+	+++	++
3	++	+++	+	+++	++
4	++	+++	+	+++	+
5	++	++	+	+++	+
6	++	++	+	++	+
7	+	++	0	++	+
8	+	+	0	++	+
9	+	++	0	+++	++
10	+	++	0	++	+
11	+	++	0	+++	++
12	+	+	0	+++	++
13	+	++	0	+++	+
14	+	+	0	++	+
15	+	+	0	++	+
16	+	+	0	++	+
17	+	+++	0	++	+
18	+	+	0	++	+
19	+	++	0	+++	+
20	+	++	0	+++	+

TABLE 2.
B. Male test subjects

Test person	Initial value on morning of 1st day untreated	Evening of 1st day		Morning of 2nd day	
		untreated	treated	untreated	treated
1	+++	+++	++	+++	++
2	+++	+++	+	+++	++
3	+++	+++	+	+++	++
4	+++	+++	++	+++	++
5	++	++	+	+++	++
6	+	++	+	+++	++
7	0	++	0	++	+
8	0	+	0	++	+
9	0	++	0	++	+
10	+	++	+	++	+
11	0	++	0	++	+
12	+	++	+	+++	++
13	0	++	0	+++	++
14	+	+++	++	+++	++

TABLE 3.
A. Female test subjects

Test person	Initial value on morning of 1st day untreated	Evening of 1st day		Morning of 2nd day	
		1	2	1	2
1	++	+	+	+	+
2	+	+	+	+	++
3	+	0	++	+	++
4	++	++	+	++	++ (+)
5	++	++	++	++	++
6	+++	++	++	++	++
7	++	+	++	++	++
8	++	+	++	++	++ (+)
9	++	++	+++	+++	+++
10	+	++	+++	+++	+++

1 = Inhibitor of the formula I

2 = Kallikrein — Trypsin inhibitor from cattle organs (178 FIP—TIU/mg)

TABLE 4.

B. Male test subjects

Test person	Initial value on morning of 1st day untreated	Evening of 1st day		Morning of 2nd day	
		1	2	1	2
1	+++	+	+++	++	+++
2	+++	++	+++	+++	+++
3	+++	++	+++	++	+++
4	+++	+	++	++	+++
5	+++	+	+++	++	+++
6	++	+	+++	++	+++
7	+++	+	++	++	+++
8	+++	+	++	++	+++
9	+++	++	+++	+++	+++
10	+++	+	++	++	+++

1 = Inhibitor of the formula I

2 = Kallikrein — Trypsin inhibitor from cattle organs (178 FIP—TIU/mg)

The experimental results in Tables 1 and 2 show that if an 0.2% (by weight) aqueous-alcoholic inhibitor solution is used, a very good to good deodorant action results, which persists for a sufficient period. An 0.2% (by weight) aqueous inhibitor solution shows a similar action.

The results of Tables 3 and 4 of Experiment 3 show the clear superiority of the inhibitor of the formula I over proteinase inhibitors of animal and vegetable origin.

The same experimental results as were found when using the pure compound of the formula I could also be admixed when using the crude inhibitor, such as is obtained by microbial preparation without fine purification, the preparations used in the experiments containing 1% (by weight) of the crude inhibitor instead of 0.2% (by weight) of the pure inhibitor.

The preparation of the active compound of the formula I, especially the preparation of the crude inhibitor, is illustrated by the following Examples:

Example 1.

120 L of a nutrient solution containing 2% of maize starch, 1% of glucose, 1% of yeast extract, 0.5% of casein hydrolysate and 0.4% of CaCO_3 , to which 0.1% by volume of silicone defoamer has also been added for defoaming before sterilisation, are inoculated in a fermenter, under sterile conditions, with 1 L of pre-culture of *Streptomyces michiganensis* strain CBS 538.68 (obtained in the same nutrient solution in a shaking flask), and are incubated at 28°C with vigorous stirring and aeration; after a period of fermentation of 41 hours a culture broth which gives a result of 120 FIP—TIU/ml in the inhibition test described below, is obtained.

After separating off the mycelium by centrifuging, active charcoal of 3 mm particle size (75 mg/ml) is added to the culture broth (100 L) and the mixture is stirred for 30 minutes at room temperature. The active charcoal is separated from the culture solution and the inactive supernatant liquid is discarded.

The inhibitor is desorbed from the active charcoal with 40 L of 80% strength ethanol containing hydrochloric acid (pH 2) at 60°C, whilst stirring, and the desorption step is again repeated with 30 L of ethanol containing hydrochloric acid

(pH 2). The combined desorbates are adjusted to pH 6.8 with NaOH and concentrated to about 2.5 L. 2 Kg of pretreated macroporous polystyrene resin (washed with pure methanol) are added to 10 L of an active charcoal extract which has been diluted 1:10 with water, and the mixture is stirred for 30 minutes at room temperature. After filtering off the inactive supernatant liquid, the inhibitor is eluted stepwise with an ethanol/water gradient. The bulk of the inhibitor is desorbed from the resin with 30% strength ethanol (3 x 10 L). For decolouration, the dark brown concentrate is treated batchwise with a weakly basic ion exchanger in the OH⁻ form and the eluate is concentrated *in vacuo* and lyophilised.

The specific activity of the inhibitor obtained by this process is 182 FIP units/mg.

Yield: 7 g of a solid which contains the inhibitor of the formula I and which can be used directly as a deodorant.

Example 2.

A 1 litre Erlenmeyer flask which contains 120 ml of a nutrient solution containing 2% of maize starch, 1% of glucose, 1% of yeast extract, 0.5% of casein hydrolysate and 0.4% of CaCO₃, sterilised for 1 hour at 121°C, is inoculated with 1 ml of a 3 day old pre-culture of the strain CBS 538.68 in the same nutrient solution and the mixture is incubated for 3 days on a rotary shaking machine at 28°C; a culture broth which shows 100 FIP-Trypsin inhibitor units/ml in the inhibition test described below, is obtained.

Working up is effected as in Example 1.

Example 3.

A 1 litre Erlenmeyer flask which contains 120 ml of a nutrient solution containing 2% of maize starch, 1% of glucose, 1% of yeast extract, 0.5% of casein hydrolysate and 0.4% of CaCO₃, sterilised for 1 hour at 121°C, is inoculated with 1 ml of a 3 day old pre-culture of the strain Ami 734=CBS 193.73 in the same nutrient solution and the mixture is incubated for 2 days on a rotary shaking machine at 28°C; a culture broth which shows 2.75 FIP-Trypsin inhibitor units/ml in the inhibition test described below, is obtained. Working up is effected as in Example 1.

Example 4.

A 1 litre Erlenmeyer flask which contains 120 ml of a nutrient solution containing 2% of maize starch, 1% of glucose, 1% of yeast extract, 0.5% of casein hydrolysate and 0.4% of CaCO₃, sterilised for 1 hour at 121°C, is inoculated with 1 ml of a 3 day old pre-culture of the strain Halde 1160=CBS 193.73 in the same nutrient solution and the mixture is incubated for 3 days on a rotary shaking machine at 28°C; a culture broth which shows 130 FIP-Trypsin inhibitor units/ml in the inhibition test described below, is obtained.

Working up is effected as in Example 1.

Example 5.

A 1 litre Erlenmeyer flask which contains 120 ml of a nutrient solution containing 3% of soya flour, 3% of glycerine and 0.2% of CaCO₃, sterilised for 1 hour at 121°C, is inoculated with 1 ml of a 3 day old pre-culture of the strain CBS 538.68 in the same nutrient solution and incubated for 3 days on a rotary shaking machine at 28°C; a culture broth containing 90 FIP-TIU/ml is obtained.

Working up is effected as in Example 1.

Example 6.

A 1 litre Erlenmeyer flask which contains 120 ml of a nutrient solution containing 0.5% of peptone, 0.5% of meat extract, 0.2% of yeast extract, 0.03% of casein hydrolysate, 1% of inositol, 1% of sorbitol, 1% of mannitol, 1% of glucose, 0.1% of K₂HPO₄, 0.5% of KCl, 0.05% of MgSO₄ and 0.01% of FeSO₄, sterilised for 1 hour at 121°C, is inoculated with 1 ml of a 3 day old pre-culture of the strain CBS 538.68 in the same nutrient solution and incubated for 3 days on a rotary shaking machine at 28°C; a culture broth which shows 30 FIP-TIU/ml in the inhibition test described below, is obtained.

Working up is effected as in Example 1.

The substances obtained in Examples 1 to 6 contain the inhibitor of the formula I, which can be isolated from these substances by customary methods.

Since the impurities do not interfere, it is however also possible to use the crude products in accordance with the invention.

The enzyme-inhibitory action of the crude inhibitor can be determined by the following test method:

Trypsin and Trypsin inhibition test according to FIP—R. Ruysen, "Symposium on Pharmaceutical Enzymes and their Assay", May 24, 1968.

Reagents:

0.02 M of BAEE (N- α -benzoyl-L-arginine ethyl ester hydrochloride)
0.0015 M of borate buffer, pH 8.0

0.1 N NaOH

Trypsin 100 FIP units/ml

Inhibitor solution

Method:

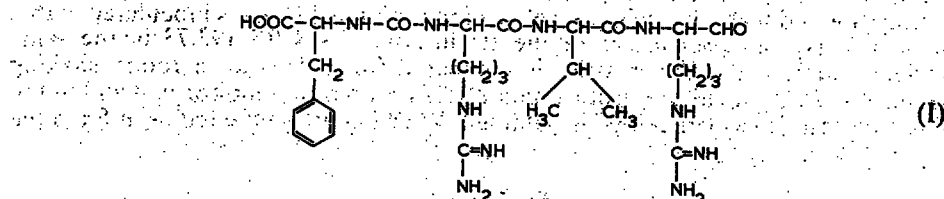
The inhibition of the esterolytic activity at constant temperature (25°C) and constant pH (8.0) is measured in an auto-titrator.

Definition of the FIP unit:

A Trypsin inhibitor FIP unit is defined as the amount (in mg) which completely inhibits one FIP Trypsin unit (expressed as the rate of hydrolysis of one micromol of BAEE per minute).

WHAT WE CLAIM IS:—

1. A deodorizing composition containing as a deodorizing ingredient the proteinase inhibitor of the formula:—



mixed with a semi-solid or solid diluent or carrier.

2. A deodorizing composition containing the ingredient of formula (I) mixed with a liquid diluent or carrier and a solubilizing agent or emulsifier.

3. A deodorizing composition containing 0.05—10 wt% of the ingredient of formula (I) mixed with a liquid diluent or carrier.

4. A composition according to claim 1 or 2 containing 0.05–10 wt% of the said ingredient.

5. A composition according to claim 3 or 4 containing 0.1—1.0 wt% of the said ingredient.

6. A composition according to claim 5 containing 0.2—0.5 wt% of the said ingredient.

7. A composition according to any preceding claim comprising another deodorizing ingredient.

8. A composition according to any preceding claim comprising also a perfume.

9. A composition according to any preceding claim in which the proteinase inhibitor is in crude form as hereinbefore defined.

10. A composition according to any of claims 1—8 in which the proteinase inhibitor is in purified form.

11. A composition according to claim 2 or 3 and any of claims 4 to 10 when dependent on claim 2 or 3 in which the diluent or carrier comprises water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl-formamide, animal and vegetable oils, glycerine, glycerine-formal, tetrahydrofurfuryl alcohol, polyethylene glycol, a fatty acid ester of sorbitane, or a mixture of two or more thereof.

12. A composition according to claim 2, 3 or 11 in which the diluent or carrier comprises an aerosol propellant.

Since the impurities do not interfere, it is however also possible to use the crude products in accordance with the invention.

The enzyme-inhibitory action of the crude inhibitor can be determined by the following test method:

Trypsin and Trypsin inhibition test according to FIP—R. Ruyssen, "Symposium on Pharmaceutical Enzymes and their Assay", May 24, 1968.

Reagents:

0.02 M of BAEE (N- α -benzoyl-L-arginine ethyl ester hydrochloride)

0.0015 M of borate buffer, pH 8.0

0.1 N NaOH

Trypsin 100 FIP units/ml

Inhibitor solution

Method:

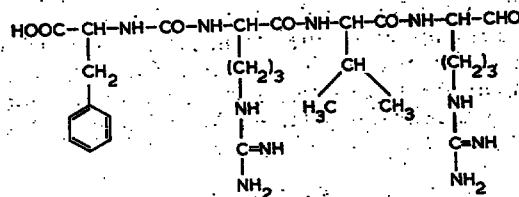
The inhibition of the esterolytic activity at constant temperature (25°C) and constant pH (8.0) is measured in an auto-titrator.

Definition of the FIP unit:

A Trypsin inhibitor FIP unit is defined as the amount (in mg) which completely inhibits one FIP Trypsin unit (expressed as the rate of hydrolysis of one micromol of BAEE per minute).

WHAT WE CLAIM IS:—

1. A deodorizing composition containing as a deodorizing ingredient the proteinase inhibitor of the formula:—



mixed with a semi-solid or solid diluent or carrier.

2. A deodorizing composition containing the ingredient of formula (I) mixed with a liquid diluent or carrier and a solubilizing agent or emulsifier.

3. A deodorizing composition containing 0.05—10 wt% of the ingredient of formula (I) mixed with a liquid diluent or carrier.

4. A composition according to claim 1 or 2 containing 0.05–10 wt% of the said ingredient.

5. A composition according to claim 3 or 4 containing 0.1—1.0 wt% of the said ingredient.

6. A composition according to claim 5 containing 0.2—0.5 wt% of the said ingredient.

7. A composition according to any preceding claim comprising another deodorizing ingredient.

8. A composition according to any preceding claim comprising also a perfume.

9. A composition according to any preceding claim in which the proteinase inhibitor is in crude form as hereinbefore defined.

10. A composition according to any of claims 1—8 in which the proteinase inhibitor is in purified form.

11. A composition according to claim 2 or 3 and any of claims 4 to 10 when dependent on claim 2 or 3 in which the diluent or carrier comprises water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl-formamide, animal and vegetable oils, glycerine, glycerine-formal, tetrahydrofurfuryl alcohol, polyethylene glycol, a fatty acid ester of sorbitane, or a mixture of two or more thereof.

12. A composition according to claim 2, 3 or 11 in which the diluent or carrier comprises an aerosol propellant.



13. A composition according to claim 12 that is anhydrous and contains:—
(i) as the propellant, a fluorochlorohydrocarbon; and
(ii) ethanol or isopropanol; and
(iii) a viscous or tacky ester.
- 5 14. An aerosol dispenser containing the composition of claim 12 or 13. 5
15. A composition according to claim 2 or 3 in the form of an emulsion.
16. A semi-solid composition according to claim 1 and any of claims 4—10
when dependent on claim 1.
- 10 17. A composition according to claim 16 in which the diluent or carrier
comprises a hydrophilic semi-solid vehicle. 10
18. A composition according to claim 16 or 17 in which the diluent or carrier
comprises an animal vegetable fat, a wax, a semi-solid paraffin, starch, tragacanth,
a cellulose derivative, polyethylene glycol, a silicone, bentonite, silica, talc, or zinc
oxide, stearic acid or a stearate, or a mixture of two or more thereof.
- 15 19. A composition according to claim 17 or 18 which is a deodorant stick 15
composition comprising a stearate and water.
20. A deodorant stick comprising the composition of claim 19 moulded into a
bar and mounted in a holder.
- 20 21. A composition according to claim 1 and any of claims 4 to 10 when 20
dependent on claim 1 in the form of a coherent solid or a powder.
22. A composition according to claim 21 which is a powder and in which the
diluent or carrier comprises lactose, talc, zinc oxide, alumina, aluminium
hydroxide, calcium silicate, zinc oxide, or polyamide powder or a mixture of two
or more thereof.
- 25 23. A solid composition of claim 21 in which the diluent or carrier comprises a 25
solid soap.
24. A moulded soap tablet of the composition of claim 23.
25. Deodorizing compositions substantially as hereinbefore described in any
of the Examples 1—7.
- 30 26. A method of combating body odour in human and non-human animals 30
comprising applying the proteinase inhibitor of formula I, either alone or as a
composition of any of claims 1—13, 15—19, 21—23 and 25 locally to a human or
non-human animal.
27. The method of claim 26 substantially as hereinbefore described.

For the Applicants,
CARPMAELS & RANSFORD,
Chartered Patent Agents,
43 Bloomsbury Square,
London WC1A 2RA.

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