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(54) **Title:** SKIN CARE METHOD AND COMPOSITION

(57) **Abstract**

A composition for topical application to the skin for alleviation or prevention of dry flaky skin conditions, dandruff or acne comprising one or more stratum corneum cathepsin-D-like enzymes. The composition may further comprise an additional enzyme selected from glycosidases, other proteases, lipases and mixtures thereof. Optional additional active ingredients include sunscreens, lipids, hydroxy carboxylic acids and ketocarboxylic acids.

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SKIN CARE METHOD AND COMPOSITION

The present invention relates to compositions for application to the skin and their cosmetic and/or pharmaceutical use. In particular, the invention relates to compositions comprising stratum corneum cathepsin-D-like enzymes and their use in alleviating or preventing conditions involving abnormal desquamation by facilitating desmosomal degradtion.

In normal, healthy epidermis the continuous production of new stratum corneum is balanced by a well-regulated shedding of corneocytes from the skin surface. Little is known about this desquamation process at the molecular level.

It has been shown by A. Lundstrom and T. Egelrud (J. Invest Dermatol, (1988) 91 340-343; Arch Dermatol Res (1990) 282 234-237; J. Invest Dermatol (1990) 94 216-220) that cohesion between cells in the stratum corneum is dependant on protein structures. These structures must be degraded before cell dissociation can occur.

Furthermore, evidence has been provided to show that cell dissociation is preceded by a degradation of the extracellular parts of desmosomes. (T. Egelrud (1992) European Journal of Dermatology 2 46-49).

It is thought that the process of desquamation involves proteolytic degradation of desmosomes, causing the cohesive links between the cells to break down and thereby allowing detachment of peripheral corneocytes.

Little is known about the proteases thought to be involved in the desquamatory process. One particular protease, the serine protease stratum corneum chymotryptic-like enzyme

(SCCE) has been implicated as a putative desmosomal degrading enzyme (see A. Lundstrom and T. Egelrud, Acta Derm Venersol (Stockh) (1991) 71, 471-474).

The present inventors have found that the stratum corneum additionally contains proteases having cathepsin-D-like substrate specificity, hereinafter referred to as stratum corneum cathepsin-D-like enzymes which may be involved in the process of cell dissociation (desquamation) and desmosomal degradation. These enzymes are therefore of interest in the treatment of conditions where the underlying aetiology indicates that assisting the processes of desmosomal degradation and/or desquamation would be beneficial.

Accordingly the invention provides a composition for topical application to the skin, comprising one or more stratum corneum cathepsin-D-like enzymes.

As used herein, the term "stratum corneum cathepsin-D-like enzyme" means a protease, or a pro-enzyme thereof, which in its active form exhibits similar substrate sensitivity and inhibitor sensitivity to cathepsin-D. More specifically, the term "stratum corneum cathepsin-D-like enzyme" means a protease, or a pro-enzyme thereof, which in its active form is inhibited by pepstatin, a specific inhibitor of aspartic proteases such as cathepsin-D.

It will be appreciated that a pro-enzyme is an inactive form of the enzyme which may be activated by appropriate proteolytic cleavage to give the active form.

Suitable stratum corneum cathepsin-D-like enzymes for use according to the present invention have apparent molecular weights, as determined by the method of gel filtration of 31kDA and 60kDa.

Preferably the composition comprises 0.00001 to 50% more preferably 0.001 to 20% and even more preferably 0.001 to 0.1% by weight of the composition stratum corneum thiol proteases.

Compositions according to the invention may further comprise an additional enzyme selected from glycosidases, other proteases, lipases or similar lipid modifying enzymes, ceramidases and mixtures thereof. Preferably the composition comprises 0.00001 to 50%, more preferably 0.001 to 20%, even more preferably 0.001 to 0.1% by weight of the composition of the additional enzyme.

Stratum corneum cathepsin-D-like enzymes may be extracted from human or animal skin or callus by high salt solution (e.g. 2M NaCl), detergent or solvent extraction, and purified by chromatography or electrophoretic techniques. Recombinant stratum corneum cathepsin-D-like enzymes may also be produced by biotechnological means by the over-expression of its gene in yeast, bacteria, plant or mammalian cells.

Glycosidases, other proteases and lipases for inclusion in the compositions according to the invention may suitably be isolated from animal, plant, fungal or bacterial sources.

Typical glycosidases include neuraminidase, mannosidase, galactosidase, glucosidase, N-acetyl glucosaminidase and N-acetyl galactosaminidase. Preferably these may be isolated from plant sources including almonds, green coffee beans, and spinach, or may be obtained commercially.

Suitable additional proteases include bromelain, papain, chymotrypsin and chymotrypsin-like enzymes, stratum corneum chymotryptic-like enzyme, serine and cysteine cathepsin and cathepsin-like enzymes, alcalase, savinase, chymopapain, clostripain, endoproteinase Asp N, protease V.8, proteinase

K, subtilisin, thermolysin, plasmin, pronase, and trypsin and trypsin-like enzyme. Preferably the protease may be isolated from plant sources including the seeds of wheat, barley, maize, oilseed rape, cocoa, linseed, illipe, shea nut, palm kernal, jojoba bean, pea, green bean, broad bean, soya bean and sunflower, and olives, papaya, pineapple, coconut, tomato and figs.

Lipases, or similar lipid modifying enzymes, may be isolated from plant, animal or bacterial sources. Suitable enzymes include lipolase, pancreatic lipases, phospholipases, ceramidase, aryl sulphatase, cholesterol esterase, candida rugosa OF360 lipase, humicola sp. lipase, pseudomonas sp. lipase and Candida antarctica A and B lipases.

Compositions according to the invention also comprise a vehicle to act as a dilutant, dispersant or carrier for the active ingredients in the composition, so as to facilitate their distribution when the composition is applied to the skin and/or hair. Preferably the vehicle is cosmetically and/or pharmaceutically acceptable.

Vehicles other than water can include liquid or solid emollients, solvents, humectants, thickeners and powders typically found in cosmetic formulations. Examples of each of these types of vehicle, which can be used singly or as mixtures of one or more vehicles, are as follows:

Emollients, such as stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, eicosanyl alcohol, behenyl alcohol, cetyl palmitate, silicone oils such as dimethylpolysiloxane, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate,

isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, cocoa butter, corn oil, cotton seed oil, tallow, lard, olive oil, palm kernel oil, rapeseed oil, safflower seed oil, evening primrose oil, soybean oil, sunflower seed oil, avocado oil, olive oil, sesame seed oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petroleum jelly, mineral oil, butyl myristate, isostearic acid, palmitatic acid, isopropyl linoleate, lauryl lactate, myristyl lactate, decyl oleate, myristyl myristate;

Propellants, such as air, propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide;

Solvents, such as squalene, squalane, ethyl alcohol, methylene chloride, isopropanol, acetone, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol monoethyl ether, dimethyl sulphoxide, dimethyl formamide, tetrahydrofuran;

Humectants, such as polyhydric alcohols including glycerol, polyalkylene glycols and alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxysorbitol, 1,3-butylene glycol, 1,2,6-hexanetriol, ethoxylated glycerol, propoxylated glycerol and mixtures thereof.

Powders, such as chalk, talc, fullers earth, kaolin, starch, gums, colloidal silica sodium polyacrylate, tetra alkyl and/or trialkyl aryl ammonium smectites, chemically modified magnesium aluminium silicate, organically modified montmorillonite clay, hydrated aluminium silicate, fumed silica, carboxyvinyl polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate.

The vehicle will usually form from 10 to 99.9%, preferably

from 50 to 99% by weight of the emulsion, and can, in the absence of other adjuncts, form the balance of the composition.

A particularly convenient form of the composition according to the invention is an emulsion, in which case an oil or oily material will normally be present, together with an emulsifier to provide either a water-in-oil emulsion or an oil-in-water emulsion, depending largely on the average hydrophillic-lyophilic balance (HLB) of the emulsifier employed.

Compositions according to the invention can optionally comprise one or more oils or other materials having the properties of an oil.

Examples of suitable oils include mineral oil and vegetable oils, and oil materials, such as those already proposed herein as emollients. Other oils or oily materials include silicone oils, both volatile and non-volatile, such as polydimethyl siloxanes.

The oil or oily material, when present for the purposes for forming an emulsion, will normally form up to 90%, preferably from 10 to 80% by volume of the composition.

Compositions according to the invention may also optionally comprise one or more emulsifiers, the choice of which will normally determine whether a water-in-oil or an oil-in-water emulsion is formed.

When a water-in-oil emulsion is required, the chosen emulsifier or emulsifiers should normally have an average HLB value of from 1 to 6. When an oil-in-water emulsion is required, a chosen emulsifier or emulsifiers should have an average HLB value of >6.

Examples of suitable emulsifiers are set out below in Table 1 in which the chemical name of the emulsifiers is given together with an example of a trade name as commercially available, and the average HLB value.

TABLE 1

Chemical Name of Emulsifier	Trade Name	HLB Value
Sorbitan trioleate	Arlacel 85	1.8
Sorbitan tristearate	Span 65	2.1
Glycerol monooleate	Aldo MD	2.7
Glycerol monostearate	Atmul 84S	2.8
Glycerol monolaurate	Aldo MC	3.3
Sorbitan sesquioleate	Arlacel 83	3.7
Sorbitan monooleate	Arlacel 80	4.3
Sorbitan monostearate	Arlacel 60	4.7
Poloxyethylene (2) stearyl ether	Brij 72	4.9
Poloxyethylene sorbitol beeswax derivative	G-1702	5
PEG 200 dilaurate	Emerest 2622	6.3
Sorbitan monopalmitate	Arlacel 40	6.7
Polyoxyethylene (3.5) nonyl phenol	Emulgen 903	7.8
PEG 200 monostearate	Tegester PEG 200 MS	8.5
Sorbitan monolaurate	Arlacel 200	8.6
PEG 400 dioleate	Tegester PEG 400-DO	8.8
Polyoxyethylene (5) monostearate	Ethofat 60-16	9.0
Polyoxyethylene (4) sorbitan monostearate	Tween 61	9.6
Polyoxyethylene (4) lauryl ether	Brij 30	9.7

Polyoxyethylene (5) sorbitan monooleate	Tween 81	10.0
PEG 300 monooleate	Neutronyx 834	10.4
Polyoxyethylene (20) sorbitan tristearate	Tween 65	10.5
Polyoxyethylene (20) sorbitan trioleate	Tween 85	11.0
Polyoxyethylene (8) monostearate	Myrj 45	11.1
PEG 400 monooleate	Emerest 2646	11.7
PEG 400 monostearate	Tegester PEG 400	11.9
Polyoxyethylene 10 monooleate	Ethofat 0/20	12.2
Polyoxyethylene (10) stearyl ether	Brij 76	12.4
Polyoxyethylene (10) cetyl ether	Brij 56	12.9
Polyoxyethylene (9.3) octyl phenol	Triton X-100	13.0
Polyoxyethylene (4) sorbitan monolaurate	Tween 21	13.3
PEG 600 monooleate	Emerest 2660	13.7
PEG 1000 dilaurate	Kessco	13.9
Polyoxyethylene sorbitol lanolin derivative	G-1441	14.0
Polyoxyethylene (12) lauryl ether	Ethosperse LA-12	14.4
PEG 1500 dioleate	Pegosperse 1500	14.6
Polyoxyethylene (14) laurate	Arosurf HFL-714	14.8
Polyoxyethylene (20) sorbitan monostearate	Tween	14.9
Polyoxyethylene 20 sorbitan monooleate	Tween 80	15.0
Polyoxyethylene (20) stearyl ether	Brij 78	15.3

Polyoxyethylene (20) sorbitan monopalmitate	Tween 40	15.6
Polyoxyethylene (20) cetyl ether	Brij 58	15.7
Polyoxyethylene (25) oxypropylene monostearate	G-2162	16.0
Polyoxyethylene (20) sorbitol monolaurate	Tween 20	16.7
Polyoxyethylene (23) lauryl ether	Brij 35	16.9
Polyoxyethylene (50) monostearate	Myrj 53	17.9
PEG 4000 monostearate	Pegosperse 4000 MS	18.7

The foregoing list of emulsifiers is not intended to be limiting and merely exemplifies selected emulsifiers which are suitable for use in accordance with the invention.

It is to be understood that two or more emulsifiers can be employed if desired.

The amount of emulsifier or mixtures thereof, to be incorporated in the composition of the invention, when appropriate is from 1 to 50%, preferably from 2 to 20% and most preferably from 2 to 10% by weight of the composition.

The compositions of the invention can also comprise water, usually up to 80%, preferably from 5 to 80% by volume.

Emulsifiers or surfactants in the form of silicone polymers may be incorporated into compositions of the present invention in place of or in addition to the optional emulsifier(s) already mentioned.

A particularly preferred silicone surfactant is cyclomethicone and dimethicone copolyol, such as DC 3225C Formulation Aid available from DOW CORNING. Another is laurylmethicone copolyol, such as DC Q2-5200, also available from Dow Corning.

The amount of silicone surfactant, when present in the composition will normally be up to 25%, preferably from 0.5 to 15% by weight of the emulsion.

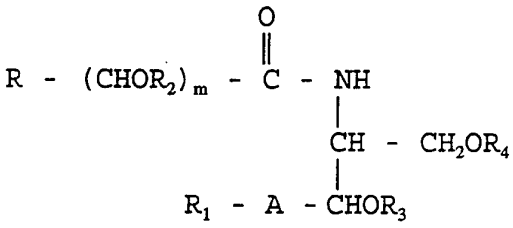
Various other adjuncts conventionally found in cosmetic or pharmaceutical formulations may optionally be present in the compositions according to the present invention. They include preservatives, such as para-hydroxy benzoate esters; antioxidants, such butyl hydroxy toluene; humectants, such as glycerol, sorbitol, 2-pyrrolidone-5-carboxylate, dibutylphthalate, gelatin, polyethylene glycol, preferably PEG 200-600; buffers, such as lactic acid together with a base such as triethanolamine or sodium hydroxide; surfactants, such as glycerol ethers, waxes, such as beeswax, ozokerite wax, paraffin wax; plant extracts, such as Aloe vera, cornflower, witch hazel, elderflower, cucumber; thickeners; activity enhancers; colourants; and perfumes.

Various types of additional skin benefit ingredients may optionally be present in the compositions according to the present invention. These include sunscreens, hydroxy carboxylic acids and ketocarboxylic acids or esters thereof and lipids such as ceramides, pseudoceramides (synthetic ceramide-like structures), polyol fatty acid polyesters, sterols, phospholipids, galactosyldiacylglycerols, glycosphingolipids, fatty acids or esters thereof, and mixtures thereof.

Suitable sunscreens include those materials commonly used to block ultraviolet light and may include inorganic

sunscreen materials, such as ultrafine titanium dioxide, or organic sunscreens such as p-aminobenzoic acid and esters thereof, ethylhexyl p-methoxycinnamate, 2-ethoxyethyl p-methoxycinnamate or butylmethoxydibenzoylmethane, and mixtures thereof. It will be appreciated that the amount of sunscreen employed will vary depending on the degree of protection required.

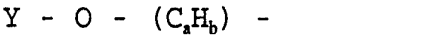
Suitable ceramides and synthetic analogues thereof which may be employed are disclosed in European patent application EP-A-587288 which is incorporated by reference herein. Preferred ceramides have the structure.



(1)

where A represents - CH_2 -; - $CHOR_5$ -; - CH=CH - or - CHOY -

R represents a linear or branched saturated or unsaturated, hydroxylated or non-hydroxylated aliphatic hydrocarbon group having from 1 to 49 carbon atoms or a subgroup (2).



(2)

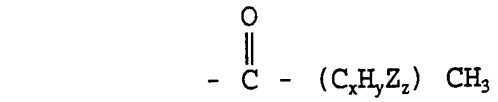
R_1 represents a linear or branched, saturated or unsaturated, hydroxylated or non-hydroxylated aliphatic hydrocarbon group having from 8 to 28 carbon atoms;

R_2, R_3 and R_5 individually represent H, a phosphate residue or a sulphate residue;

R_4 represents H, a phosphate residue, a sulphate residue or sugar residue;

a is an integer of from 1 to 49
b is an integer of from 10 to 98
m is 0 or 1

Y represents H or a residue of a C_{1-22} fatty acid having the general structure (3)



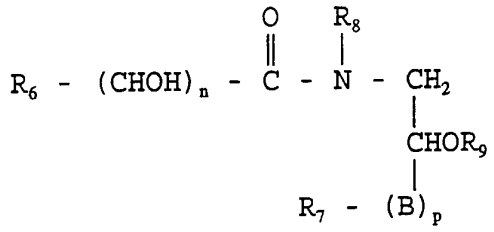
(3)

where Z is - OH or an epoxy oxygen
x is an integer of from 0 to 20
y is an integer of from 0 to 40
and z is 0 or an integer of from 1 to 4

Ceramides having the general structure (1) are naturally occurring and can be isolated from a suitable plant source or from animal tissue such as pig skin or neural tissue. Ceramides can also be synthesised.

Particular preferred examples of ceramides are ceramide-1, ceramide-2 and ceramide-3.

Pseudoceramides are preferably selected from pseudoceramides (i.e. synthetic ceramide like structures) having the general structure (4):



(4)

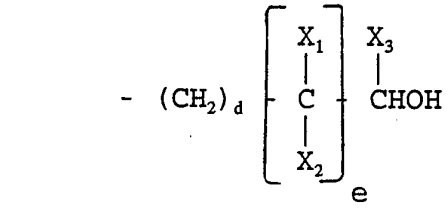
where B represents - OCH_2 - or CHOH.

R_6 represents a linear or branched, saturated or

unsaturated, hydroxylated or non-hydroxylated aliphatic hydrocarbon group having from 1 to 49 carbon atoms or the subgroup (2).

R_7 represents a linear or branched, saturated or unsaturated, hydroxylated or non-hydroxylated hydrocarbon group having from 8 to 28 carbon atoms.

R_8 represents H, or a subgroup - $(CH_2)_cCOOH$, where c is an integer of from 1 to 6, or a subgroup having the structure (5).



(5)

where X_1, X_2 and X_3 each individually represent H, a C_{1-5} alkyl or a C_{1-5} hydroxyalkyl;

d is 0 or an integer of from 1 to 4
e is 0 or 1
n is 0 or 1
and p is 0 or 1;

R_9 represents H, a phosphate residue, a sulphate residue or a sugar residue.

Polyol fatty acid polyesters are fatty acid polyesters derived from any aliphatic or aromatic polyol which has at least 4 free hydroxyl groups, of which at least 60% of these free hydroxyl groups are then esterified with one or more fatty acids having from 8 to 22 carbon atoms. The polyol is preferably chosen from sugar polyols, which comprise mono-, di- and polysaccharides. Preferred polyol fatty acid polyesters are sucrose fatty acid polyesters where the ester is derived from lauric acid or natural

oils, such as palm oil, palm kernal oil, soyabean oil, coconut oil, fish oil and mixtures thereof.

Sterols may conveniently be selected from cholesterol, pro-vitamin D_3, campesterol, stigmastanol, stigmasterol, 5-dihydrocholesterol, α-spinasterol, palysterol, clionasterol, γ-sitosterol, stigmastenol, sargasterol, avenasterol, ergostanol, sitosterol, corbisterol, chondrillasterol, poriferasterol, haliclonaseterol, neospongosterol, fucosterol, aptostanol, Ergostadienol, ergosterol, 22-dihydroergosterol, brassicasterol, 24-methylenecholesterol, 5-dihydroergosterol, dehydroergosterol, 14-dehydroergosterol, 24-dehydroergosterol, fungisterol, cholestanol, coprostanol, Zymosterol, 7-hetocholesterol, Lathosterol, 22-dehydrocholesterol, β-sitosterol, cholestatrien-3β-01, coprostanol, cholestanol, ergosterol, 7-dehydrocholesterol, 24-dehydrocholest-adione-3β-01, equilenin, equilin, estrone, 17β-estradiol, Androst-4-ene-3β, 17β-diol, dehydroepiandrosterone and mixtures thereof. Cholesterol is preferred.

Fatty acids are preferably essential fatty acids chosen from linoleic acid, γ-linolenic acid, homo-γ-linolenic acid, columbinic acid, eicosa-(n-6,9,13)-trienoic acid, arachidonic acid, α-linolenic acid, timnodonic acid, hexaenoic acid and mixtures thereof.

Lipids, such as those described above, may conveniently be incorporated in compositions according to the invention in an amount of from 0.00001 to 50%, preferably from 0.001 to 10%, more preferably from 0.1 to 10% by weight of the composition.

Suitable hydroxy acids include α-hydroxy acids, β-hydroxyacids, other hydroxycarboxylic acids and mixtures thereof. Preferably, the hydroxy acid is chosen from α-

hydroxy acids having the general structure:

$$R_bCHOHCOOR_a$$

wherein R_a and R_b are H, alkyl, aralkyl or aryl group of saturated or unsaturated, isomeric or non-isomeric, straight or branched chain or cyclic form, having 1 to 30 carbon atoms, and in addition R_2 may carry F, Cl, Br, I, N, S, OH, CHO, COOH and alkoxy group having 1 to 9 carbon atoms; and mixtures thereof.

The alpha hydroxy acids may be present as a free acid or an ester form, or in a salt form with an organic base or an inorganic alkali. The typical alkyl, aralkyl and aryl groups for R_1 and R_2 include methyl, ethyl, propyl, isopropyl, butyl, pentyl, octyl, lauryl, stearyl, benzyl and phenyl, etc.

D, DL, or L stereoisomeric forms of an alpha hydroxy acid may be employed compositions. The L form is preferred.

Suitable alpha hydroxy acids which may be used include, but are not limited to, alpha hydroxy acetic acid (also known as "glycolic acid"), alpha hydroxypropionic acid (also known as "lactic acid"), alpha hydroxyoctanoic acid (also known as "alpha hydroxy caprylic acid"), alpha hydroxydodecanoic acid (also known as "alpha hydroxy lauric acid") and mixtures thereof.

Suitable esters include, but are not limited to, alkyl esters (for example, methyl, ethyl, propyl, pentyl, hexyl, octyl esters) and mono-, di- or triglycerides, or mixtures thereof.

Suitable salts of alpha hydroxy acids include but are not limited to sodium, potassium, ammonium, triethanolamine, calcium, lithium salts. The salts may be obtained

commercially or they may be prepared by methods known in the art, e.g., neutralizing an alpha hydroxy acid with a suitable base, such as hydroxide bases of ammonium, potassium, sodium.

Conveniently, a mixture of alpha hydroxy acids may be employed. A suitable mixture comprises lactic acid, alpha hydroxy octanoic acid and alpha hydroxy lauric acid.

The preferred compositions according to the invention contain at least 60% of an alpha hydroxy acid in L-configuration, by weight of total alpha hydroxy acid.

The alpha hydroxy acid is suitably present in an amount of from 0.001% to 70%, preferably from 0.1% to 20%, most preferably from 1% to 10% by weight of the composition.

The keto acids can be chosen from α-keto acids, β-keto acids and mixtures thereof. A particularly preferred α-keto acid is 2-keto octanoic acid.

Compositions according to the invention may also include chelating agents, particularly those having high affinity with zinc and/or magnesium ions.

Suitable chelating agents may conveniently be selected from aminocarboxylic acids or salts thereof, polyphosphoric acids or salts thereof, diphosphonic acids, salts of diphosphonic acids, tertiary amines, aminophosphonic acids, iminodiacetic acid derivatives, azines, hydroxyquinolines, and amino acid esters.

Examples of suitable chelating agents include but are not limited to ethylene diamine tetraacetic acid, a salt of ethylene diamine tetraacetic acid, sodium pyrophosphate, sodium tripolyphosphate, 8-hydroxyquinoline, DL-(Methylene)dinitrolo tetra acetic acids,

trans-decahydronaphthylene-trans-2, 3-bis-iminodiacetic, aminophenyl methylene diphosphonic acid, ethylene-bis-N,N1-(2,6-carboxyl) piperdine, adenosine triphosphate, L-cysteine methyl ester and 8-hydroxyquinoline.

Preferred chelating agents are EDTA and/or pyrophosphate, and/or 8-hydroxy quinoline due to their ready availability, excellent performance, relatively low cost, and safety in use.

The chelating agent is employed in the inventive compositions in an amount effective to enhance the activity of the enzyme. It will be appreciated that the precise amount will depend on the particular chelating agent used. Typically, the amount is in the range of 0.1 to 2%, preferably from 0.2 to 2% by weight of the composition.

Compositions according to the invention are useful in treating or alleviating conditions of the skin which are characterised by hyperkeratinisation, decreased rate of desquamation or abnormal desmosomal formation.

Accordingly, the invention provides the cosmetic or pharmaceutical use of a composition comprising one or more stratum corneum cathepsin-D-like enzymes, particularly in the treatment of conditions where the underlying aetiology indicates that assisting desquamation and/or desmosomal degradation would be beneficial.

The invention further provides a method of treating skin comprising topically administering thereto a composition comprising one or more stratum corneum cathepsin-D-like enzymes.

It will be appreciated that compositions according to the invention will primarily be of use in the treatment of established symptoms although prophylaxis is not excluded.

Compositions according to the invention are of use in treating or preventing diseases of the skin such as psoriasis, ichthyosis and acne. Compositions according to the invention are of particular interest in treating dry and/or flaky skin and in smoothing and enhancing the quality of skin. The compositions may also be used to alleviate dandruff.

It will be appreciated that the amount of the composition and the frequency of its application to the skin will depend on the condition of the patient.

In use, a small quantity of the composition, for example from 1 to 5ml, is applied to areas of the skin or scalp, from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin or scalp using the hand or fingers or a suitable device.

The topical skin treatment compositions according to the invention may be formulated in conventional manner using one or more cosmetically and/or pharmaceutically acceptable carriers or excipients.

For example, the topical compositions of the invention may suitably be formulated as a lotion having a viscosity of from 4,000 to 10,000 mPas, a fluid cream having a viscosity of from 10,000 to 20,000 mPas or a cream having a viscosity of from 20,000 to 100,000 mPas, or above. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or fluid cream can be packaged in a bottle or a roll-ball applicator or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar. The composition may be used for general lotions and creams, leave-on-

creams, wash-off cleansers, face masks shampoos and bath oils.

The invention accordingly also provides a closed container containing an acceptable composition as herein defined.

CHARACTERISATION OF STRATUM CORNEUM CATHEPSIN-D-LIKE ENZYMES.

Gel Filtration

Stratum corneum cathepsin-D-like activity was derived from either human plantar or freeze scraped pig stratum corneum. The tissues were extracted in 1M sodium chloride in 0.05M sodium acetate pH6, 0.1% (v/v) Triton X-100 at 4°C. The extracts were fractionated by size using a Superose 12 gel filtration column with a buffer of 0.02M sodium acetate pH6 containing 1M sodium chloride.

Gel filtration detected two peaks of activity with apparent molecular weights of 31kDa (major peak) and 60kDa (Figure 1).

Cathepsin-D-like activity was determined by a modification of the haemoglobin (hb) hydrolysis assay described Barret, A.J. "Lysosomal Acid Proteinase of Rabbit Liver". Biochem. J. 104 601 (1967). Samples were incubated for 1h in 0.1M sodium formate pH3.5 buffer containing ^{3}Hb at 37°C. Proteolysis was determined from the radioactivity in the chloroacetic acid (TCA) soluble fraction. A unit of Haemoglobin (Hb) hydrolysis is defined as the amount of cathepsin-D-like activity which produces 1000 dpm of TCA soluble material.

Inhibition profile

Purified cathepsin-D-like activity (31kDa) was incubated in the presence and absence of specified protease inhibitors. Activity was determined and expressed as a percentage of the control value. The results obtained are presented in Figure 2.

The concentrations of inhibitors used were 1μM pepstatin, 10μM leupeptin, 10μM chymostatin, 1μM aprotinin, 2mM PMSF, 1μM E64, 5mM EDTA and 100μM zinc sulphate.

Cathepsin-D-like specificity was shown by the almost total inhibition of activity by pepstatin.

DESMOSOMAL DEGRADATION ASSAY

To assess the effect of the proteases on desmosomal degradation in stratum corneum, Superose 12 purified 31 and 60kDa stratum corneum cathepsin-D-like enzymes were incubated with chopped peelings from human sunburnt stratum corneum. The tissue was incubated in 0.1M sodium formate pH3.5 for 20h at 37°C with the enzyme variants. Incubations containing both variants and the cathepsin-D inhibitor pepstatin (10μM) were also performed.

After incubation, the skin was washed in tris buffered saline containing 0.5% (v/v) Tween 20 and incubated for 1h at 37°C with a desmosomal marker antibody (α48/46) raised against the 46 and 48kDa N-terminal fragments of human desmocollin 1 (dsc 1) (Gift from Dr I King, N.I.M.R). This was followed by incubating for 1h with an anti-rabbit IgG conjugated to FITC and the resulting fluorescence was detected by microscopy with a U.V. light source. The fluorescence was quantified from photographic negatives using an Epson GT8000 scanner coupled with Phoreti image analysis software.

The results obtained are presented graphically in Figure 3. Both the 60kDa and 31kDa variants decreased significantly the levels of dsc1, indicating desmosomal degradation. In both cases, this degradation was inhibited by pepstatin (10μM), a specific inhibitor of aspartic proteases such as cathepsin-D.

In order that the invention may be well understood, the following examples are given by way of illustration only.

EXAMPLES

Water In Oil Emulsion

	% w/w
Fully hydrogenated coconut oil	3.9
Stratum corneum cathepsin-D-like enzyme	0.5
Glycosidase	0.5
Polyoxyethylene oleyl ether	5.0
Bentone 38	0.5
Preservative	0.3
$MgSO_4$ $7H_2O$	0.3
Butylated hydroxytoluene	0.01
Perfume	qs
Water	to 100

CLAIMS

1. A topical composition comprising one or more stratum corneum cathepsin-D-like enzymes.

2. A composition according to claim 1 wherein the stratum corneum cathepsin-D-like enzyme has an apparent molecular weight of 31 or 60kDa when determined by gel filtration.

3. A composition according to claim 1 or 2 wherein the stratum corneum cathepsin-D-like enzyme is present in an amount of from 0.00001 to 50% by weight of the composition.

4. A composition according to any one of claims 1 to 3 wherein the stratum corneum cathepsin-D-like enzyme is present in an amount of from 0.001 to 20% by weight of the composition.

5. A composition according to any one of claims 1 to 4 further comprising an enzyme selected from glycosidases, other proteases, lipases, ceramidases and mixtures thereof.

6. A composition according to claim 5 wherein the additional enzyme is present in an amount of from 0.00001 to 50% by weight of the composition.

7. A composition according to claims 5 or 6 wherein the additional enzyme is present in an amount of from 0.001 to 20% by weight of the composition.

8. A composition according to any one of claims 1 to 7 further comprising one or more ingredients selected from sunscreens, hydroxycarboxylic acids, ketocarboxylic acids, ceramides, pseudoceramides, polyol fatty acid polyesters, sterols, phospholipids, galactosyldiacylglycerols, glycosphingolipids, fatty acids or esters thereof.

9. The use of a composition according to any of the preceding claims for topical application to dry skin conditions, acne and dandruff.

Fig.1.

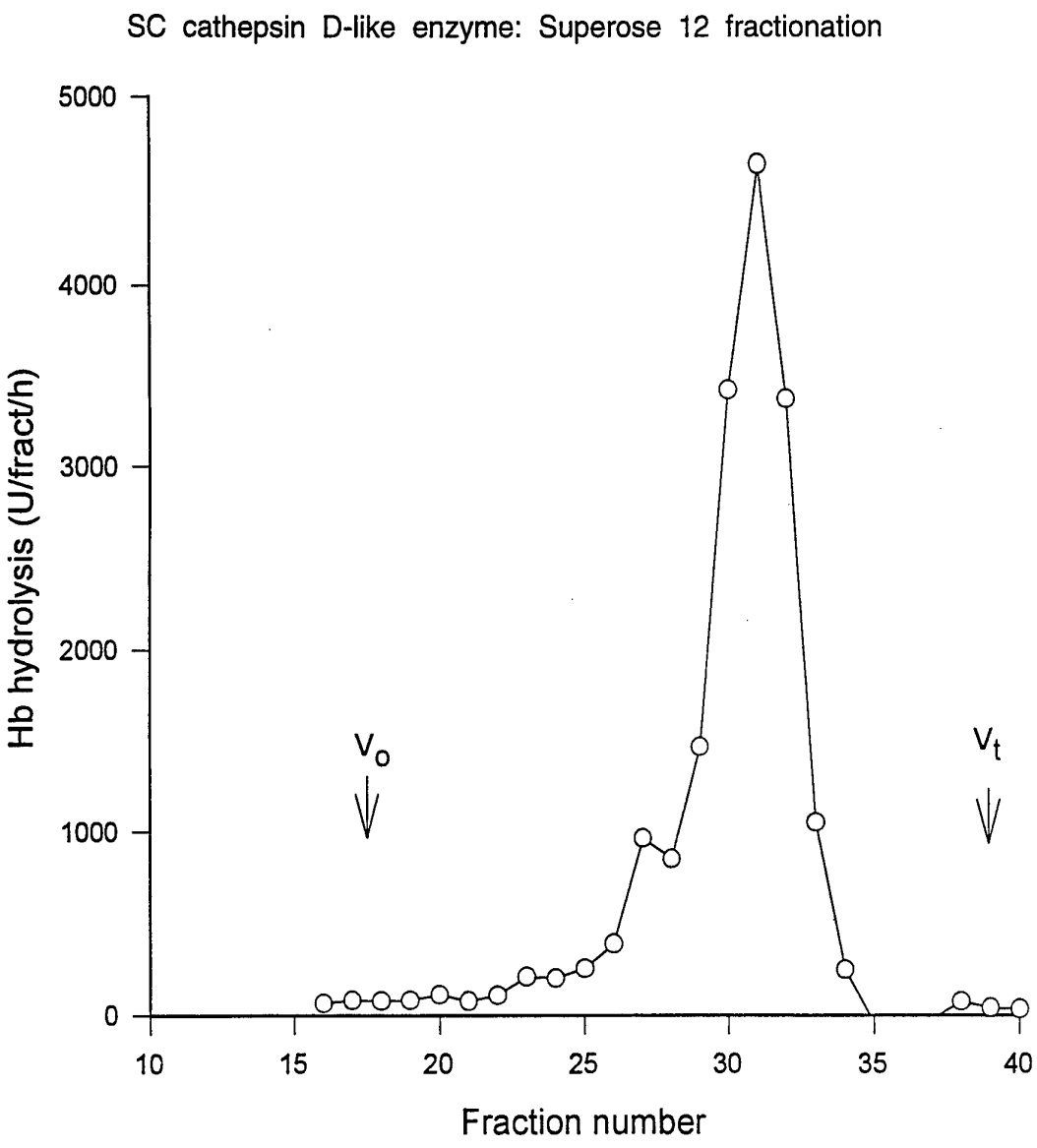

SC cathepsin D-like enzyme: Superose 12 fractionation
Hb hydrolysis (U/fract/h)
5000
4000
3000
2000
1000
0
V_o
V_t
10
15
20
25
30
35
40
Fraction number

Fig.2.

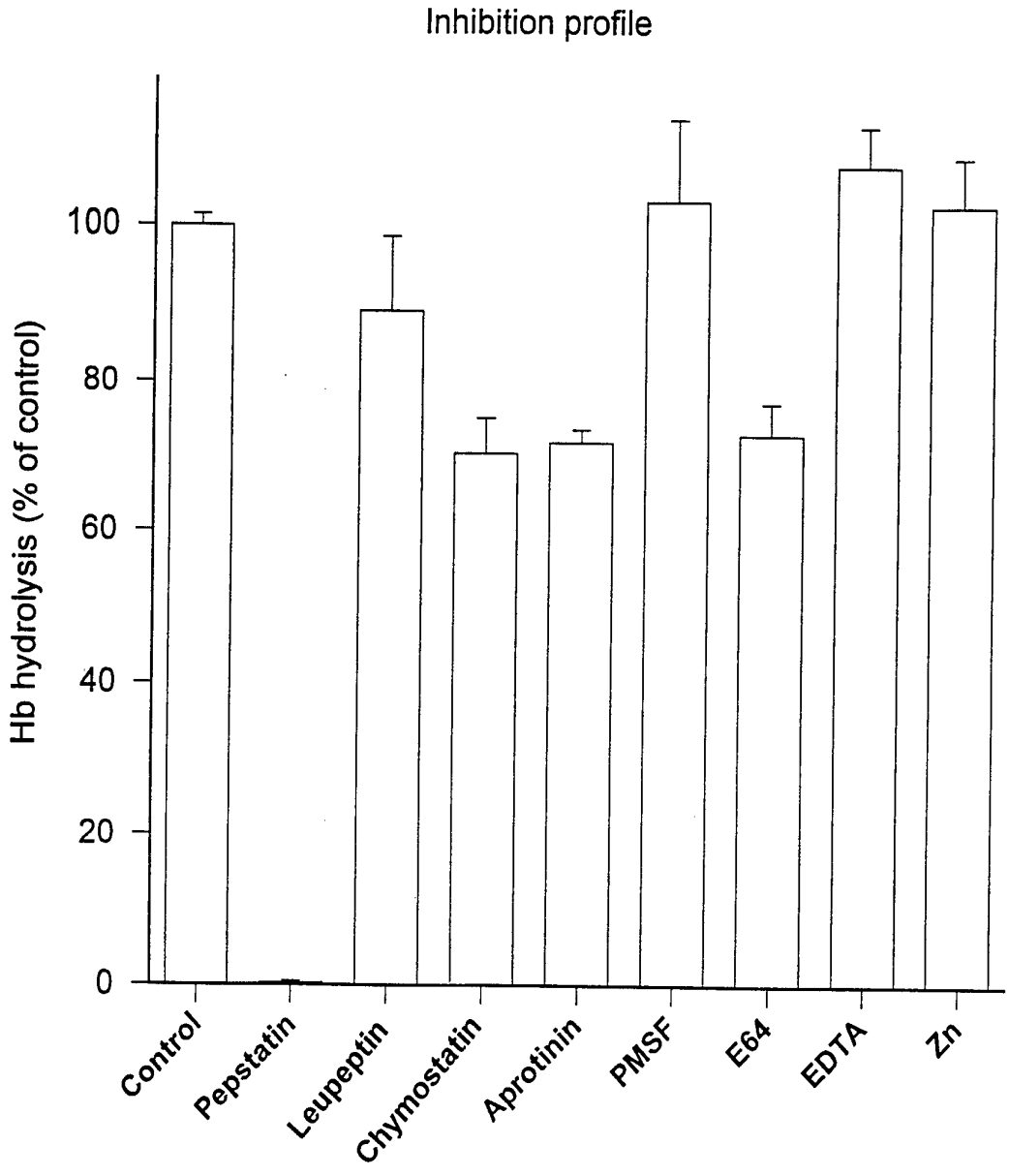
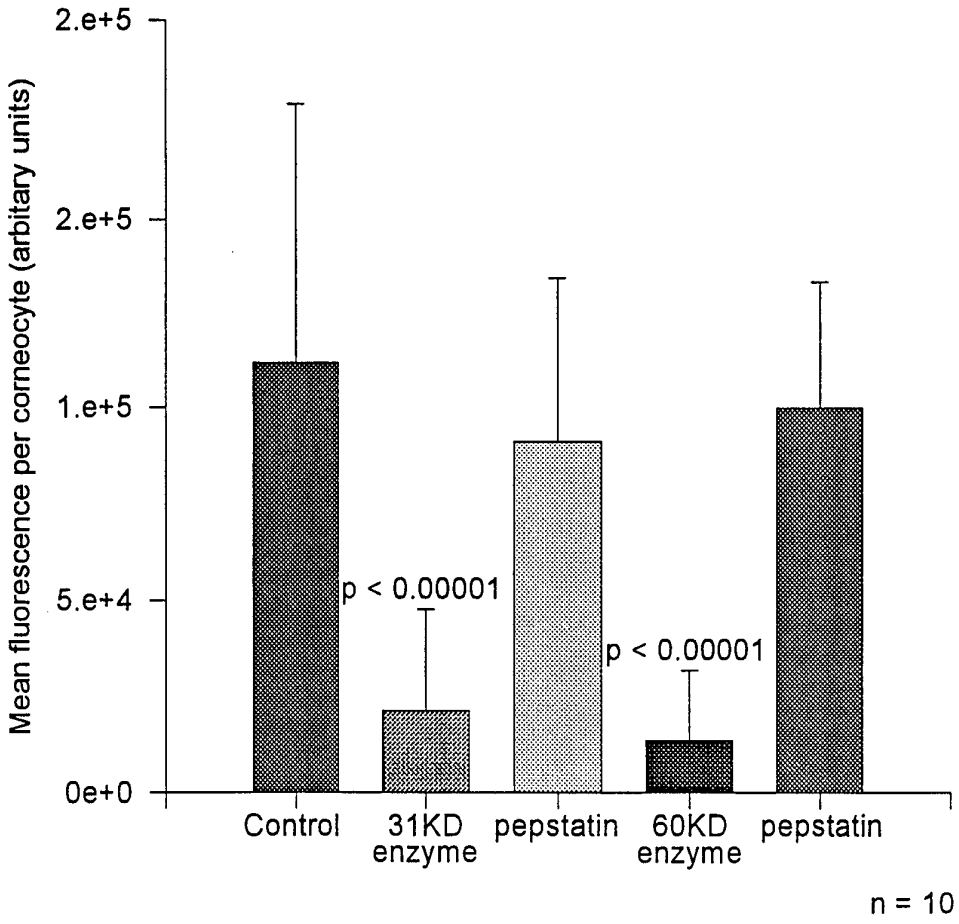

Inhibition profile
Hb hydrolysis (% of control)
0
20
40
60
80
100
Control
Pepstatin
Leupeptin
Chymostatin
Aprotinin
PMSF
E64
EDTA
Zn

Fig.3.

Effect on stratum corneum desmocollin 1 degradation



INTERNATIONAL SEARCH REPORT

Inter. ..al Application No
PCT/EP 94/03000

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K7/48 A61K38/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,93 19732 (HENKEL KGAA) 14 October 1993 see the whole document ---	1,3-9
P,X	WO,A,93 19731 (UNILEVER PLC.) 14 October 1993 see the whole document ---	1,3-9
A	ACTA DERMATO-VENEREOLOGICA, vol.71, no.6, 1991, STOCKHOLM SE pages 471 - 474 LUNDSTRÖM ET AL. 'stratum corneum chymotryptic enzyme' cited in the application --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 1995	11. 01. 95

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inter ial Application No
PCT/EP 94/03000

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 8, no. 106 (C-223) & JP,A,59 020 211 (AYUKAWA TAIZOU) 1 February 1984 see abstract -----	1-9

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INTERNATIONAL SEARCH REPORT

information on patent family members

Inter ıal Application No
PCT/EP 94/03000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9319732	14-10-93	AU-B- 3896293	08-11-93
WO-A-9319731	14-10-93	AU-B- 3896193	08-11-93