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(54) Title: SKIN CARE COMPOSITION CONTAINING AN AMIDE AND RETINOL OR RETINYL ESTER

(57) Abstract

N-substituted fatty acid amides of specific structure sufficiently inhibit LRAT or ARAT catalyzed esterification of retinol into inactive retinyl esters, thus having the same effect on keratinocytes as retinoic acid. Effects of retinol or retinyl esters in combination with these N-substituted fatty acid amides are analogous to treatment with retinoic acid.

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SKIN CARE COMPOSITION CONTAINING AN AMIDE AND RETINOL OR RETINYL ESTER

FIELD OF THE INVENTION

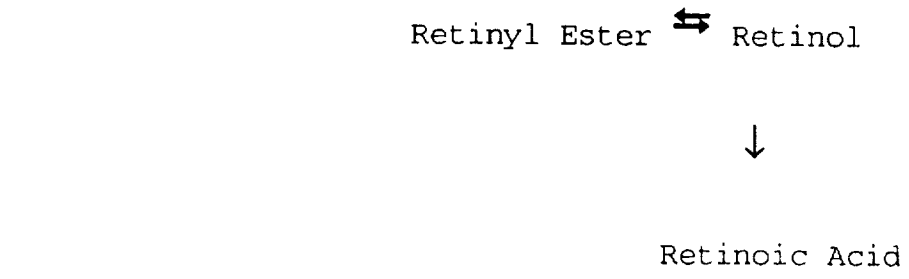
The present invention relates to skin care compositions containing a fatty acid amide and retinol or retinyl ester and to methods involving applying such compositions to the skin.

BACKGROUND OF THE INVENTION

Retinol (vitamin A) is an endogenous compound which occurs naturally in the human body and is essential for normal epithelial cell differentiation. Natural and synthetic vitamin A derivatives have been used extensively in the treatment of a variety of skin disorders and have been used as skin repair or renewal agents. Retinoic acid has been employed to treat a variety of skin conditions, e.g., acne, wrinkles, psoriasis, age spots and discoloration. See e.g., Vahlquist, A. et al., *J. Invest. Dermatol.*, Vol. 94, Holland D.B. and Cunliffe, W.J. (1990), pp. 496-498; Ellis, C.N. et al., "Pharmacology of Retinols in Skin", Vasel, Karger, Vol. 3, (1989), pp. 249-252; Lowe, N.J. et al., "Pharmacology of Retinols in Skin", Vol. 3, (1989), pp. 240-248; PCT Patent Application No. WO 93/19743.

It is believed that the use of retinol or esters of retinol would be preferred over retinoic acid. Retinol occurs naturally in the human body and is considered much safer than retinoic acid. Esters of retinol hydrolyze in-vivo to produce

retinol. It is believed that retinol esters and retinol are metabolically converted in the skin into retinoic acid according to the following mechanism:



However, most of the endogenously applied retinol is rapidly converted into inactive fatty esters for storage in epidermal cells (keratinocytes). Esterification of retinol into inactive retinyl esters is achieved in cells by transfer of a fatty acyl group from an acyl CoA, catalyzed by the enzyme acyl CoA retinol transferase (ARAT), or by the transfer of an acyl group from phosphatidyl choline, catalyzed by the enzyme lecithin retinol acyl transferase (LRAT). These esterification reactions are very efficient in keratinocytes--the majority (95%) of cellular retinoids are in the form of retinyl fatty esters. Thus, unfortunately, although retinol and retinyl esters are safer to use than retinoic acid, they are less effective than retinoic acid at providing skin benefits.

The present invention is based, in part, on the discovery that certain N-substituted fatty acid amides inhibit these esterification reactions and thus potentiate the action of retinol by increasing the amount of retinol available for conversion to retinoic acid. Thus, a mixture of these N-

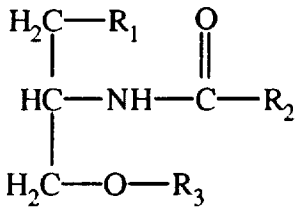
substituted fatty acid amides with retinol or retinyl esters mimics retinoic acid yet is safer to use than retinoic acid. An earlier filed European Patent Application EP 0 742 005 (Unilever; priority date May 8, 1995), published November 13, 1996 (after the priority date of the present application), discloses combinations of fatty acid amides with retinol or retinyl esters. EP '005 however does not teach specific N-substituted fatty acid amides of the present invention or any fatty acid amide with a branched, alkoxy-containing substitution at the nitrogen atom.

SUMMARY OF THE INVENTION

The present invention includes, in part, a skin conditioning composition containing:

(a) from 0.001% to 10% of retinol or retinyl ester;

(b) from 0.0001% to 50% of an N-substituted fatty acid amide of Formula I:



(I)

wherein R_1 = alkyl or alkoxy having from 1 to 10 carbon atoms;

R_2 = alkyl or alkenyl having from 8 to 25 carbon atoms;

R_3 = alkyl containing 1 to 5 carbon atoms, or a phosphate ester; and

(c) a cosmetically acceptable vehicle.

The invention also provides a cosmetic method of conditioning skin comprising topically applying the present composition to the skin. It further provides a cosmetic method mimicking the effect of retinoic acid on skin comprising topically applying the present composition to the skin.

The term "conditioning" as used herein means any one or more of the following: prevention and/or treatment of dry skin, photodamaged skin, appearance of wrinkles, age spots and/or aged skin, increasing stratum corneum flexibility, lightening skin color, controlling sebum excretion and generally increasing the quality of skin.

It has been found as part of the present invention that N-substituted fatty acid amides of Formula I preferably inhibits, at 100 μM concentration, at least 20% of LRAT or ARAT catalyzed retinol esterification as measured by an in vitro Microsomal Assay (described below). Thus, the presence of an N-substituted fatty acid amide of Formula I in the inventive compositions substantially improves the performance of retinol or a retinyl ester in cosmetic compositions.

DESCRIPTION OF THE PREFERRED EMBODIMENT

All amounts are by weight of the final composition, unless otherwise indicated.

The inventive compositions contain, as a first essential ingredient, a compound selected from the group consisting of retinol and retinyl ester. The term "retinol" includes amongst others the following isomers of retinol: all-trans-retinol, 13-cis-retinol, 11-cis-retinol, 9-cis-retinol, 3,4-didehydro-retinol. Preferred isomers are all-trans-retinol, 13-cis-retinol, 3,4-didehydro-retinol, 9-cis-retinol. Most preferred is all-trans-retinol, due to its wide commercial availability.

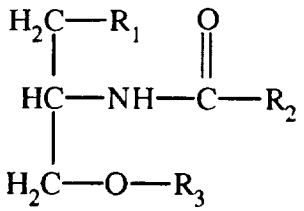
Retinyl ester is an ester of retinol. The term "retinol" has been defined above. Retinyl esters suitable for use in the present invention are C_{1}-C_{30} esters of retinol, preferably C_{2}-C_{20} esters, and most preferably C_{2}, C_{3}, and C_{16} esters because they are more commonly available. Examples of retinyl esters include but are not limited to: retinyl palmitate, retinyl formate, retinyl acetate, retinyl propionate, retinyl butyrate, retinyl valerate, retinyl isovalerate, retinyl hexanoate, retinyl heptanoate, retinyl octanoate, retinyl nonanoate, retinyl decanoate, retinyl undecandate, retinyl laurate, retinyl tridecanoate, retinyl myristate, retinyl pentadecanoate, retinyl heptadeconoate, retinyl stearate, retinyl isostearate, retinyl nonadecanoate, retinyl arachidonate, retinyl behenate, retinyl linoleate, retinyl oleate.

The preferred ester for use in the present invention is selected from retinyl palmitate, retinyl acetate and retinyl

propionate, because these are the most commercially available and therefore the cheapest. Retinyl linoleate is also preferred due to its efficacy.

Retinol and/or retinyl ester is employed in the inventive composition in an amount of from 0.001% to 10%, preferably in an amount of from 0.01% to 1%, most preferably in an amount of from 0.01% to 0.5%.

The second essential ingredient of the inventive compositions is an N-substituted fatty acid amide of Formula I:



(I)

wherein R_1 = alkyl or alkoxy having from 1 to 10 carbon atoms;

R_2 = alkyl or alkenyl having from 8 to 25 carbon atoms;

R_3 = alkyl containing 1 to 5 carbon atoms, or a phosphate ester.

Preferably, R_1 is a linear saturated alkyl or alkoxy group containing 1 to 5 carbon atoms, most preferably 1 or 4 carbon atoms.

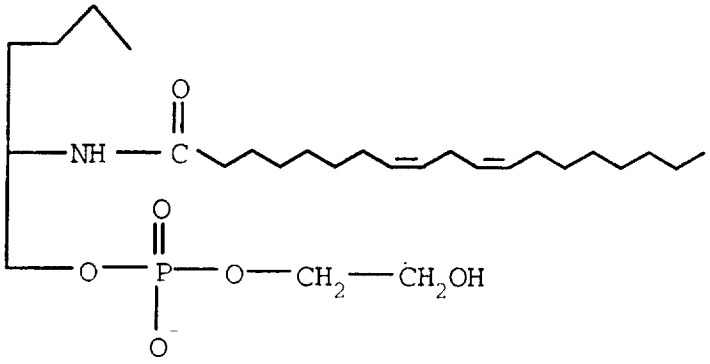
R_2 is preferably a linear unsaturated alkenyl group containing from 10 to 20 carbon atoms, most preferably from 10 to 18 carbon atoms.

Optimally, R_2 is the linoleic acid residue ($C_{18:2}$).

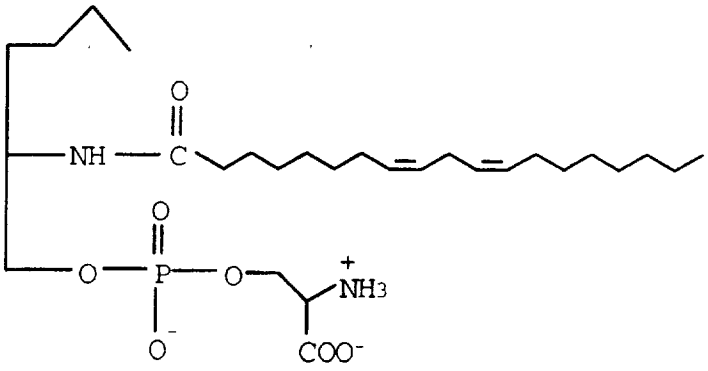
R_3 is preferably either a methyl group or a phosphate ester, most preferably phosphate ester of choline or glycol.

Most preferably, the N-substituted fatty acid amide is selected from the compounds having formulae A and B below.

Formula A



Formula B



The N-substituted fatty acid amide is included in the inventive compositions in an amount ranging from 0.0001% to 50%, preferably from 0.01% to 10%, most preferably from 0.1% to 5%.

The N-substituted fatty acid amide of Formula I preferably inhibits, at 100μM concentration, at least 20% of LRAT or ARAT catalyzed retinol esterification as measured by in vitro Microsomal Assay:

In vitro Microsomal Assay:

Microsomes are obtained as described in: J.C. Saari and D.L. Bredberg, "CoA and Non-CoA Dependent Retinol Esterification in Retinal Figment Epithelium" J. Biol. Chem. 263, 8084-90 (1988).

A solution containing 0.1M sodium phosphate pH 7 buffer, 5mM dithiothreitol, 2 mg/ml bovine serum albumin, 40 micromolar palmitoyl CoA, 40 micromolar dilauroyl phosphatidyl choline, 10 micromolar retinol and a test compound or a solvent blank, is incubated for 1 hour at 37°C with a microsomal fraction isolated from bovine retinal pigment epithelial cells. After incubation, the reaction is quenched by addition of an equal volume of ethanol, and the retinyl esters formed (retinyl laurate from the LRAT catalyzed reaction and retinyl palmitate from ARAT catalyzed reaction) are extracted with hexane. The hexane layer is removed, evaporated under nitrogen, and the residue analyzed by HPLC on a 3.9x300 mm C_{18} reversed phase column using a 80% methanol in tetrahydrofuran mobile phase and fluorescence detection (325 nm excitation, 480 nm emission) to quantitate the retinyl ester. The quantity of

ester formed in the presence of the solvent blank is taken as 100%, and this is used to calculate the percent inhibition of ester formation for the compounds tested. As a control, an aliquot of microsomes is inactivated by boiling for 5 minutes, which results in at least 95% inhibition of ester formation.

In a preferred embodiment of the invention, N-substituted fatty acid amide is selected which, at a 100 µM concentration, inhibits at least 40% of LRAT or ARAT catalyzed retinol esterification.

Cosmetically Acceptable Vehicle

The composition according to the invention also comprises a cosmetically acceptable vehicle to act as a dilutant, dispersant or carrier for the retinol and/or retinyl ester and the N-substituted fatty acid amide, so as to facilitate its distribution when the composition is applied to the skin.

Vehicles other than or in addition to water can include liquid or solid emollients, solvents, humectants, thickeners and powders. An especially preferred nonaqueous carrier is a polydimethyl siloxane and/or a polydimethyl phenyl siloxane. Silicones of this invention may be those with viscosities ranging anywhere from 10 to 10,000,000mm^2/s(centistokes) at 25°C. Especially desirable are mixtures of low and high viscosity silicones. These silicones are available from the General Electric Company under trademarks Vicasil, SE and SF and from the Dow Corning Company under the 200 and 550 Series. Amounts of silicone which can be utilized in the compositions of this invention range anywhere from 5% to 95%, preferably from 25% to 90% by weight of the composition.

The cosmetically acceptable vehicle will usually form from 5% to 99.9%, preferably from 25% to 80% by weight of the composition, and can, in the absence of other cosmetic adjuncts, form the balance of the composition. Preferably, the vehicle is at least 50%, more preferably at least 80 wt.% water, by weight of the vehicle. Preferably, water comprises at least 50 wt.% of the inventive composition, most preferably from 60 to 80 wt.%, by weight of the composition.

Optional Skin Benefit Materials and Cosmetic Adjuncts

An oil or oily material may 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 hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

The inventive compositions preferably include sunscreens. Sunscreens include those materials commonly employed to block ultraviolet light. Illustrative compounds are the derivatives of PABA, cinnamate and salicylate. For example, octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone) can be used. Octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trademarks, Parsol MCX and Benzophenone-3, respectively. The exact amount of sunscreen employed in the emulsions can vary depending upon the degree of protection desired from the sun's UV radiation.

Another preferred optional ingredient is selected from essential fatty acids (EFAs), i.e., those fatty acids which are essential for the plasma membrane formation of all cells,

in keratinocytes EFA deficiency makes cells hyperproliferative. Supplementation of EFA corrects this. EFAs also enhance lipid biosynthesis of epidermis and provide lipids for the barrier formation of the epidermis. The essential fatty acids are preferably chosen from linoleic acid, γ-linolenic acid, homo-γ-linolenic acid, columbinic acid, eicosa-(n-6,9,13)-trienoic acid, arachidonic acid, timnodonic acid, hexaenoic acid and mixtures thereof.

Yet another preferred optional ingredient is selected from azoles, e.g., climbazole, bifonazole, clotrimazole, ketoconazole, miconazole, econazole, itraconazole, fluconazole, terconazole, butoconazole, sulconazole, lionazole and mixtures thereof. The azole may be included in the inventive compositions in an amount of from 0.001 to 50 wt. %, preferably from 0.001 to 10 wt.%, most preferably from 0.1 to 5%.

Emollients are often incorporated into cosmetic compositions of the present invention. Levels of such emollients may range from 0.5% to 50%, preferably between 5% and 30% by weight of the total composition. Emollients may be classified under such general chemical categories as esters, fatty acids and alcohols, polyols and hydrocarbons.

Esters may be mono- or di-esters. Acceptable examples of fatty di-esters include dibutyl adipate, diethyl sebacate, diisopropyl dimerate, and dioctyl succinate. Acceptable branched chain fatty esters include 2-ethyl-hexyl myristate, isopropyl stearate and isostearyl palmitate. Acceptable tribasic acid esters include triisopropyl trilinoleate and trilauryl citrate. Acceptable straight chain fatty esters include lauryl palmitate, myristyl lactate, oleyl eurcate and

stearyl oleate. Preferred esters include coco-caprylate/caprate (a blend of coco-caprylate and coco-caprate), propylene glycol myristyl ether acetate, diisopropyl adipate and cetyl octanoate.

Suitable fatty alcohols and acids include those compounds having from 10 to 20 carbon atoms. Especially preferred are such compounds as cetyl, myristyl, palmitic and stearyl alcohols and acids.

Among the polyols which may serve as emollients are linear and branched chain alkyl polyhydroxyl compounds. For example, propylene glycol, sorbitol and glycerin are preferred. Also useful may be polymeric polyols such as poly-propylene glycol and polyethylene glycol. Butylene and propylene glycol are also especially preferred as penetration enhancers.

Exemplary hydrocarbons which may serve as emollients are those having hydrocarbon chains anywhere from 12 to 30 carbon atoms. Specific examples include mineral oil, petroleum jelly, squalene and isoparaffins.

Another category of functional ingredients within the cosmetic compositions of the present invention are thickeners. A thickener will usually be present in amounts anywhere from 0.1 to 20% by weight, preferably from 0.5% to 10% by weight of the composition. Exemplary thickeners are cross-linked polyacrylate materials available under the trademark Carbopol from the B.F. Goodrich Company. Gums may be employed such as xanthan, carrageenan, gelatin, karaya, pectin and locust bean gum. Under certain circumstances the thickening function may be accomplished by a material also serving as a silicone or emollient. For instance, silicone gums with viscosity in

excess of 10 centistokes and esters such as glycerol stearate have dual functionality.

Powders may be incorporated into the cosmetic composition of the invention. These powders include chalk, talc, kaolin, starch, smectite clays, chemically modified magnesium aluminum silicate, organically modified montmorillonite clay, hydrated aluminum silicate, fumed silica, aluminum starch octenyl succinate and mixtures thereof.

Other adjunct minor components may also be incorporated into the cosmetic compositions. These ingredients may include coloring agents, opacifiers and perfumes. Amounts of these other adjunct minor components may range anywhere from 0.001% up to 20% by weight of the composition.

Use of the Composition

The composition according to the invention is intended primarily as a product for topical application to human skin, especially as an agent for conditioning and smoothening the skin, and preventing or reducing the appearance of wrinkled or aged skin.

In use, a small quantity of the composition, for example from 1 to 100ml, is applied to exposed areas of the skin, from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device.

Product Form and Packaging

The topical skin treatment composition of the invention can suitably be formulated as a lotion, a cream or a gel. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or 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 also be included in capsules such as those described in U.S. Patent 5,063,057.

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

The following specific examples further illustrate the invention.

MATERIALS AND METHODS

Cell Culture:

Human keratinocytes, isolated from neonatal foreskin by trypsin treatment were grown in Dulbecco Modification Eagle (DME) Hams F12 (1:1) medium/10% fetal calf serum in the presence of irradiated 3T3 mouse fibroblasts for establishing dividing keratinocyte colonies. Cells were grown under the above condition until their second passage and kept frozen for future use. Frozen second passage keratinocytes were thawed

and plated into the above medium and grown for five days before they were switched to a serum-free MCDB 153-based medium keratinocyte growth medium (KGM) from Clonetics Corporation, San Diego, CA, containing 0.15 mM Ca, or keratinocyte serum-free media (KSFM) from GIBCO containing 0.09 mM Ca). On day 7, when the cells were 80-90% confluent, they were trypsinized and plated in the serum-free medium for the various experiments.

TRANSGLUTAMINASE ASSAY

Transglutaminase Assay and Keratinocyte Differentiation

During the process of terminal differentiation in the epidermis, a 15nm thick layer of protein, known as the cornified envelope (CE) is formed on the inner surface of the cell periphery. The CE is composed of numerous distinct proteins which have been cross-linked together by the formation of N^{ε}-(γ-glutamyl) lysine isodipeptide bonds catalyzed by the action of at least two different transglutaminases (TGases) expressed in the epidermis. Transglutaminase I (TGase I) is expressed in abundance in the differentiated layers of the epidermis, especially the granular layer, but is absent in the undifferentiated basal epidermis. Thus TGase I is a useful marker of epidermal keratinocyte differentiation with high TGase I levels indicating a more differentiated state. An ELISA based TGase I assay, using a TGase I antibody, was used to assess the state of differentiation of the cultured keratinocytes in the examples that follow.

For Example 1, the following procedure was used:

Keratinocytes (cultured as described above) were plated in 96 well plates at a density of 3,000 cells per well in 200 µl media. After incubation for four days the media was changed to media containing test compounds (six replicates per test). The cells were cultured for a further 72 hours after which time the media was aspirated and the plates stored at -70°C. Plates were removed from the freezer, and the cells washed with PBS. 100 µl sterile water was added and the cells were freeze fractured by freezing at -70°C then thawing. The cells were incubated for one hour at room temperature (R/T) with PBS/3% BSA (wash buffer, bovine serum albumin), then rinsed with a fresh aliquot of wash buffer. Cells were incubated with 50 µl of primary antibodies monoclonal anti-human transglutaminase mouse antibody (IgG) obtained from Biomedical Industries diluted 1:2,000 in wash buffer for one hour, 37°C then rinsed two times with wash buffer. Cells were then incubated with 50 µl of secondary antibody (Fab fragment, peroxidase conjugated anti-mouse IgG obtaining from Amersham) diluted 1:4,000 in wash buffer for one hour at 37°C, then rinsed two times with wash buffer. Cells were incubated with substrate solution (4 mg ophenylene diamine and 3.3 µl 30% H_2O_2 in 10ml 0.1M citrate buffer pH 5.0) for five minutes, R/T, in darkness (under aluminum foil). The reaction was stopped by the addition of 50 µl 4N H_2SO_4. The absorbance of samples was read at 492nm in the plate reader. Out of the six replicates, four were treated with both antibodies, two were treated only with the secondary antibody (i.e., to determine background binding of enzyme conjugated Ab). TGase levels were determined by subtracting background from the readings

from each treatment and determining mean ± s.d. for the replicates exposed to both antibodies.

For Example 3, the following procedure was used:

Keratinocytes (cultured as described above) were plated in 96 well plates at a density of 3,000 cells per well in 200µl of cell culture media. After incubation for 4 days, the media was changed to media containing test compounds (six replicates per test). The cells were cultured for a further 72 hours after which time the media was aspirated and the plates stored at -70°C. After the plates were removed from the freezer, the cells were further freezed fractured by freezing and thawing and then washed 3x with PBS. The cells were incubated for one hour at room temperature (R/T) with TBS/5% BSA buffer. Cells were then incubated with 100µl of monoclonal anti-human transglutaminase (IgG) mouse antibody (primary antibody) obtained from Biomedical Technologies Inc. diluted 1:2000 in TBS/1% BSA buffer for two hours at 37°C, and then rinsed six times with wash buffer (TBS/1% BSA/0.05% Tween-20). Cells were next incubated with 100µl of Fab fragment, peroxidase conjugated anti-mouse IgG antibody (secondary antibody) from Amersham diluted 1:4,000 in wash buffer for two hours at 37°C and then rinsed three times with wash buffer and three times with PBS. Cells were incubated with substrate solution (4mg o-phenylene diamine and 3.3:1 30% H_2O_2 in 10mL 0.1M citrate buffer, pH 5.0) for five minutes at R/T and in darkness (under aluminum foil). The reaction was stopped by the addition of 50µl 4N H2SO4. The absorbance of samples was read at 492nm in the plate reader. Out of the six replicates, four were treated with both antibodies, two were treated only with the secondary antibody (i.e., to determine the background binding of the

enzyme conjugated antibody). Transglutaminase I (Tgase I) levels were determined by subtracted background from the readings from each treatment and determining the mean ± s.d. for the replicates exposed to both antibodies.

DNA Assay

The level of Tgase I detected after treatment of the cells could be influenced by cell number, i.e., the greater the number of cells the greater the level of TGase I detected. The level of Tgase I was normalized to DNA content of the cells in the same well thus eliminating variation due to differences in cell number. DNA quantitation is a particularly useful indicator of cell number, including keratinocyte cell number, because each cell has to all intents and purposes an identical genome and therefore an identical quantity of DNA. The total DNA content of a well of cells therefore is directly proportional to the cell number in that well. Quantitation of DNA was used to normalize the TGase data to cell number.

Keratinocytes were plated in 96 well plates at a density of 3,000 cells per well in 200 µl media. After incubation for four days the media was changed for media containing test compounds (6 replicates per test). The cells were cultured for a further 72 hours after which time the media was aspirated and the plates stored for at least 1.5 hours at -70°C. Plates were removed from the freezer and thawed for 30 minutes. 100 µl/well of Hoechst dye (1 µg/ml final concentration) was added and this was incubated for 15 minutes, covered and then read in a fluorimeter (ex. 360nm and

em. 460nm). The dye solution was removed and the wells were rinsed with PBS in preparation for the TGase assay.

EXAMPLE 1

Retinoic acid is more effective than retinol at altering keratinocyte differentiation state

The effect on Transglutaminase levels normalized to DNA content of the cells after addition of retinoic acid (RA) and retinol (ROH) was examined and the results are shown in Table 1.

TABLE 1

Treatment	mean TGase/ DNA x 10^{-4} ± s.d (% control)	p value vs Control	p value vs 2.5x 10^{-7} M ROH	p value vs 2.5x 10^{-8} M ROH	p value vs 2.5x 10^{-9} M ROH
Control	2.44 ± 0.24 (100%)	-	0.001	0.001	0.001
2.5x10^{-7}M RA	0.16 ± 0.11 (7%)	0.001	0.001	0.001	0.001
2.5x10^{-7}M ROH	1.14 ± 0.22 (47%)	0.001	-	0.001	0.001
2.5x10^{-8}M RA	1.34 ± 0.40 (55%)	0.001	0.2	0.001	0.001
2.5x10^{-8}M ROH	1.89 ± 0.30 (77%)	0.001	0.001	-	0.001
2.5x10^{-9}M RA	1.87 ± 0.49 (77%)	0.001	0.001	0.784	0.001
2.5x10^{-9}M ROH	2.70 ± 0.59 (>100%)	0.001	0.001	0.001	-

n = 3

All concentrations of retinoic acid tested, i.e., 2.5×10^{-7}M, 2.5×10^{-8}M and 2.5×10^{-9}M decreased keratinocyte differentiation to a significantly greater extent than each of the corresponding 2.5×10^{-7}M, 2.5×10^{-8}M and 2.5×10^{-9}M retinol treatments. The decrease in transglutaminase level was dose dependent for both retinoic acid and retinol. This is consistent with retinoic acid having a greater inhibitory effect on epithelial differentiation than retinol.

EXAMPLE 2

In vitro microsomal esterification of retinol:

Microsomes are obtained as described in: J. C. Saari and D. L. Bredberg, "CoA and Non-CoA Dependent Retinol Esterification in Retinal Pigment Epithelium" J. Biol. Chem. 23, 8084-90 (1988).

A solution containing 0.1M sodium phosphate pH 7 buffer, 5mM dithiothreitol, 2 mg/ml bovine serum albumin, 40 micromolar palmitoyl CoA, 40 micromolar dilauroyl phosphatidyl choline, 10 micromolar retinol and a test compound or solvent blank, was incubated for 1 hour at 37°C with a microsomal fraction isolated from bovine retinal pigment epithelial cells. After incubation, the reaction was quenched by addition of an equal volume of ethanol, and the retinyl esters formed (retinyl palmitate from the ARAT catalyzed reaction, and retinyl laurate from the LRAT catalyzed reaction) were extracted with hexane. The hexane layer was removed, evaporated under nitrogen, and the residue analyzed by HPLC on a 3.9x300 mm C18 reversed phase column using a 80% methanol in tetrahydrofuran

mobile phase and fluorescence detection (325 nm excitation, 480 nm emission) to quantitate the retinyl esters. The quantity of ester formed in the presence of the solvent blank was taken as 100%, and this was used to calculate the percent inhibition of ester formation for the compounds tested. As a control, an aliquot of microsomes was inactivated by boiling for 5 minutes, which resulted in at least 95% inhibition of ester formation.

The results that were obtained are summarized in Tables 2A and 2B.

The compounds in Table 2A were tested at a 100 µM concentration. The compounds in Table 2B were tested at a 10 µM concentration.

Compound of Formula C which doesn't fall within the scope of the present invention was also tested.

Formula C

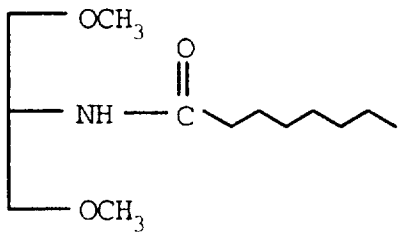


TABLE 2A		
COMPOUND	**% INHIBITION, ARAT**	**% INHIBITION, LRAT**
Control	0	0
Formula C	0	0
Formula B	83	92
Formula A	54	48

TABLE 2B		
COMPOUND	**% INHIBITION, ARAT**	**% INHIBITION, LRAT**
Control	0	0
Formula C	N/D	N/D
Formula B	42	51
Formula A	43	0

It can be seen from the results in Tables 2A and 2B that N-substituted fatty acid amides, wherein R_2 has more than 7 carbon atoms (i.e., Formulae A and B), are potent inhibitors of LRAT and ARAT catalyzed retinol esterification.

EXAMPLE 3

The effect on keratinocyte differentiation of compounds and combinations listed in Table 3 was examined. The results were expressed as % of control. Transglutaminase level was normalized to DNA.

TABLE 3

Effect of Retinol and Amide of Formula A on Keratinocyte TGase/DNA

Treatment	mean TGase/ DNA $x10^5 \pm$ s.d (% control)	p value vs Control	p value vs 2.5x 10^{-7} M ROH	p value vs 2.5x 10^{-7} M RA	p value vs 10^{-4} M Formula A
Control	57.80 ± 8.61 (100%)	-	0.020	0.004	0.003
2.5x10^{-7}M RA	39.99 ± 5.28 (69%)	0.004	0.093	-	0.525
2.5x10^{-7}M Retinol	45.60 ± 3.92 (79%)	0.020	-	0.093	0.054
10^{-4}M Formula A	41.57 ± 0.75 (72%)	0.003	0.054	0.525	-
2.5x10^{-7}M ROH + 10^{-4}M Formula A	33.27 ± 3.97 (58%)	0.001	0.001	0.052	0.002

n = 3

$2.5x10^{-7}M$ retinoic acid was effective at repressing keratinocyte TG1 levels (to 69% of control level). $2.5x10^{-7}M$ retinol and $10^{-4}M$ Formula A compound were less effective at inhibiting keratinocyte Tgase I level when used alone. However, combined $2.5x10^{-7}M$ retinol + $10^{-4}M$ Formula A compound repressed keratinocyte Tgase I to 58% of control levels. This example also establishes a good correlation between microsomal assay and cell culture data.

Examples 4-9 illustrate topical compositions according to the present invention. The compositions can be processed in conventional manner. They are suitable for cosmetic use. In particular the compositions are suitable for application to wrinkled, rough, dry, flaky, aged and/or UV-damaged skin to improve the appearance and the feel thereof as well as for application to healthy skin to prevent or retard deterioration thereof.

EXAMPLE 4

This example illustrates a high internal phase water-in-oil emulsion incorporating the inventive composition.

	% w/w
Retinol	0.5
Fully hydrogenated coconut oil	3.9
Formula A	5
Brij 92*	5
Bentone 38	0.5
$MgSO_4 7H_2O$	0.3
Butylated hydroxy toluene	0.01
Perfume	qs
Water	to 100

* Brij 92 is polyoxyethylene (2) oleyl ether

EXAMPLE 5

This example illustrates an oil-in-water cream incorporating the inventive composition.

	% w/w
Retinol	0.15
Mineral oil	4
Formula B	2
Brij 56*	4
Alfol 16RD*	4
Triethanolamine	0.75
Butane-1,3-diol	3
Xanthan gum	0.3
Butylated hydroxy toluene	0.01
Water	to 100

* Brij 56 is cetyl alcohol POE (10)
Alfol 16RD is cetyl alcohol

EXAMPLE 6

This example illustrates an alcoholic lotion incorporating the composition according to the invention.

	% w/w
Retinyl palmitate	0.15
Formula B	0.5
Ethanol	40
Butylated hydroxy toluene	0.01
Water	to 100

EXAMPLE 7

This example illustrates another alcoholic lotion containing the inventive composition.

	% w/w
Retinol	0.15
Formula A	0.2
Ethanol	40
Antioxidant	0.1
Water	to 100

EXAMPLE 8

This example illustrates a suncare cream incorporating the composition of the invention:

	% w/w
Retinol	0.01
Formula A	0.3
Silicone oil 200 cts	7.5
Glycerylmonostearate	3
Cetosteryl alcohol	1.6
Polyoxyethylene-(20)-cetyl alcohol	1.4
Xanthan gum	0.5
Parsol 1789	1.5
Octyl methoxycinnate (PARSOL MCX)	7
Perfume	qs
Color	qs
Water	to 100

EXAMPLE 9

This example illustrates a non-aqueous skin care composition incorporating the inventive combination.

	% w/w
Retinyl palmitate	0.15
Formula B	1
Silicone gum SE-30[1]	10
Silicone fluid 345[2]	20
Silicone fluid 344[3]	55.79
Squalene	10
Linoleic acid	0.01
Cholesterol	0.03
2-hydroxy-n-octanoic acid	0.7
Vitamin E linoleate	0.5
Herbal oil	0.5
Ethanol	2

[1] A dimethyl silicone polymer having a molecular weight of at least 50,000 and a viscosity of at least 10,000 centistokes at 25°C, available from GEC

[2] Dimethyl siloxane cyclic pentamer, available from Dow Corning Corp.

[3] Dimethyl siloxane tetramer, available from Dow Corning Corp.

Compounds tested in the Examples were obtained from the following sources:

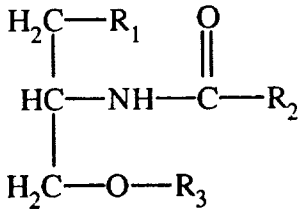
COMPOUND	SOURCE
Retinol	Sigma
Retinyl Palmitate	Sigma
Retinoic Acid	Sigma
N-substituted fatty acid amides	University of Utrecht, Netherlands

CLAIMS:

1. A skin care composition comprising:

(a) from 0.001% to 10% of a compound selected from the group consisting of retinol, retinyl ester and mixtures thereof;

(b) from 0.0001% to 50% of an N-substituted fatty acid amide of the following structure:



wherein R_1 = alkyl or alkoxy having from 1 to 10 carbon atoms;

R_2 = alkyl or alkenyl having from 8 to 25 carbon atoms;

R_3 = alkyl containing 1 to 5 carbon atoms, or a phosphate ester;

(c) a cosmetically acceptable vehicle.

2. The composition of claim 1 wherein the N-substituted fatty acid amide at 100 μM concentration inhibits at least 20% of LRAT or ARAT catalyzed retinol

esterification as measured by an in vitro Microsomal Assay.

3. The composition of claim 2, wherein the N-substituted fatty acid amide at 100 μM concentration inhibits at least 40% of LRAT or ARAT catalyzed retinol esterification as measured by an in vitro Microsomal Assay.

4. The composition of any one of claims 1 - 3 wherein the retinyl ester is selected from the group consisting of retinyl palmitate, retinyl acetate, retinyl propionate, and mixtures thereof.

5. The composition of any one of claims 1 - 3 wherein the retinyl ester is retinyl linoleate.

6. The composition of any one of claims 1 - 3 wherein compound (a) is retinol.

7. A cosmetic method of conditioning skin the method comprising applying topically to the skin a composition according to any one of claims 1-6.

8. A cosmetic method of mimicking the effect on skin of retinoic acid, the method comprising applying to the skin a composition according to any one of claims 1 - 6.

INTERNATIONAL SEARCH REPORT

Internat .l Application No
PCT/EP 97/05138

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K7/48

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.
A	WO 93 09805 A (LABORATOIRES INOCOSM) 27 May 1993 see example 1 ---	1
A	WO 94 23694 A (UNILEVER PLC ;UNILEVER NV (NL)) 27 October 1994 see page 3, line 30 - line 35 see page 7, line 2 - line 30 see page 21, line 1 - line 23 ---	1,4-8
P,A	EP 0 742 005 A (UNILEVER PLC ;UNILEVER NV (NL)) 13 November 1996 cited in the application see claims -----	1,2,7,8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"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
20 January 1998	04. 02. 98

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

Information on patent family members

Interna ıl Application No
PCT/EP 97/05138

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