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(54) Title: METHOD OF SYNTHESISING PHYTOSPHINGOSINE-CONTAINING CERAMIDES AND COSMETIC COMPOSITIONS COMPRISING THEM		
$ \begin{array}{c} \text{CH}_3 (\text{C}_x\text{H}_y\text{Z}_z) - \overset{\text{O}}{\parallel} \text{C} - \text{O} - (\text{C}_3\text{H}_5) - (\text{CHOH})_m - \overset{\text{O}}{\parallel} \text{C} - \text{NH} \\ \\ \text{CH} - \text{CH}_2\text{OR}_1 \\ \\ \text{R} - \text{CHOH} - \text{CHOH} \end{array} \quad (1) $		
(57) Abstract An efficient method of producing phytosphingosine-containing ceramide one having general structure (1), comprising: (a) obtaining a phytosphingosine base from tetraacetylphytosphingosine (TAPS) by a deacetylation reaction wherein the TAPS is produced by fermentation of cells of the F-60-10 mating type strain of <i>Hansenula ciferrii</i> using a fed-batch mode and a non-fermentable carbon source; and (b) coupling together the phytosphingosine base and a fatty acid/ ω -hydroxy fatty acid component wherein the ω -hydroxy fatty acid component is prepared by a process which includes Kolb \acute{e} synthesis.		

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Method of synthesising phytosphingosine-containing ceramides and cosmetic compositions comprising them

FIELD OF THE INVENTION

5

This invention relates to a method of synthesising phytosphingosine-containing ceramide one structures plus cosmetic compositions comprising these structures.

10

BACKGROUND TO THE INVENTION

Ceramides are an important group of lipids, members of which are found in the epidermis of mammals. Skin ceramides are believed to play an important role in the water permeability properties of the skin, providing an epidermal water-barrier which functions to give increased strength to the skin structure and to decrease water loss and so improve the condition of the skin.

20

Ceramides are N-acylated sphingosine bases. Sphingosine bases are of variable chain length and have the general formula (i):



25

where A is $-\text{CH}=\text{CH}-$ (sphingosine), $-\text{CH}_2\text{CHOH}-$ (phytosphingosine) or $-\text{CH}_2\text{CH}_2-$ (dihydrosphingosine), and where x is generally in the broad range 7 to 27, more typically in the range 10 to 16. It should be noted that sphingosines contain asymmetric carbon atoms and so various stereoisomers are possible. Sphingosine/ceramides from especially mammalian sources are all the D-erythro isomer and phytosphingosine/phytoceramides the D-D-erythro isomer. Seven distinguishable groups of ceramides have been identified in pig and human epidermis. Each group consists of molecules of varying fatty acid chain length. The structures of typical skin ceramides are described in the

30

35

paper entitled "Ceramides of Pig Epidermis: Structure Determination" by P W Wertz and D T Downing in Journal of Lipid Research, Vol 24, 1983, pages 759-765.

5 Because of their properties it is known to use ceramides, ceramide derivatives and also pseudoceramides (synthetic molecules which have properties similar to those of naturally occurring ceramides) as components of skin care compositions.

10

It is difficult to extract ceramides from natural sources, and in some cases the resulting product is not acceptable for cosmetic compositions. Furthermore, ceramides are difficult and expensive to synthesise chemically.

15

It has been proposed in EP 0 097 059 to synthesise N-[omega-(O-linoleoyl) 23-cis-dotriacontenoyl] sphingosine by chemically synthesising an appropriate sphingosine component and linking this to an appropriate acid.

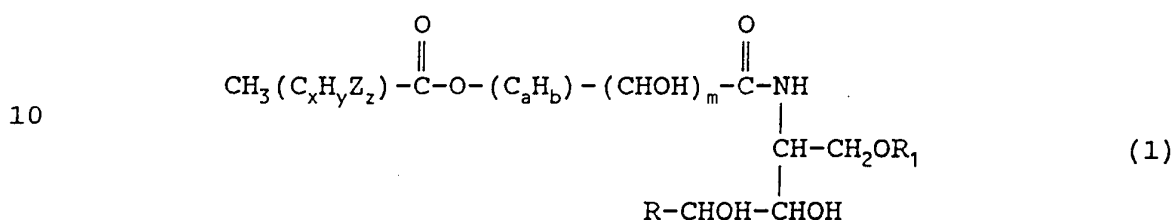
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The paper "Formation of Extracellular Sphingolipids by Microorganisms" by H G Maister et al in Appl Microbiol vol 10, page 401 to 406 describes a process for producing tetraacetyl phytosphingosine (TAPS) from the F-60-10 mating
25 type strain of the yeast Hansenula ciferrii. This process uses glucose as a carbon source in a batch mode fermentation at 25°C. The process, however, is not very efficient, and while it can yield sufficient TAPS for experimental purposes the yield is too low to be
30 practicable for commercial purposes. The TAPS produced is the D-D-erythro isomer, the same as occurs in human skin.

The present inventors have derived a modified process for producing TAPS from Hansenula ciferrii that is much more
35 efficient and which is then used as a component of commercial production of phytosphingosine-containing ceramide one.

DISCLOSURE OF THE INVENTION

Accordingly the present invention provides a method of producing a phytoshingosine-containing ceramide one having
5 the general structure (1):



15 where

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

R₁ represents H, a phosphate residue, a sulphate residue or a sugar residue

Z is -OH or an epoxy oxygen

```
25     a is an integer of from 7 to 50
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b is an integer of from 10 to 100
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m is 0 or 1

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x is an integer of from 12 to 20
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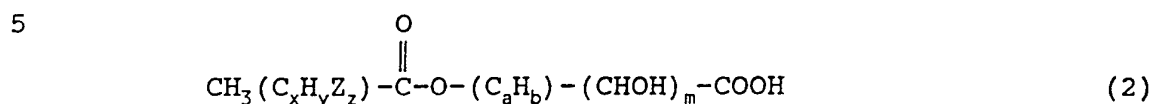
y is an integer of from 20 to 40

```
30      z is 0 or an integer of from 1 to 4
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comprising:

35 (a) obtaining a phytoshingosine base from TAPS by a deacetylation reaction wherein the TAPS is produced by fermentation of cells of the F-60-10 mating type strain of Hansenula ciferrii using a fed-batch mode and a non-fermentable carbon source; and

(b) coupling together the phytosphingosine base and a fatty acid/hydroxy fatty acid component having the general structure (2) via an amide linkage:



where

10

Z is -OH or an epoxy oxygen

a is an integer of from 7 to 50

b is an integer of from 10 to 100

m is 0 or 1

15

x is an integer of from 12 to 20

y is an integer of from 20 to 40

z is 0 or an integer of from 1 to 4

20

wherein the fatty acid/hydroxy fatty acid component having the general structure (2) is prepared by linking together an ω -hydroxy fatty acid having the structure (3)



25

and a fatty acid having the general structure (4)

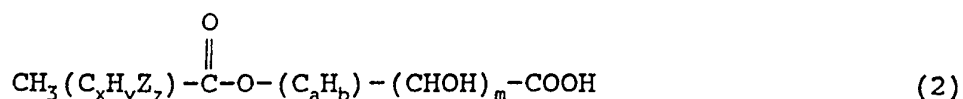
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the ω -hydroxy fatty acid (3) being prepared by a process which includes Kolb  synthesis.

35

The present invention also provides a method of producing a fatty acid/ ω -hydroxy acid component having the general structure (2):



5 where

Z is -OH or an epoxy oxygen

a is an integer of from 7 to 50

b is an integer of from 10 to 100

10 m is 0 or 1

x is an integer of from 12 to 20

y is an integer of from 20 to 40

z is 0 or an integer of from 1 to 4

15 wherein an ω -hydroxy fatty acid having the structure (3):



is linked to a fatty acid having the general structure (4):

20



25 the ω -hydroxy fatty acid (3) being prepared by a process which includes Kolbé synthesis.

Preferably the fermentation of Hansenula ciferrii to produce TAPS is conducted at 30°C.

30

Surprisingly, the inventors have discovered that yields are further increased when the fermentation of Hansenula ciferrii is carried out in the presence of a solvent selected from ethanol, methanol and mixtures thereof. The fermentation is therefore preferably conducted in the presence of such solvents.

35

Additionally yields are noted to be improved when the fermentation of Hansenula ciferrii is carried out in the presence of a surfactant.

5 Examples of suitable surfactants are Tween and Triton. The fermentation is therefore preferably conducted in the presence of such surfactants.

10 Furthermore, yields are noted to be improved when the fermentation of Hansenula ciferrii is carried out in the presence of selected precursors eg palmitic acid, serine and mixtures thereof. The fermentation is therefore preferably conducted in the presence of such precursors.

15 Preferably the non-fermentable carbon source is glycerol. Use of a non-fermentable carbon source improves the biomass titres (g cells/litre fermentation broth) and hence the productivity of the reaction (eg TAPS/litre). TAPS is conveniently extracted from the fermentation products by
20 solvent extraction.

The fermentation process produces a mixture of products, namely phytosphingosines with some sphingosines, of mixed chain lengths. The main product is TAPS, with some
25 triacetylphytosphingosine and also some triacetylsphingosine. The products are a mixture of C₁₆₋₂₀ of odd and even chain length, mainly straight chain but possibly with some branched chain products. The main product is C₁₈ straight chain TAPS.

30 TAPS can be readily converted to phytosphingosine by a suitable deacetylation reaction, as is well known to those skilled in the field, eg by base catalysed hydrolysis for example using potassium hydroxide.

35 The phytosphingosine obtained via fermentation of Hansenula ciferrii is then used for the preparation of

phytosphingosine-containing ceramide one. The use of phytosphingosine obtained by fermentation for this preparation is particularly advantageous to the use of other sources of phytosphingosine (eg chemically synthesised) because the phytosphingosine obtained by fermentation is the D-D-erythro isomer, the same as occurs in human skin. Other methods of preparation of phytosphingosine produce a mixture of stereoisomers which must be separated prior to use. Accordingly use of phytosphingosine obtained by fermentation for the preparation of ceramide one provides a very simple, cost effective method.

Alpha hydroxy fatty acids are also produced in the fermentation reaction. The acids are produced in a range of chain lengths from about C₁₆₋₂₄. The amount of acids produced can be controlled by varying the carbon source: more is produced from glucose than glycerol. The acids can be separated and used in the production of component (2) for production of ceramide one.

The fatty acid/hydroxy fatty acid component of ceramide one has the general structure (2) shown above. Component (2) can be produced by esterifying ω -hydroxy acid with fatty acid or fatty acyl chloride.

Synthesis of ω -hydroxy fatty acid of suitable chain length can be effected, for example, by using Kolbé synthesis to link together the half esters of two dioic acids, to produce the diester of a longer chain dioic acid. After hydrolysis to the half ester the molecule can then be reduced to produce an omega hydroxy long chain fatty acid. The two dioic acids used can be the same or different. For example, two C₁₆ dioic acids can be linked to form a C₃₀ acid, two C₁₂ dioic acids can be linked to form a C₂₂ acid, etc. The reaction scheme is shown in Figure 4.

Alternatively, a dioic acid half ester can be linked to an ω -hydroxy monocarboxylic acid by Kolbé synthesis to produce a longer chain ω -hydroxy acid. For example C_{16} dioic acid half ester and C_{16} omega hydroxy monocarboxylic acid produce
5 C_{30} omega hydroxy acid.

Alternatively two ω -hydroxy fatty acids may be coupled to give a long chain diol, followed by partial oxidation to give a long chain ω -hydroxy fatty acid.
10

The long chain fatty acid/ ω -hydroxy fatty acid component (2) of ceramide 1 can be made in a two stage process, for example by first linking two C_{16} dioic (dicarboxylic) acid half esters followed by reduction to produce a C_{30} omega hydroxy acid and then linking linoleic acid (which is commercially available, eg derived from plant oil such as sunflower oils) by an esterification reaction to produce
15 the long chain acid. The linoleic acid is preferably activated, for example, in the form of an acyl chloride.

20 It will thus be seen that this approach is very versatile and can enable production of a wide range of long chain fatty acid/ ω -hydroxy fatty acid components of general structure (2).

25 Kolbé synthesis reactions are well known to those skilled in the art, and details will not be given here.

Dioic acids are conveniently obtained by biochemical
30 oxidation of monocarboxylic fatty acids, eg using Candida cloacae, for example as disclosed in EP 0 341 796.

The phytosphingosine base and fatty acid component/hydroxy fatty acid (2) can be readily linked by chemical or enzymic
35 routes, eg using conventional N acylation reactions.

For example, a simple amidation reaction can be carried out, possibly acid or base catalysed or possibly non-catalysed after carboxyl group activation. For instance, the fatty acid component can be converted to the methyl ester and reacted, under vacuum with heating, with the phytosphingosine base and sodium methoxide catalyst. This reaction is found to be simple, straightforward and effective, giving a good yield of product, and furthermore does not require the presence of a solvent.

Component (2) may alternatively be reacted in the form of the acid chloride or using an activating reagent such as 2-chloromethyl pyridium iodide.

Enzymic routes are also possible.

The order of reaction steps is not critical. For example a component (2) may be synthesised from shorter components, as described above, and then coupled to the phytosphingosine base. Alternatively, a ω -hydroxy fatty acid component may be linked to the phytosphingosine base prior to esterification of the ω -hydroxyl group.

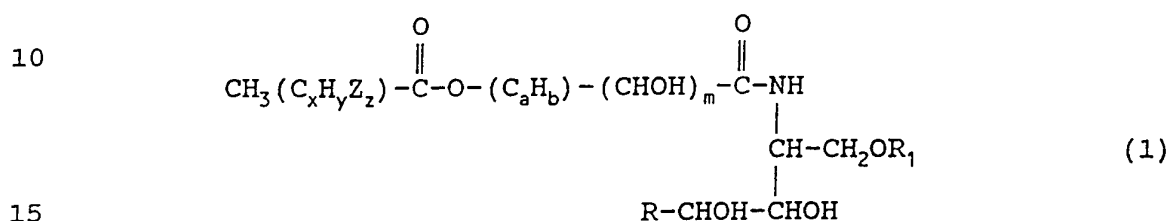
It will be apparent that the invention provides the potential for production of a wide range of phytosphingosine-containing ceramide one structures, both identical to those found in nature and of novel structure.

In a further aspect, the present invention provides a phytosphingosine-containing ceramide one produced by the method of the invention, and derivatives thereof.

The phytosphingosine-containing ceramide one structures find particular application in the treatment of skin, hair and nails.

In a further aspect, the invention thus provides a cosmetic composition suitable for topical application to skin, hair or nails comprising:

5 (i) an effective amount of from 0.00001 to 50% by weight of a phytosphingosine-containing ceramide one having the general structure (1):



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

R₁ represents H, a phosphate residue, a sulphate residue or a sugar residue

Z is -OH or an epoxy oxygen

```
25     a is an integer of from 7 to 50
```

b is an integer of from 10 to 100

m is 0 or 1

x is an integer of from 12 to 20

y is an integer of from 20 to 40

```
30      z is 0 or an integer of from 1 to 4
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wherein the phytoshingosine-containing ceramide one is synthesised according to the invention; and

35 (ii) a cosmetically acceptable vehicle for the
phytosphingosine-containing ceramide one.

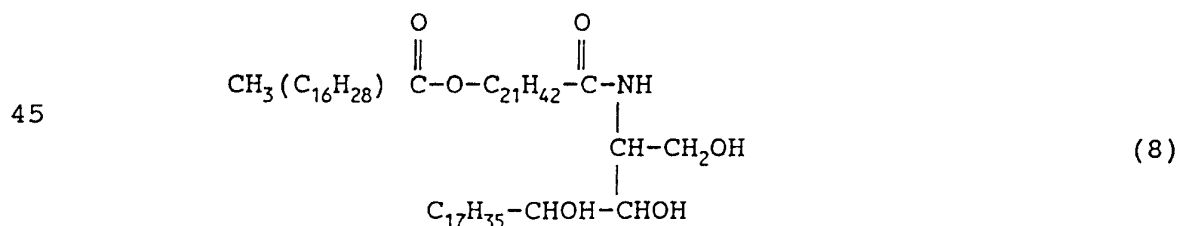
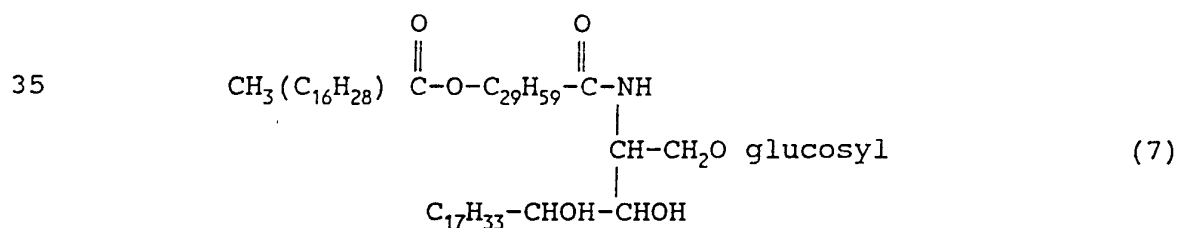
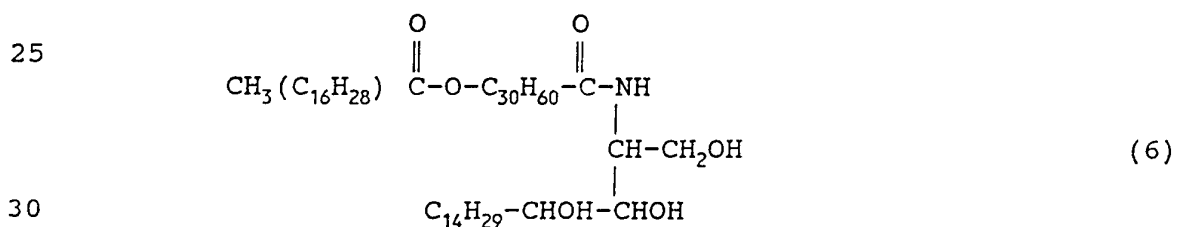
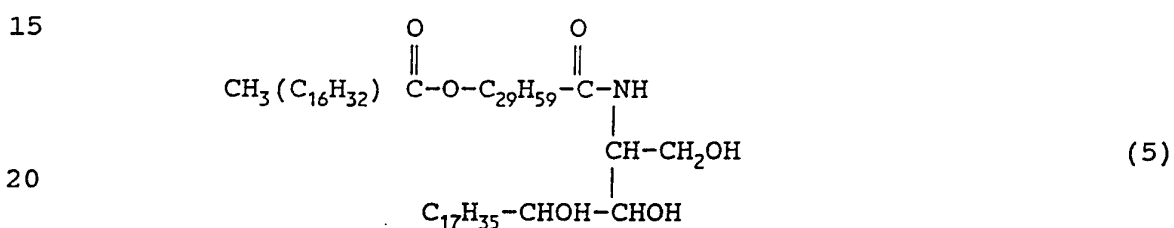
With reference to structure (1) the group R preferably represents an aliphatic hydrocarbon group having from 12 to

22 carbon atoms.

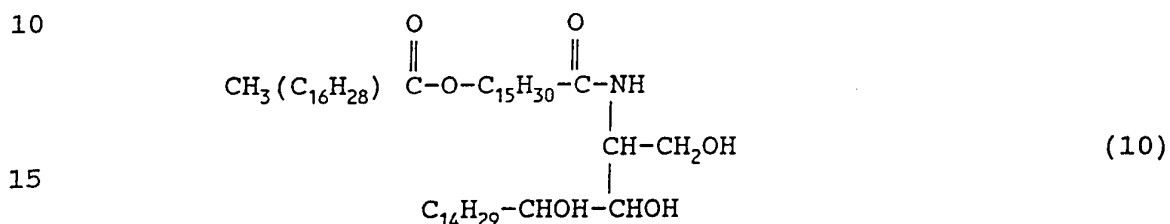
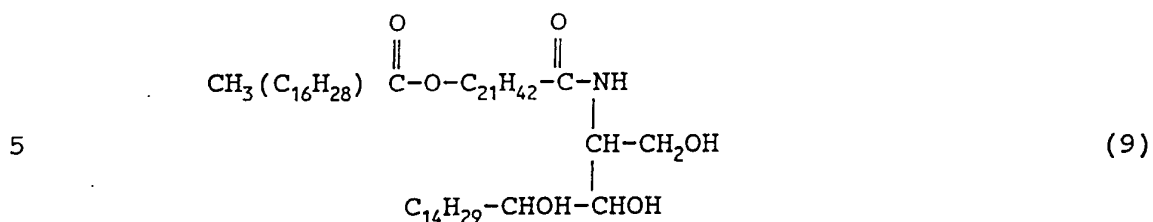
With reference to structure (1), the value of "a" is preferably an integer of from 24 to 30 and the value of "b" is preferably an integer of from 44 to 60.

Structure (4) preferably represents a straight chain saturated C₁₆₋₁₈, fatty acid residue or a straight chain all cis n-6,9 di-unsaturated C₁₆₋₁₈ fatty acid residue.

Specific examples of these phytosphingosine-containing ceramide one structures are those having the structures (5) to (10):



12



The amount of the phytosphingosine-containing ceramide one present in the composition according to the invention is from 0.00001 to 50%, preferably from 0.001 to 20% and most preferably from 0.1 to 10% by weight.

The 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 phytosphingosine-containing ceramide one, so as to facilitate its distribution when the composition is applied to the skin and/or hair.

Vehicles other than water can include liquid or solid emollients, solvents, humectants, thickeners and powders. 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
5 seed oil, tallow, lard, olive oil, passion flower 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,
10 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,
15 dimethyl ether, carbon dioxide, nitrous oxide;

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

Powders, such as chalk, talc, fullers earth, kaolin,
starch, gums, colloidal silica sodium polyacrylate, tetra
25 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.

30 The cosmetically acceptable 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 cosmetic
adjuncts, form the balance of the composition.

OPTIONAL SKIN BENEFIT MATERIALS AND COSMETIC ADJUNCTS

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 hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

Oil or oily material

The composition 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.

Emulsifier

The composition according to the invention can also optionally comprise one or more emulsifiers the choice of which will normally determine whether a water-in-oil or and 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 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

10	Chemical Name of Emulsifier	Trade Name	HLB Value
	Sorbitan trioleate	Arlacel 85	1.8
	Sorbitan tristearate	Span 65	2.1
15	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
20	Sorbitan monostearate	Arlacel 60	4.7
	Poloxyethylene (2) stearyl ether	Brij 72	4.9
	Poloxyethylene sorbitol beeswax derivative	G-1702	5
25	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
30	Sorbitan monolaurate	Arlacel 200	8.6
	PEG 400 dioleate	Tegester PEG 400-DO	8.8
	Polyoxyethylene (5) monostearate	Ethofat 60-16	9.0
35	Polyoxyethylene (4) sorbitan monostearate	Tween 61	9.6

	Polyoxyethylene (4) lauryl ether	Brij 30	9.7
	Polyoxyethylene (5) sorbitan monooleate	Tween 81	10.0
5	PEG 300 monooleate	Neutronyx 834	10.4
	Polyoxyethylene (20) sorbitan tristearate	Tween 65	10.5
	Polyoxyethylene (20) sorbitan trioleate	Tween 85	11.0
10	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
15	Polyoxyethylene (10) stearyl ether	Brij 76	12.4
	Polyoxyethylene (10) cetyl ether	Brij 56	12.9
20	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
25	PEG 1000 dilaurate	Kessco	13.9
	Polyoxyethylene sorbitol lanolin derivative	G-1441	14.0
	Polyoxyethylene (12) lauryl ether	Ethosperse LA-12	14.4
30	PEG 1500 dioleate	Pegosperse 1500	14.6
	Polyoxyethylene (14) laurate	Arosurf HFL-714	14.8
	Polyoxyethylene (20) sorbitan monostearate	Tween	14.9
35	Polyoxyethylene 20 sorbitan monooleate	Tween 80	15.0

	Polyoxyethylene (20)		
	stearyl ether	Brij 78	15.3
	Polyoxyethylene (20)		
	sorbitan monopalmitate	Tween 40	15.6
5	Polyoxyethylene (20) cetyl		
	ether	Brij 58	15.7
	Polyoxyethylene (25)		
	oxypropylene	G-2162	16.0
	monostearate		
10	Polyoxyethylene (20)		
	sorbitol monolaurate	Tween 20	16.7
	Polyoxyethylene (23)		
	lauryl ether	Brij 35	16.9
	Polyoxyethylene (50)		
15	monostearate	Myrj 53	17.9
	PEG 4000 monostearate	Pegosperse 4000	
		MS	18.7

20 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.

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

30 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.

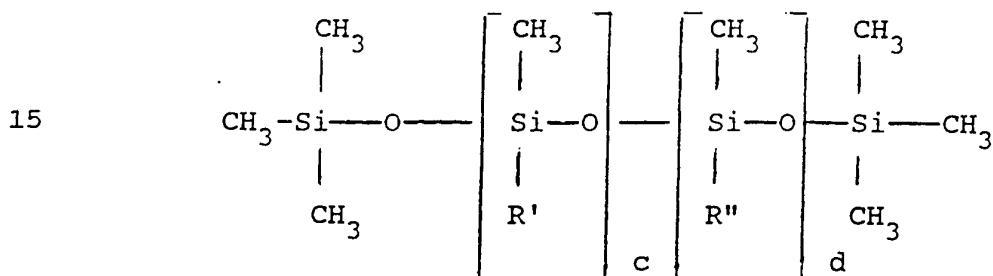
Water

35 The composition of the invention can also comprise water, usually up to 98%, preferably from 5 to 80% by volume.

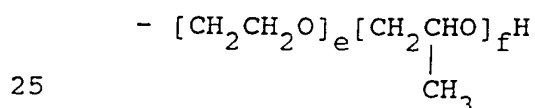
Silicone Surfactant

The composition of the invention can also optionally comprise a high molecular weight silicone surfactant which can also act as an emulsifier, in place of or in addition to the optional emulsifier(s) already mentioned.

The silicone surfactant is a high molecular weight polymer of dimethyl polysiloxane with polyoxyethylene and/or polyoxypropylene side chains having a molecular weight of from 10,000 to 50,000 and having the structure:



where the groups R' and R'' are each chosen from -H, C₁₋₁₈ alkyl and



e has a value of from 9 to 115,

f has a value of from 0 to 50,

c has a value of from 133 to 673,

d has a value of from 25 to 0.25.

Preferably, the dimethyl polysiloxane polymer is one in which:

e has a value of from 10 to 114

f has a value of from 0 to 49

c has a value of from 388 to 402

d has a value of from 15 to 0.75

one of groups R' and R" being lauryl, and the other having a molecular weight of from 1000 to 5000.

5 A particularly preferred dimethyl polysiloxane polymer is one in which:

e has the value 14

f has the value 13

c has the value 249

10 d has the value 1.25

15 The dimethyl polysiloxane polymer is conveniently provided as a dispersion in a volatile siloxane, the dispersion comprising, for example, from 1 to 20% by volume of the polymer and from 80 to 99% by volume of the volatile siloxane. Ideally, the dispersion consists of a 10% by volume of the polymer dispersed in the volatile siloxane.

20 Examples of the volatile siloxanes in which the polysiloxane polymer can be dispersed include polydimethyl siloxane (pentamer and/or hexamer).

25 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.

30 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.

Other Cosmetic Adjuncts

35 Examples of conventional adjuncts which can optionally be employed 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
5 hydroxide; surfactants, such as glycerol ethers and other ceramides of synthetic, animal or plant origin; phospholipids; waxes, such as beeswax, ozokerite wax, paraffin wax, plant extracts, such as Aloe vera, cornflower, witch hazel, elderflower, cucumber; thickeners;
10 activity enhancers; colourants; perfumes; and sunscreen materials such as ultrafine titanium dioxide and organic sunscreens such as p-aminobenzoic acid and esters thereof, ethylhexyl p-methoxycinnamate, 2-ethoxyethyl p-methoxycinnamate and butyl methoxydibenzoylmethane, and
15 mixtures thereof.

In a further preferred composition, the phytosphingosine-containing ceramide one is combined with conventional ceramides, pseudoceramides, polyol fatty acid polyesters,
20 sterols particularly cholesterol, galactosyldiacylglycerols, glycosphingolipids, fatty acids and esters thereof, triglycerides, cerebroside, phospholipid and other ingredients well known to those skilled in the art to produce a liposomal dispersion or bilayer structure.

25 A preferred composition may also contain, in combination with the phytosphingosine containing ceramide one and optional additional ingredients disclosed above, an organic acid component chosen from hydroxy carboxylic acids, keto
30 carboxylic acids, esters thereof and mixtures thereof.

In yet another preferred composition, the phytosphingosine-containing ceramide one is dissolved in squalene or squalane, optionally together with conventional ceramides,
35 and formulated with volatile and non-volatile silicones to produce an anhydrous or nearly anhydrous single phase system.

Cosmetic adjuncts can form the balance of the composition.

Use of the Composition

5 The composition according to the invention is intended primarily as a product for topical application to human skin, especially as an agent for reducing the permeability to water of the skin, particularly when the skin is dry or damaged, in order to reduce moisture loss and generally to
10 enhance the quality and flexibility of skin. The composition can also be applied to hair and nails.

15 The composition may therefore be used as a product for topical application to human skin to treat dry, detergent-damaged or ageing skin.

20 In use, a small quantity of the composition, for example from 1 to 5ml, 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

25 The topical skin, hair or nail treatment composition of the invention can 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
30 composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer.

35 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.

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

EXAMPLES

10 The invention will be further described by way of illustration, in the following examples and by reference to the accompanying drawings in which:

15 Figure 1 is a flow chart illustrating the isolation and purification of TAPS;

Figure 2 and 3 are graphs of TAPS production versus increasing cell mass in a fermenter showing that TAPS formation is growth-associated, ie the amount of TAPS formed is directly proportional to the amount of cells grown;

20

Figure 4 illustrates the half ester route for production of component 2; and

25 Figure 5 illustrates schematically production of ceramide 1 by the method of the invention.

Example 1

30 Synthesis of C₂₂ hydroxy fatty acid from dodecanedioic acid

A solution of dodecanedioic acid (100g) in methanol (400ml) was reacted at 70°C with a solution of borontrifluoride in methanol (14%, 70ml). After 1 hour excess methanol was removed and the dioic diester product recrystallised from petroleum ether (60-80°) (200ml). The product was more than 99% pure in a yield of 95%.

35

Diester (100g) was hydrolysed with half mole equivalent of KOH (400 ml, 90:5 methanol:water) at 70°C for 1 hour. The reaction mix was subsequently neutralised with 1N HCl and then the aqueous phase removed by filtration. The reaction product consisting of monoester (1/2 acid ester), diester and diacid was dissolved in 60°-80° petroleum ether. Diacid was found to be barely soluble and was removed by filtration. The half acid ester was recrystallised twice as it was only 85% pure from one recrystallisation. The yield of half acid ester recovered pure was 52%. The reaction product was analysed by GC and IR spectroscopy. Remaining diacid, diester and impure half acid ester was recycled to give an overall yield after one recycle of 67%.

With pure half acid ester available it was possible to effect a Kolbé electrolysis to synthesise the C₂₂ dimethyl ester.

A solution of dodecanedioic acid half ester (10g in 80 ml methanol) was partially neutralised with 0.1 equivalent of KOH in water (8 ml). The resulting solution was electrolysed at 120 volts and an initial current of 0.9 amps between 2 platinum electrodes 4cm x 0.5cm x 0.1mm. The electrodes were set 2-3 mm apart and were constructed with a platinum/wire junction sealed in glass to isolate it from the reaction medium. On cooling to 0°C product was found to precipitate. Reaction was carried out for 4-6 hours. The collected product was recrystallised from petroleum ether 60°-80°. The final yield of C₂₂ diester was 5 g (yield 60%, purity 80%) in this case.

With the long chain ester available the next step is selectively to reduce one end of the molecule. This was achieved by partial hydrolysis as before, followed by reduction of the ester moiety using lithium borohydride.

C₂₂ diester (1g) was initially reacted with a half mole

equivalent of KOH in methanol/water 90:10 (50ml) at 70°C. The half acid ester was purified as before. Yield of isolated half acid ester was 300mg (80% pure, total yield 31%).

5

The half ester obtained was dissolved in dry diethyl ether (5ml) and reacted with 1 mole equivalent of lithium borohydride in dry diethyl ether (5ml, 10% w/v). After 24 hours, reaction was stopped by addition of 1N HCl (2ml).

10

Diethyl ether was removed and the product washed with water and dried under vacuum. The crude mix was dissolved in ethanol (5ml, 60°C) and water added until turbidity was present. The C₂₂ product crystallised out and was filtered

15

off. GCMS of this product showed that it was predominantly omega-hydroxy C₂₂ acid. Purity by GC was 74%-84%. C¹³ and H¹ NMR also confirmed the authenticity of the product.

Example 2

20

Synthesis of Phytosphingosine-containing ceramide one

Preparation of Phytosphingosine

The yeast Hansenula (Pichia) ciferrii (mating type F-60-10) obtained from the Northern Regional Research Labs, Peoria, Illinois, USA was grown up under suitable conditions. A 1% v/v inoculum was added to the fermenter (working volume 3.5l) containing a growth medium comprising:

30

Glycerol, 30 gl⁻¹; KH₂PO₄, 6.4 gl⁻¹; (NH₄)₂HPO₄, 4 gl⁻¹; and Na₂SO₄, 1.5 gl⁻¹; together with the trace nutrients pantothenate, 6 mg l⁻¹; thiamine, 8mg l⁻¹; nicotinic acid 30 mg l⁻¹; pyridoxine, 20 mg l⁻¹ and biotin, 100 µg l⁻¹. In

35

addition some yeast extract was required, particularly to maintain cell growth during extended fermenter runs. Typically 3 gl⁻¹ yeast extract was added.

Fermentation was carried out at 30°C with 0.5 v/v/min air supplied. The fermentation was characterised by the production of copious foam which was combatted by the addition of antifoam and/or by the use of a lower fermenter working volume. The course of the fermentation was continuously monitored by gas analysis, and also by glc analysis of timed samples for remaining glycerol and tetraacetylphytosphingosine (TAPS) and other metabolites produced. Additional glycerol was added at intervals, as indicated by the gas analyses, so as to maintain growth and produce as high biomass concentration as possible (greater than or equal to 50-55 gdw l⁻¹).

Once these high biomass concentrations were reached the broth was centrifuged at 3500 rpm at 10°C or 20°C, and the cell pellet freeze-dried so as to facilitate solvent extraction.

Isolation and Purification of TAPS

The freeze-dried cells were ground with MeOH/EtOAc (1/1, v/v) at 60°C and then filtered, and this extraction and filtration repeated. (85% of the TAPS was recovered in the first extraction.) The combined filtrates were evaporated under reduced pressure to produce a crude solid extract (30g from 174gd cells, equivalent to 690 gw cells). This solid extract was redissolved in hot EtOAc (0.5 l) and partitioned with 0.5 l of water, so as to remove water-soluble impurities; these include a sugar, probably xylitol, in significant amounts. The EtOAc phase was evaporated to dryness to produce 16.9g of lipid extract containing alpha-HPA, cholesterol and ergosterol as metabolic side-products, plus large amounts of silicone antifoam carried over from the fermentation.

Optionally the fermentation supernatant (2.69 l) could also be extracted with EtOAc (2.5 l) to produce 1.49g of lipid

extract with a similar TAPS composition to that of the cell extract.

5 These extracts could be combined and dissolved in 75ml of warm light petroleum (60-80°C) and chromatographed on a column of 100g of 70-230 mesh silica gel. Elution with more petroleum (400ml) eluted the antifoam. Elution with light petroleum - 30% diethyl ether (800ml) removed the alpha-HPA and sterols, and then finally light petroleum - 10 60% diethyl ether (1.2 l) eluted with the TAPS in 94% yield and over 95% purity. Phytosphingosines with varying side chains, some triacetylsphingosine and also some triacetylphytosphingosine were also present.

15 The isolation and purification of TAPS is illustrated in Figure 1. TAPS production is illustrated graphically in Figures 2 and 3 including results for glycerol and glucose carbon sources. Figure 3 gives results using glycerol only, and shows that the amount of TAPS formed is directly 20 proportional to the amount of cells grown.

The process described is much more efficient than the prior art process. Results are shown in Tables 2 and 3.

Table 2Comparison of Prior Art (Best-Case) & Present TAPS
Fermentations

5		PRESENT INVENTION		
		PRIOR ART	BATCH	FED-BATCH
10	Yield of TAPS from glucose/glycerol (mg/g)	5	20	22
	Yield of TAPS on biomass (mg/gdw)	15	40	44
15	Max broth concentration (mg l ⁻¹)	307	600	2700
20	Volumetric productivity (mg TAPS l ⁻¹ h ⁻¹)	3.2*	13.3	22.5

* Excludes 65h post-fermentation incubation required to facilitate extraction of TAPS, otherwise 2.25 mg l⁻¹h⁻¹.

Table 3

TAPS Fermentation Productivities

CARBON SOURCE	TEMPERATURE (°C)	BATCH VS FED-BATCH	TAPS TITRE (mg l ⁻¹)	BIOMASS CONC (gdw l ⁻¹)	SPECIFIC YIELD OF TAPS (mg/gdw cells)
Glucose	25	Batch	30	6-8	6.5
Glucose (pure strain of H ciferrii used)	25	Batch	100	8.3	12
Glycerol	25	Batch	200-250	12	17-21
Glycerol	30	Batch	1,000	22	59
Glycerol	30	Fed-Batch	2,700	50-55	45-55
Glycerol plus methanol	30	Batch	720	12	60
Glycerol plus methanol	30	Fed-Batch	2083	34	61
Glycerol, methanol 0.2% w/v palmitic acid 0.2% w/v serine	30	Batch	1207	14	86

Deacetylation

0.9g TAPS was refluxed with 1g KOH in ethanol/water (9/L
v/v) (20ml) for 5h. The solvent was removed under vacuum
5 and the phytosphingosine produced extracted into diethyl
ether Glc and nmr analysis showed that the phytosphingosine
was obtained in 95% purity at a yield of 81%.

Coupling Phytosphingosine to Component (2)

10

The phytosphingosine as produced above was coupled to a
fatty acid/ ω -hydroxy fatty acid component (2). The
component (2) was synthesised via ester formation. A broad
range of types of component (2) are possible, long chain
15 hydroxy acids being obtained as in Example 1. For ease of
analysis of the final phytosphingosine-containing ceramide
1 product, in this example we synthesised a ceramide where
component (2) was produced from decanoyl chloride and 12-
hydroxy dodecanoic acid to give the $C_{10}C_{12}$ acid ester.

20

12-hydroxydodecanoic acid (2.5g) was dissolved in petrol
60-80° (25ml) and to this solution was added decanoyl
chloride (2.2g) and pyridine (1ml). After 3 hours the
reaction was stopped. The mixture was run through basic
25 alumina II (45g) which removed any shorter chain reactants
remaining. The alumina was washed with diethyl ether.
1.5g of product (99% by GC) was recovered (yield 35%).
Confirmation of structure was by GCMS which was entirely
consistent with the expected ion fragmentation pattern for
30 the $C_{10}C_{12}$ ester.

The $C_{10}C_{12}$ acid ester (5mg) was dissolved in dichloromethane
(0.5ml) together with phytosphingosine (4mg) and 2-
chloromethyl pyridium iodide (4mg). To this mixture was
35 added 5 μ l triethylamine. The reaction was left at RT for
1 hour then washed 3 times with water (5ml). The
dichloromethane layer was dried over 4A molecular sieve and

evaporated to dryness. The residue 9.7mg of tan white powder was analyzed by GCMS. Yield 115%, purity 85% by GC. The major product was the C₁₀C₁₂ ceramide 1 as evidenced by GCMS ion fragmentation patterns.

5

Example 3

This example illustrates a high internal phase water-in-oil emulsion in accordance with the invention.

10

A high internal phase water-in-oil emulsion having the following formulation was prepared:

	<u>% w/w</u>
15 Fully hydrogenated coconut oil	3.9
Phytosphingosine-containing ceramide one	
having the structure (5)	0.1
Brij 92*	5
20 Bentone 38	0.5
Preservative	0.3
MgSO ₄ ·7H ₂ O	0.3
Butylated hydroxy toluene	0.01
Perfume	qs
25 Water	to 100

*Brij 92 is polyoxyethylene (2) oleyl ether

Example 4

This example also illustrates a high internal phase water-in-oil emulsion in accordance with the invention in which
 5 the formulation of Example 3 was prepared but with the following changes:

- i. liquid paraffin replaced the fully hydrogenated coconut oil, and
 10
- ii. the phytosphingosine-containing ceramide one had the structure (6).

Example 5

15

This example illustrates an oil-in-water cream emulsion having the following formulation:

	<u>% w/w</u>
20 Mineral oil	4
Phytosphingosine-containing ceramide one having the structure (7)	0.1
Brij 56*	4
25 Alfol 16RD*	4
Triethanolamine	0.75
Butane-1,3-diol	3
Xanthan gum	0.3
Preservative	0.4
30 Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100
*Brij 56 is cetyl alcohol POE (10)	
Alfol 16RD is cetyl alcohol	

35

Example 6

This example illustrates an alcoholic lotion. The lotion had the following formulation:

5

	<u>% w/w</u>
Phytosphingosine-containing ceramide one having the structure (8)	0.2
10 Ethanol	40
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

15

Example 7

This example illustrates an alcoholic lotion. The lotion had the following formulation:

20

	<u>% w/w</u>
Phytosphingosine-containing ceramide one having the structure (9)	0.2
Dimethylsulphoxide	10
25 Ethanol	40
Antioxidant	0.1
Perfume	qs
Water	to 100

Example 8

The following composition according to the invention represent a lotion which can be used in the treatment of dry or ageing skin:

		<u>% w/w</u>
	Phytosphingosine-containing ceramide one	
10	having the structure (10)	1.0
	Sphingosine-containing ceramide	0.5
	Perfume	0.1
	Hydroxyethyl cellulose	0.4
	Absolute ethanol	25
15	p-methyl benzoate	0.2
	Sterilised demineralised water	to 100

Example 9

The following compositions according to the invention represent lotions which can be used in the treatment of dry or ageing skin:

		<u>% w/w</u>
25	The phytosphingosine-containing ceramide one	
	having the structure (5)	0.08
	Pseudo ceramide	0.15
	Ethanol	10
30	Perfume	0.5
	Distilled water	to 100

Example 10

This example illustrates a high internal phase water-in-oil emulsion in accordance with the invention.

5

A high internal phase water-in-oil emulsion having the following formulation was prepared:

	<u>% w/w</u>
10 Fully hydrogenated coconut oil	3.9
Phytosphingosine-containing ceramide one having the structure (6)	0.1
Brij 92*	5
15 Bentone 38	0.5
Preservative	0.3
MgSO ₄ ·7H ₂ O	0.3
Butylated hydroxy toluene	0.01
Perfume	qs
20 Water	to 100

*Brij 92 is polyoxyethylene (2) oleyl ether

Example 11

25

This example also illustrates a high internal phase water-in-oil emulsion in accordance with the invention in which the formulation of Example 3 was prepared but with the following changes:

30

- i. liquid paraffin replaced the fully hydrogenated coconut oil, and
- ii. the phytosphingosine-containing ceramide one had

35

the structure (6).

Example 12

This example illustrates an oil-in-water cream emulsion having the following formulation:

5		<u>% w/w</u>
	Mineral oil	4
10	Phytosphingosine-containing ceramide one having the structure (7)	0.05
	Phytosphingosine-containing ceramide one having the structure (8)	0.05
	Brij 56*	4
	Alfol 16RD*	4
15	Triethanolamine	0.75
	Butane-1,3-diol	3
	Xanthan gum	0.3
	Preservative	0.4
	Perfume	qs
20	Butylated hydroxy toluene	0.01
	Water	to 100

*Brij 56 is cetyl alcohol POE (10)

Alfol 16RD is cetyl alcohol

Example 13

This example illustrates an alcoholic lotion containing a phytosphingosine-containing ceramide one of the invention.

5

The lotion had the following formulation:

	<u>% w/w</u>
10 Phytosphingosine-containing ceramide one having the structure (9)	0.2
Ethanol	40
Perfume	qs
Butylated hydroxy toluene	0.01
15 Water	to 100

Example 14

This example illustrates an alcoholic lotion which is suitable for application to nails.

20

The lotion had the following formulation:

	<u>% w/w</u>
25 Phytosphingosine-containing ceramide one having the structure (10)	0.2
Dimethylsulphoxide	10
Ethanol	40
30 Antioxidant	0.1
Perfume	qs
Water	to 100

Example 15

The following composition according to the invention represent a lotion which can be used in the treatment of dry, unmanageable hair.

		<u>% w/w</u>
	Phytosphingosine-containing ceramide one	
10	having the structure (9)	1.0
	Pseudoceramide	0.5
	Perfume	0.1
	Hydroxyethyl cellulose	0.4
	Absolute ethanol	25
15	p-methyl benzoate	0.2
	Sterilised demineralised water	to 100

Example 16

The following compositions according to the invention represent lotions which can be used in the treatment of dry skin, hair or nails:

		<u>% w/w</u>
25	The phytosphingosine-containing ceramide one	
	having the structure (10)	0.08
	Ethanol	10
	Perfume	0.5
30	Distilled water	to 100

Example 17; Comparative Example AIn vitro efficacy studies - water vapour transmission rate (WVTR)

5

C₃₀ linoleic phytosphingosine-containing ceramide 1 (structure 5) was prepared according to the invention. The reduction in water permeability through "Acetate Plus" membranes (from Micron Separation Inc, having 25mm diameter and 5µm pore size) following topical application of a composition comprising C₃₀ linoleic phytosphingosine-containing ceramide was determined by in vitro measurement of the water vapour transmission rate (WVTR) using a similar system to that described by Blank et al (J Invest Dermatol 18 (1952) 433-440.

15

The C₃₀ linoleic phytosphingosine-containing ceramide was formulated in an oil in water emulsion containing cholesterol, stearic acid and sodium stearate (1:2:0.7:0.3 wt%) together with glycerol (1%) (Example 17) and compared with an identical emulsion except C₃₀ linoleoyl phytosphingosine-containing ceramide 1 was replaced by cholesterol (ie cholesterol:stearic acid and sodium stearate 3:0.7:0.3 wt% plus glycerol 1%) (comparative Example A).

25

Approximately 15 mgs of each aqueous formulation was applied to the membrane and allowed to dry. The membranes are weighed, and amounts of applied formulations adjusted accordingly, to ensure comparable amounts of material are applied to each membrane. Experiments are performed in triplicate.

30

Membranes with and without product applied are then applied to the WVTR cells with water in the wells of the cells, weighed and placed in a desiccator for 20 hours. The rate and amount of water loss is then determined from the change

35

in weight of the diffusion cells. Barrier efficiency is demonstrated by the difference in weight loss of the treated and nontreated membranes represented as a percentage improvement in barrier efficiency.

5

Results are shown in Table 4.

Table 4

10

Example	% improvement in barrier function
17	$9.74 \pm 0.31^*$
A	6.50 ± 0.24

* $P < 0.05$

5



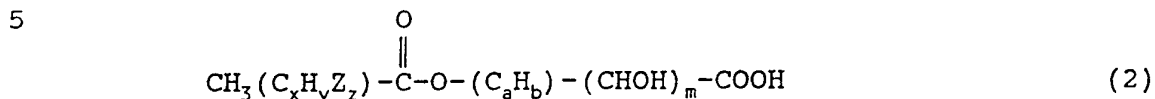
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30

(a) obtaining a phytoshingosine base from TAPS by a deacetylation reaction wherein the TAPS is produced by fermentation of cells of the F-60-10 mating type strain of Hansenula ciferrii using a fed-batch mode and a non-fermentable carbon source; and

(b) coupling together the phytosphingosine base and a fatty acid/ ω -hydroxy fatty acid component having the general structure (2) via an amide linkage:



where

10

Z is -OH or an epoxy oxygen

a is an integer of from 7 to 50

b is an integer of from 10 to 100

m is 0 or 1

15

x is an integer of from 12 to 20

y is an integer of from 20 to 40

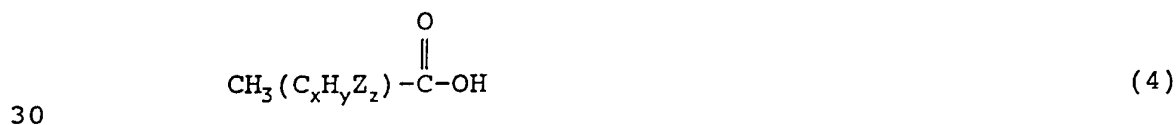
z is 0 or an integer of from 1 to 4

wherein the fatty acid/hydroxy acid component having the general structure (2) is prepared by linking together an ω -hydroxy fatty acid having the structure (3):

20



25 and a fatty acid having the general structure (4)



30

the ω -hydroxy fatty acid (3) being prepared by a process which includes Kolb  synthesis.

35 2. A method according to claim 1 wherein the fermentation of cells of the F-60-10 mating type strain of Hansenula ciferrii (step (a)) is carried out in the presence of a solvent chosen from ethanol, methanol and mixtures thereof.

3. A method according to claim 1 or claim 2 wherein the fermentation of cells of the F-60-10 mating type strain of Hansenula ciferrii is carried out in the presence of a surfactant.

5

4. A method according to any preceding claim wherein the of cells of the F-60-10 mating type strain of Hansenula ciferrii is carried using the non-fermentable carbon source glycerol.

10

5. A method according to any preceding claim wherein the fermentation of cells of the F-60-10 mating type strain of Hansenula ciferrii is carried out in the presence of a precursor selected from palmitic acid, serine and mixtures thereof.

15

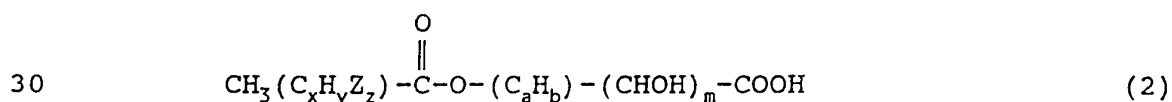
6. A method according to any preceding claim wherein the fermentation of cells of the F-60-10 mating type strain of Hansenula ciferrii is carried out at 30°C.

20

7. A method according to any preceding claim wherein the phytosphingosine base and component (2) are linked by chemical or enzymatic routes.

25

8. A method of producing a fatty acid/ ω -hydroxy fatty acid component having the general structure (2):



30

where

Z is -OH or an epoxy oxygen

35

a is an integer of from 7 to 50

b is an integer of from 10 to 100

m is 0 or 1

x is an integer of from 12 to 20

y is an integer of from 20 to 40

z is 0 or an integer of from 1 to 4

wherein an ω -hydroxy fatty acid having the structure (3):

5



is linked to a fatty acid having the general structure (4):

10



the ω -hydroxy fatty acid (3) being prepared by a process which includes Kolbé synthesis.

15

9. A cosmetic composition suitable for topical application to skin, hair or nails comprising:

20 (i) an effective amount of from 0.00001 to 50% by weight of a phytosphingosine-containing ceramide one produced by the method according to any one of claims 1 to 7; and

25 (ii) a cosmetically acceptable vehicle for the phytosphingosine-containing ceramide one.

10. A composition according to claim 9 in which the phytosphingosine-containing ceramide one forms from 0.01 to 20% by weight of the composition.

30

11. A composition according to claims 9 or 10 which further comprises a surfactant.

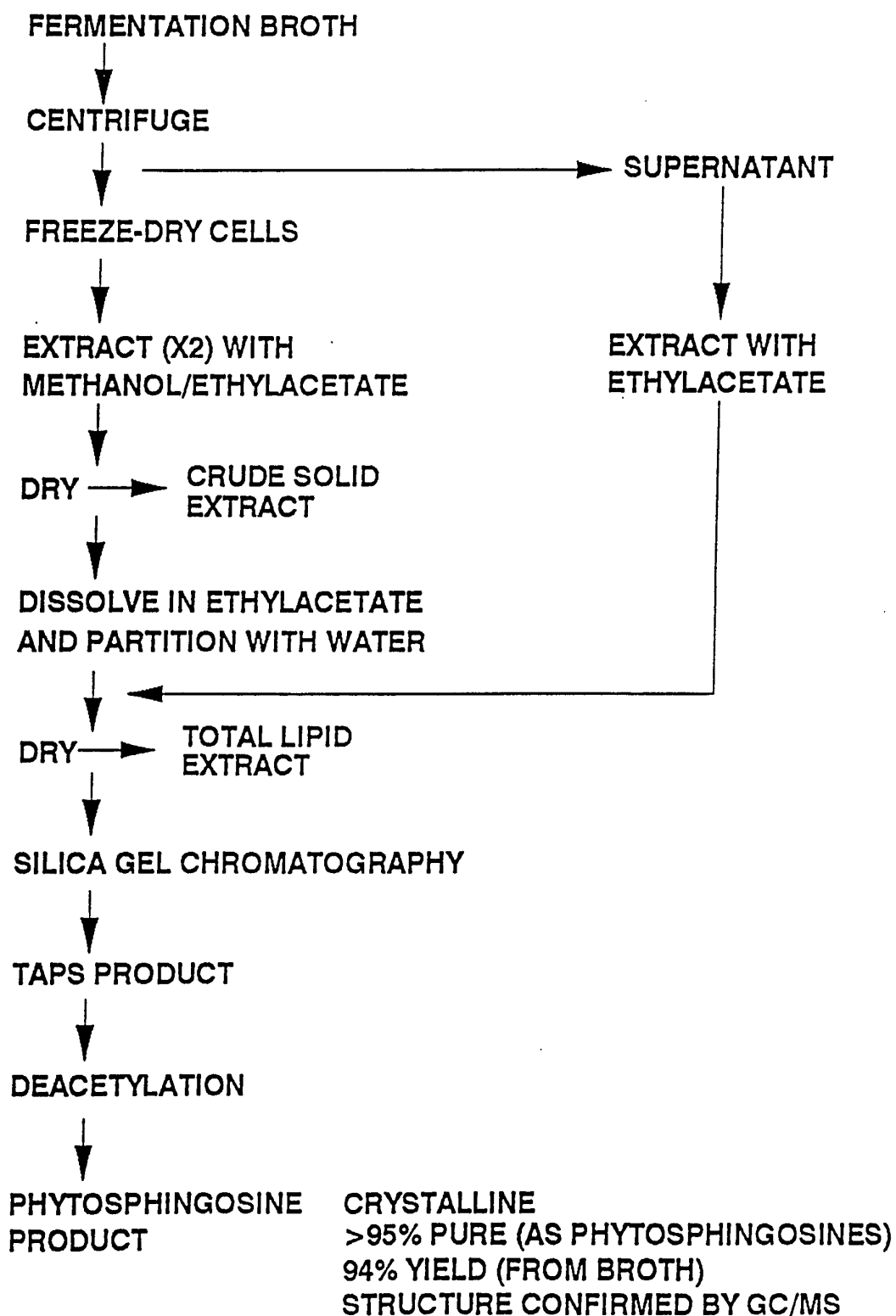
35 12. A composition according to claim 11 in which the surfactant is a glycerol ether.

13. A composition according to any one of claims 9 to 12 which further comprises a sphingosine containing ceramide.

14. A composition according to any one of claims 9 to 13 which further comprises a pseudoceramide.
- 5 15. A composition according to any one of claims 9 to 14 which includes a liposomal dispersion comprising the phytosphingosine-containing ceramide one.
- 10 16. A composition according to any one of claims 9 to 15 which further comprises a solvent for the phytosphingosine-containing ceramide one chosen from squalene, squalane and mixtures thereof.
- 15 17. A composition according to any one of claims 9 to 16 which further comprises a silicone chosen from volatile silicones, non-volatile silicones and mixtures thereof.
18. The use of a composition according to any one of claims 9 to 17 in the topical treatment of dry, ageing or detergent-damaged human skin or hair.

1/5

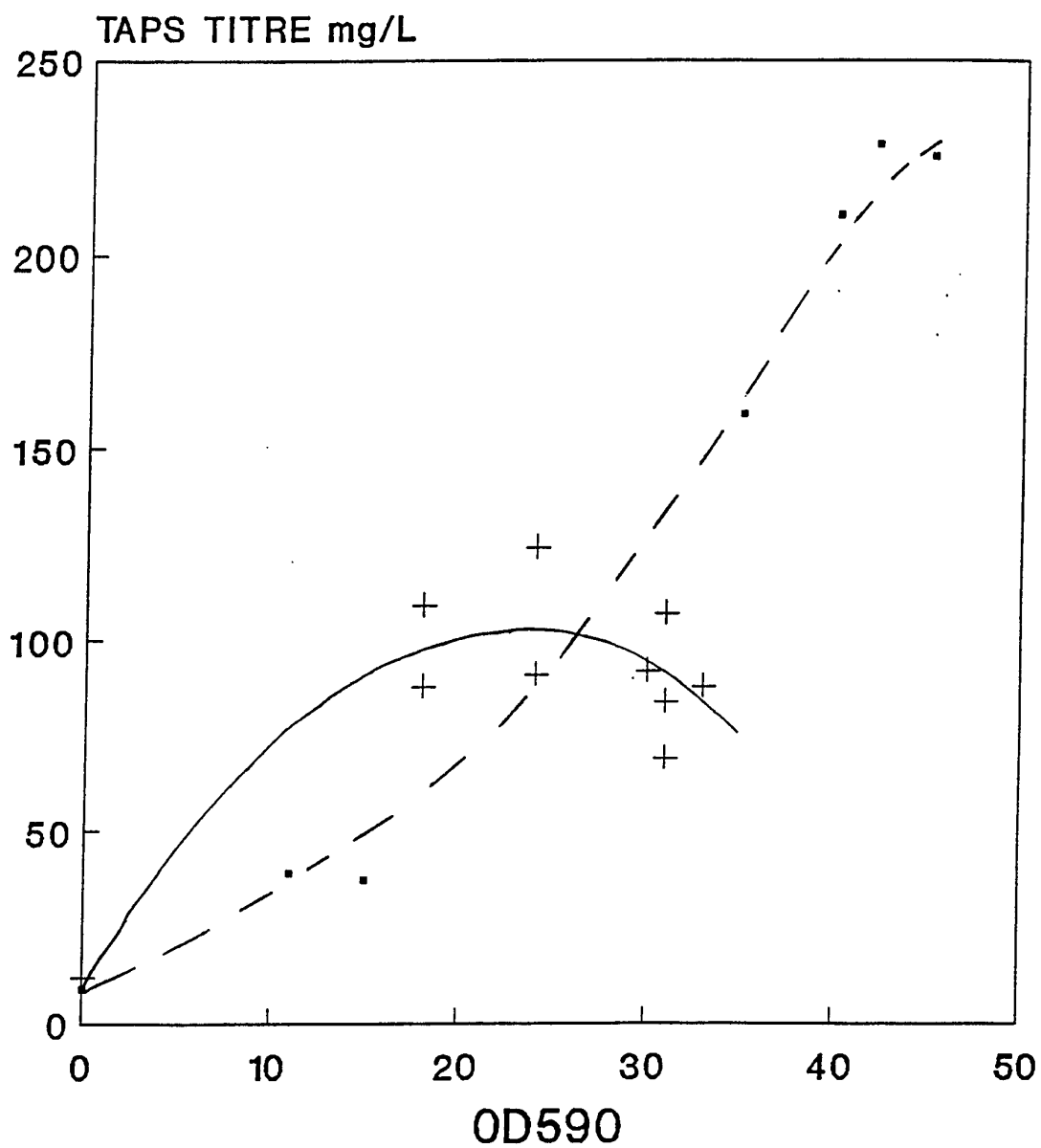
FIGURE 1
(TETRAACETYL)PHYTOSPHINGOSINE
ISOLATION AND PURIFICATION



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2/5

FIG.2

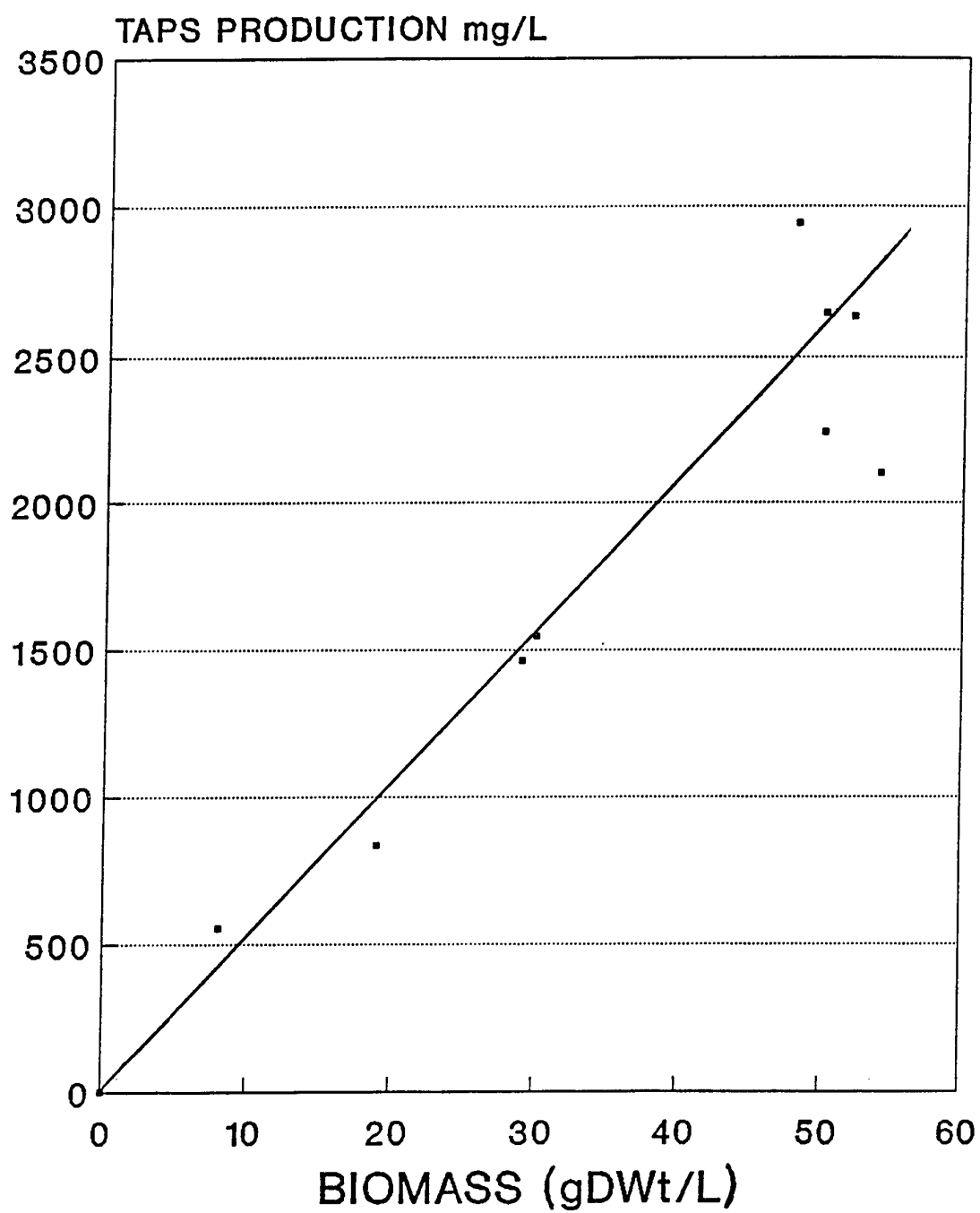


• GLYCEROL + GLUCOSE

SUBSTITUTE SHEET

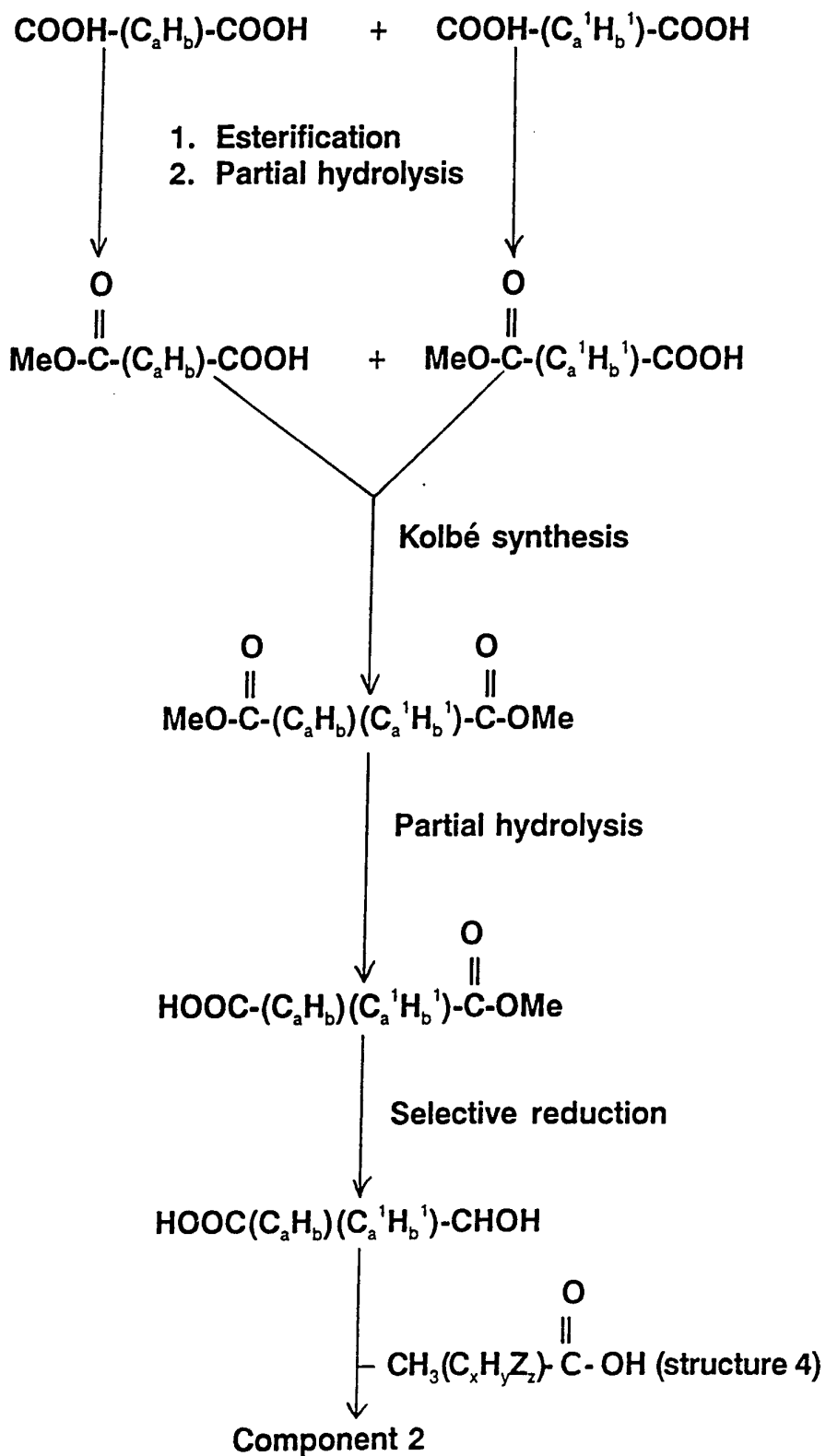
3/5

FIG.3



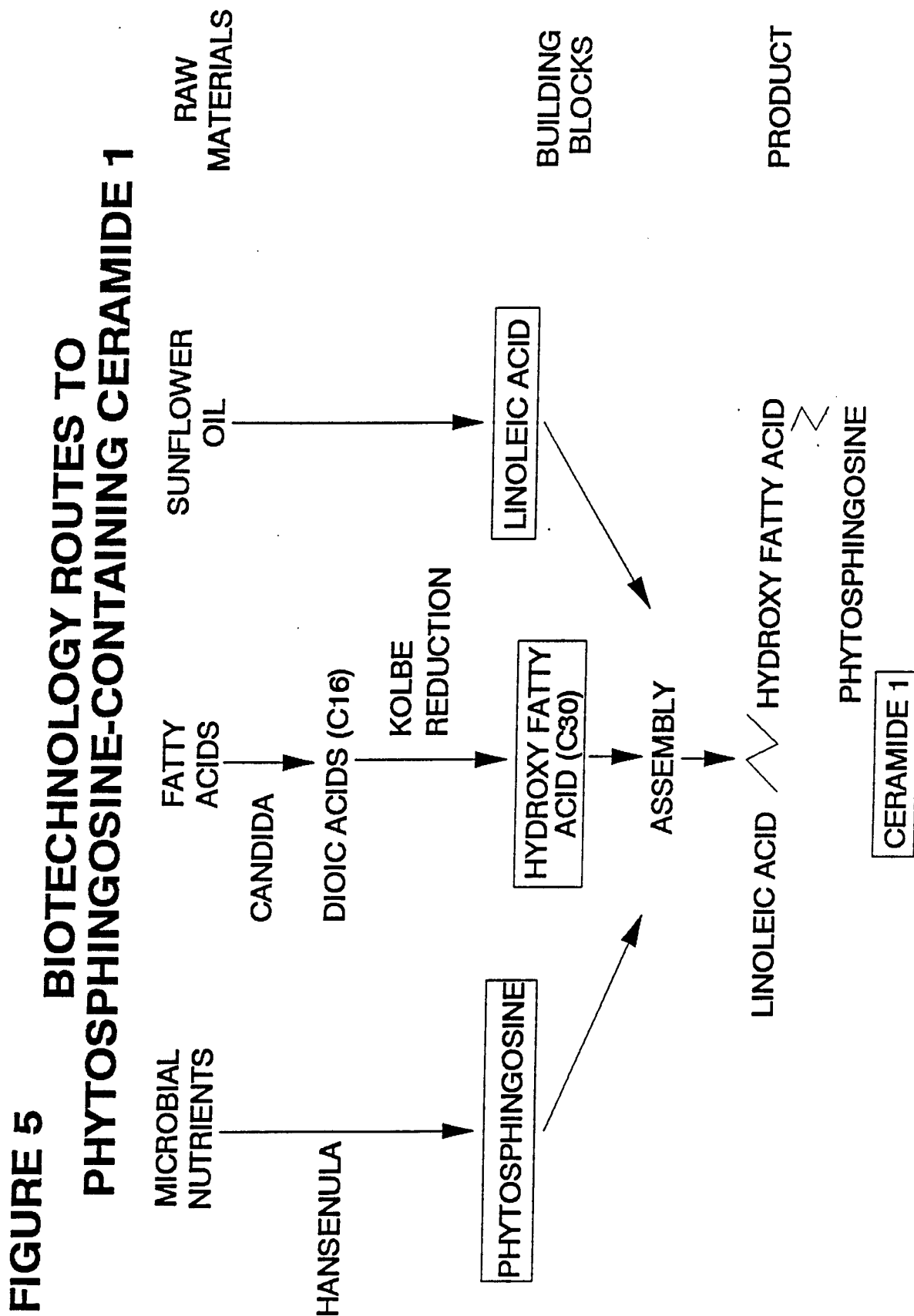
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4/5

FIGURE 4 SYNTHESIS OF COMPONENT 2

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5/5



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02230

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07C235/08 C07C235/28 C07C231/02 C07H15/04 A61K7/48
C12P13/00 //(C12P13/00,C12R1:78)

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 5 C07C C07H

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	CHEMISTRY AND PHYSICS OF LIPIDES vol. 12, no. 4 , 1974 pages 303 - 315 I. PASCHER 'Synthesis of Galactosylphytosphingosine and Galactosylceramides containing phytosphingosine' see page 304, line 16 - line 20; page 307, examples H, I; page 311, line 10 - line 19 ---	1-7
A	EP,A,0 097 059 (UNILEVER) 28 December 1983 see page 18, line 10 - 30; page 19, line 25 - 35; page 20, line 1 - 15, line 25 - 35; page 21, line 25 - 35; page 22, line 10 - 20; page 23, line 1 - 15; page 49, line 1 - 5; claims --- -/--	1-18

☒ 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
 "E" earlier document but published on or after the international filing date
 "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

20 January 1994

Date of mailing of the international search report

- 4. 02. 94

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Seufert, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02230

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. BIOLOG. CHEM. vol. 235, no. 9 , September 1960 pages 2584 - 2585 F. H. STODOLA, L. J. WICKERHAM 'Formation of extracellular sphingolipids by microorganisms' see page 2584, left column, last paragraph - right column, line 32 ---	2-7
A	J. BACTERIOL. vol. 80 , 1960 pages 484 - 491 L. J. WICKERHAM, F. H. STODOLA 'Formation of extracellular sphingolipides by microorganisms' see page 484, left column, line 25 - right column, line 4 ---	2-7
A	BIOCHIM. BIOPHYS. ACTA vol. 98 , 1965 pages 582 - 588 M. L. GREEN ET AL. 'Studies on the production of sphingolipid bases by the yeast, Hansenula ciferii' see page 587, discussion, line 1 - line 5, line 10 - line 18 ---	2,5
A	EP,A,0 482 860 (UNILEVER) 29 April 1992 see claims -----	9-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 93/02230

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		JP-B- 1045442	03-10-89
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		JP-A- 59007118	14-01-84
		US-A- 4950688	21-08-90
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		US-A- 5198210	30-03-93
