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(54) UNSATURATED ALIPHATIC DICARBOXYLIC ACIDS
UNGESÄTTIGTE ALIPHATISCHE DICARBONSÄUREN
ACIDES DICARBOXYLIQUES ALIPHATIQUES INSATURÉS

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CAMBRIDGE pages 196 - 201 D.A.OTIENO ET AL.
' THERMAL ACID-CATALYSED
REARRANGEMENTS OF NATURAL
CHRYSANTHEMIC ACIDS'

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Description

This invention relates to unsaturated dioic acids, that is, unsaturated aliphatic dicarboxylic acids, especially C₇-C₁₂ compounds, particularly but not exclusively, C₁₀ and C₁₂ unsaturated dioic acids (i.e. those having 16 or 18 carbon atoms), and concerns a method of producing dioic acids, and their use in the treatment of skin for medical and cosmetic purposes, such as acne treatment and skin lightening.

Background of the invention

It is known to use saturated dioic acids (having the general formula COOH(CH₂)ₓCOOH) for the treatment of skin for medical and cosmetic purposes. For example, US 4366104 (Nazzaro-Porro) discloses saturated dioic acids, containing 7 to 13 carbon atoms, for the treatment of acne and other skin conditions.

In particular, the C₉ saturated dioic acid ("azelaic" acid) having the formula COOH(CH₂)₇COOH, is frequently cited as being effective in the treatment of acne and other skin conditions, and in the lightening of skin.

Thus far, unsaturated dioic acids have not been readily commercially available, although a method of synthesising certain unsaturated dioic acids is disclosed in EP 0229252. Indeed some of this class of molecules have not previously been described. However, it is known that certain unsaturated dioic acids, particularly certain C₆, C₈, C₁₀ and C₁₂ mono-unsaturated dioic acids can be detected in the urine of patients with medium chain acyl-CoA dehydrogenase deficiency (Jin & Tseng [1989] Journal of Lipid Research 30, 1612-1619 and Tseng et al., [1990] Journal of Lipid Research 31, 763-771). Certain other unsaturated dioic acids are disclosed in various other publications.

EP 0341796 discloses a microbial route using Candida cloacae beta-oxidation mutants for the production of saturated dioic acids from longer chain saturated fatty acids (monocarboxylic acids) or triglycerides. The present inventors have now produced, using the mutants disclosed in EP0341796, certain unsaturated dioic acids, some of which are novel per se, which have been found to have surprisingly enhanced properties for use in the treatment of skin for medical and cosmetic purposes.

Summary of the invention

Surprisingly, it has been found by the present inventors that unsaturated dioic acids in general (especially C₁₀ and C₁₂ unsaturated dioic acids) possess much greater activity than their saturated dioic acid counterparts, as anti-microbial agents and as cosmetic agents.

The prior art teaches that conditions susceptible to treatment with dioic acids include: acne (US 4366104); wrinkles (EP 336680); malignant melanoma (US 4818766); dermatoses (EP 229654); hyperpigmented dermatosis and eczema (US 4292326); rosacea (EP 890308); lentigo (JP 91024412) and seborrhoea (DE 3133425) and impetigo.

Similarly, the prior art teaches that some dioic acid derivatives are also effective in the treatment of certain conditions. Such compounds include esters and salts (US 4818766) and mercapto-derivatives of dioic acids (US 4292326).

Other references to the utility of dioic acid derivatives may be found, for example, in JP 56170713, EP 0297436 and EP 0305407. German patent application No. DE 40 33 567 discloses, inter alia, mono ester derivatives of C₁₀-C₁₄ (main hydrocarbon chain) dioic acids (which may be saturated or unsaturated) as sebosuppressive agents for use in cosmetic or pharmaceutical applications for topical use on the hair and skin.

Thus in a first aspect the invention provides a method of treating human skin for cosmetic purposes, comprising the use of an unsaturated dioic acid and/or a derivative of an unsaturated dioic acid, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain.

The term "main hydrocarbon chain", used with respect to dioic acid derivatives, is intended to refer to that part of the molecule situated between the oxygen atoms of the two carboxylic acid groups (or the derivatised remnants thereof). Thus, for example, derivatives having the formulae R-OOC-CH₂-COO-R¹ and R-OOC-CH₂-CH₂-COO-R¹ would be described as having C₃ and C₄ main hydrocarbon chains respectively.

The derivatives may be, for example, alcohols, substituted or unsubstituted amides, mono- or diesters (aryl or alkyl, especially lower alkyl esters).

Preferably, the unsaturated dioic acids employed in this method aspect of the invention contain 8 to 22 carbon atoms, most preferably 16 or 18 carbon atoms. The unsaturated dioic acid derivative preferably contains 16 or 18 carbon atoms in the main hydrocarbon chain.

Certain unsaturated dioic acids are found to be particularly active against Propionibacterium acnes (P. acnes) and Staphylococcus aureus (Scah. aureus). Thus, in general, the method of the invention may also be found useful in treating any condition where P. acnes and/or Staph. aureus is known, or suspected, to be involved in causation, maintenance or exacerbation of that condition.

In accordance with the invention, unsaturated dioic acids can be used in the treatment of a wide range of skin conditions, such as acne etc., as discussed above in connection with the prior art. Similarly, references to derivatives
are intended to refer to derivatives such as esters and salts, as discussed in connection with the prior art.

Other conditions which may be susceptible to improvement by the use of unsaturated diocic acids or their derivatives include dandruff and the presence of body odour.

In addition it has been shown by the present inventors that unsaturated diocic acids are surprisingly effective as inhibitors of tyrosinase, and as inhibitors of melanin synthesis by cultivated melanoma cells, in tests used to screen compounds for activity as skin-lightening agents.

Therefore the invention provides, in a second aspect, a method of skin lightening, comprising the use of an unsaturated diocic acid or a derivative thereof.

In a third aspect, the invention provides a pharmaceutical or cosmetic composition comprising an unsaturated diocic acid and/or a derivative of an unsaturated diocic acid, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain.

Typically such compositions will be formulated for topical application to the skin. The unsaturated diocic acids and their derivatives are readily incorporated into such compositions which, for example, may take the form of creams, lotions or gels. Suitable formulations are very well known to those skilled in the art and are disclosed, for example, in US 4818768.

Another aspect of the invention thus provides a method of making an anti-microbial or skin-lightening composition for topical application to the skin, comprising mixing an effective amount of an unsaturated diocic acid or a derivative thereof with a dermatologically acceptable cosmetic or pharmaceutical carrier, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain.

The invention further provides an unsaturated diocic acid, and/or a derivative of an unsaturated diocic acid, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain, for use as an active therapeutic or cosmetic substance.

The invention also provides an unsaturated diocic acid, or a derivative thereof, for use as a skin-lightening and/or anti-microbial agent.

The diocic acids used in such compositions may be prepared by the novel method disclosed below. Alternatively they may be prepared using known methods, for example as disclosed in EP 296 506, or as taught by Uemura et al. (1988, Proceedings of World Conference on Biotechnology for the Fats and Oil Industry, American Oil Chemists Society); Buhler & Schlinder (1984, in *Aliphatic Hydrocarbons in Biotechnology, Rehm & Reed (Eds.) 169, Verlag Chemie, Weinheim) or by Picataggio et al. (1992, Biotechnology 10, 894-898).

Certain unsaturated diocic acids may conveniently be produced from longer chain unsaturated substrates using the mutants disclosed in EP 0341796, which previously have been used to make saturated compounds. Therefore, in a further aspect, the invention provides a method of preparing unsaturated diocic acids by limited chain-shortening beta oxidation of unsaturated substrates using a yeast propagated in a growth medium.

Preferably the unsaturated diocic acids produced are C_{16}-C_{22}, most preferably C_{16} or C_{18}.

Yeast suitable for the purpose are disclosed in EP 0341796 and in Casey et al., [(1990) *Journal of General Microbiology* 136, 1197-1202]. Such strains (eg *Candida cloacae* 5GLA12, abbreviated to "LA12") exhibit limited or reduced beta-oxidation activity.

Conveniently the yeasts are supplied with unsaturated fatty acids in the form of esters, preferably as triglyceride esters such as oil. Particularly suitable examples include unsaturated oils such as sunflower oil and olive oil.

Preferably, the oils used as starting materials are triglycerides in which the predominant unsaturated long chain fatty acid is a C_{16}-C_{22}, or more preferably, a C_{20} or C_{18} compound. Preferably the substrate material is predominantly poly-unsaturated. Fermentation by yeast strains such as LA12 can result in the production of mixtures of chain-shortened, unsaturated diocic acids (typically C_{16}-C_{22} compounds). These mixed products can be separated into fractions, for example by differential solvent extraction.

If one assumes that there is random removal of C_{2} units during beta-oxidation, and that no isomerisation of the products occurs, the following products may be predicted to be formed when using oleic acid as a substrate:

cis-6, 9-hexadecadienoic diocic acid; cis-4, 7-hexadecadienoic diocic acid; cis-5, 8-hexadecadienoic diocic acid; cis-4, 7-tetradecadienoic diocic acid; cis-4, 7-tetradecadienoic diocic acid; cis-5, 8-tetradecadienoic diocic acid; cis-3, 6-dodecadienoic diocic acid; cis-4, 7-dodecadienoic diocic acid; cis-2, 5-dodecadienoic diocic acid; cis-3, 6-dodecadienoic diocic acid; cis-2, 5-dodecadienoic diocic acid; cis-2, 5-ocadienoic diocic acid; cis-4, 7-ocadienoic diocic acid and cis-2, 6-ocadienoic diocic acid.

From linoleic acid, the following products may be expected:

cis-6, 9-hexadecadienoic diocic acid; cis-4, 7-hexadecadienoic diocic acid; cis-5, 8-tetradecadienoic diocic acid; cis-4, 7-tetradecadienoic diocic acid; cis-5, 8-tetradecadienoic diocic acid; cis-2, 5-tetradecadienoic diocic acid; cis-3, 6-dodecadienoic diocic acid; cis-4, 7-dodecadienoic diocic acid; cis-2, 5-dodecadienoic diocic acid; cis-3, 6-dodecadienoic diocic acid; cis-2, 5-dodecadienoic diocic acid; cis-2, 5-ocadienoic diocic acid; cis-4, 7-ocadienoic diocic acid and cis-2, 6-ocadienoic diocic acid.

Likewise, the predicted products using linolenic acid as a starting material are as follows:

cis-4, 7, 10-hexadecatriene diocic acid; cis-6, 9, 12-hexadecatriene diocic acid; cis-2, 5, 8-tetradecatriene diocic acid; cis-4, 7, 10-tetradecatriene diocic acid; cis-2, 5, 8-tetradecatriene diocic acid; cis-3, 6-dodecadienoic diocic acid; cis-2, 5, 8-dodecatriene diocic acid; cis-3, 6-dodecadienoic diocic acid; cis-4, 7-ocadienoic diocic acid; cis-2, 5, 8-ocadienoic diocic acid; cis-4-oc-tetradecene diocic acid and cis-2-oc-tetradecene diocic acid.
In all cases, the product mixture will contain small amounts of products of the same chain length as the starting compound.

Indeed, whilst the preferred substrates are fatty acid esters (particularly C₁₈ fatty acid esters), the products of the fermentation depend upon the starting substrate. Thus, by varying the substrate, a whole range of unsaturated dioic acids may be prepared.

Some suitable substrates are identified in EP 0 229 252 and include C₁₀⁻C₂₄ alkenes and other unsaturated hydrocarbons such as unsaturated alkanols (especially C₁₆, C₁₈ and C₂₂ unsaturated alkanols), the corresponding monocarboxylic acids, or their hydroxy-carboxylic acid derivatives.

Naturally, where trans-unsaturated compounds are the starting compounds, trans-unsaturated products will result.

It is a highly preferred feature that the yeast employed for the process is not propagated under conditions of nitrogen limitation. Instead, (unlike the method described in EP 0341796), the yeast is grown under conditions which are comparatively enriched for nitrogen, wherein alteration of pH affects the chain shortening beta oxidation activity of the organism.

Thus, it is found that the product profile of the fermentation process may conveniently be modified by alteration of the pH of the fermentation medium during the production of the unsaturated dioic acids. In particular, it is possible to alter the relative concentrations of the different lengths of dioic acid molecules in this way. For example, by reducing the pH from 7.5 to 7.1 during fermentation of olive oil, it is possible to increase the relative amount of the C₁₂ unsaturated dioic acid.

This is significant because certain fractions of the fermentation products may have especially advantageous properties for particular intended uses.

The different fractions of different products may be obtained from the culture medium by extracting with diethyl ether after adjustment of the aqueous phase to various different acidic pHs.

The different aspects of the invention can be better understood by reference to the following illustrative examples and drawings in which:

Figure 1 is a graph of dioic acid concentration (grams per litre) against time, using sunflower oil as a substrate;

Figure 2 is a graph of dioic acid concentration (grams per litre) against time, using olive oil as a substrate;

Figure 3 is a graph of dioic acid concentration (grams per litre) against time, using olive oil as a substrate with altered pH conditions; and

Figure 4 is a bar chart showing percentage melanin reduction for medium chain dioic acids obtained from sunflower oil.

**Example I - Production of Medium Chain unsaturated dioic acids by fermentation**

A beta-oxidation mutant of *Candida cloacae* produced by mutagensis using nitrosoquinidine (mutant LA12, see EP0341796 and see also Casey et al. J. Gen. Microbiol (1990), 136, 1197-1202) was used to produce C₃⁻C₁₄ unsaturated dioic acids from triglycerides such as olive oil and sunflower oil which contain high levels of unsaturated fatty acids.

A chemically defined medium was used as shown below:
Sucrose 20g/l 
\((\text{NH}_4\text{)}_2\text{HPO}_4\) 6g/l 
K\(_2\)PO\(_4\) 6.4g/l 
Na\(_2\)SO\(_4\) 1.5g/l 
Triglyceride 10-40ml/l 

\(\text{eg olive oil or sunflower oil}\)

\(\begin{align*} 
\text{ZnSO}_4 \cdot 7\text{H}_2\text{O} & \quad 20\text{mg/l} \\
\text{MnSO}_4 \cdot 4\text{H}_2\text{O} & \quad 20\text{mg/l} \\
\text{FeSO}_4 \cdot 7\text{H}_2\text{O} & \quad 20\text{mg/l} \\
\text{MgCl}_2 \cdot 6\text{H}_2\text{O} & \quad 2/\text{gl} \\
\text{Biotin} & \quad 100\text{mcg/l} \\
\text{Pantothenate} & \quad 6\text{mg/l} \\
\text{Thiamine} & \quad 8\text{mg/l} \\
\text{Nicotinic acid} & \quad 30\text{mg/l} \\
\text{Pyridoxine} & \quad 20\text{mg/l} 
\end{align*}\)

The fermenter conditions were:

\(\begin{align*} 
\text{Growth pH:} & \quad 6.8 \\
\text{Production pH:} & \quad 7.4-7.6 \\
\text{Temperature:} & \quad 30^\circ\text{C} \\
\text{Aeration:} & \quad 0.1 \text{v/v/m air} \\
\text{Impeller speed:} & \quad 800-1000 \text{ rpm} \\
\text{Fermenter volume:} & \quad 2.5\text{L} \\
\text{Inoculum:} & \quad 2\% \\
\text{Fermenter type:} & \quad \text{LSL fitted with foam breaker} 
\end{align*}\)

The medium (2.5L) was inoculated with 2\% (v/v) of a 24 hr culture of Candida cloacae beta-oxidation mutant LA12 grown on yeast extract (5g/l), sucrose(10g/l), peptone (5g/l) medium. The culture was grown for 20 hrs at pH 6.8 then 20ml of oil was added and the pH increased to 7.4-7.6 to initiate production of the medium chain unsaturated dioic acids. The oil was either sunflower oil or silica-purified olive oil. During production of the dioic acids, the RQ (respiratory quotient) value fell to about 0.6. Aliquots (10-20ml) of fermenter broth were removed daily for lipid analysis and additional oil was added as required.

The fermentation was harvested when production ceased at 8-12 days.

Medium chain unsaturated dioic acids were isolated from fermenter broths by acidification to pH 6 with HCl then extraction with diethyl ether to isolate a C\(_{12}\)-C\(_{14}\) rich fraction. The broth was then further acidified with HCl to ca. pH 2.0 and further extracted with diethyl ether to isolate a C\(_{8}\)-C\(_{10}\) rich fraction. For isolation of the mixed acids the broth pH was decreased from 7.5 to ca. 2.0 in one step then extracted with diethyl ether.

Solvent was removed from the dioic acid fractions by rotary evaporation.

A time course of medium chain unsaturated dioic acid production from sunflower oil (SFO) and silica-purified olive
oil (OO) is shown in Figures 1 and 2 respectively.

Figure 1 shows the production of C14 unsaturated dioic acids individually and in total, using sunflower oil as the substrate. The rate of production of unsaturated medium chain dioic acids increased rapidly between days 3 and 4 but declined virtually to zero by day 8, such that by that time the concentration of dioic acids was more or less constant.

Figure 2 shows the production of C6-C14 unsaturated dioic acids individually and in total, using olive oil as the substrate. In this instance, the rate of production of dioic acids showed a less sudden increase but was continuing to rise at day 8.

In both cases, the larger dioic acids (C14, C12) constituted a greater percentage of the total than did the shorter chain dioic acids (C10, C8), although the precise product profile did vary between the two substrates (eg relatively more C10 product was obtained using sunflower oil as the substrate).

These data are also represented in tabular form in Table 1.

**Example II - Use of pH to alter product profile**

At a production pH of 7.4-7.6 the dominant species from oils (eg olive oil) containing C18 unsaturated fatty acids is the C14 unsaturated dioic acid.

However, if the production pH is decreased from 7.4-7.6 to around 7.1, the C12 unsaturated dioic acid becomes the dominant species. Fermentation was performed as detailed in the above examples until fermentation day 8 when the pH was dropped to 7.1 resulting in ‘turn-over’ at the C14 species and an increase in C12 production. The results are illustrated in Figure 3.

<table>
<thead>
<tr>
<th>Fermentation Time (Days)</th>
<th>C14</th>
<th>C12</th>
<th>C10</th>
<th>C8</th>
<th>TOTAL C8-C14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFO</td>
<td>OO</td>
<td>SFO</td>
<td>OO</td>
<td>SFO</td>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>1.5</td>
<td>0.9</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>5.7</td>
<td>3.0</td>
<td>2.3</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>7.4</td>
<td>3.9</td>
<td>3.4</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>8.4</td>
<td>5.1</td>
<td>4.2</td>
<td>3.7</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>8.4</td>
<td>6.8</td>
<td>4.5</td>
<td>3.6</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>8.7</td>
<td>8.8</td>
<td>4.7</td>
<td>5.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Figure 3 shows the production of C6-C14 unsaturated dioic acids individually and in total, using olive oil as the substrate where the pH is adjusted on day 8 from 7.5 to 7.1. As noted in Figure 2, the total production of dioic acids continues to increase after day 8 when using olive oil as a substrate. However the product profile is significantly affected.

Until day 8, the concentration of the C14 dioic acid continued to increase and was the most-concentrated dioic acid product. However, after that point, in the conditions of reduced pH, the concentration started to decline, whereas the C12 product continued to increase, such that after day 10 the C12 dioic acid represented the major product.

These data are also shown in tabular form in Table II.

This experiment shows that the product profile can be controlled to some extent by the production pH. The rate of C6-C16 dioic production remains substantially linear after alteration of the production pH.

**Example III - Tyrosinase Inhibition Assay**

Inhibition of tyrosinase activity is used to identify potential skin whitening agents. Assays of tyrosinase inhibition were performed according to the methods of Humada and Mishima (Br. J. Derm. (1972) 96, 385-394).

All solutions were freshly prepared using 0.1M sodium phosphate buffer (pH 6.8) as diluent. These were: 40mM Inhibitor stock solution: from which serial dilutions were made to obtain the following concentrations of 4.0, 0.4 and 0.04mM inhibitor;
Table II

<table>
<thead>
<tr>
<th>Fermentation Day</th>
<th>Dioic Acid (g/l)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₁₆</td>
<td>C₁₄</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>0.52</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>2.1</td>
<td>4.4</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>5.5</td>
</tr>
<tr>
<td>7</td>
<td>0.6</td>
<td>7.1</td>
</tr>
<tr>
<td>8 Change of pH from 7.5-7.1</td>
<td>0.6</td>
<td>8.8</td>
</tr>
<tr>
<td>9</td>
<td>0.4</td>
<td>8.2</td>
</tr>
<tr>
<td>10</td>
<td>0.13</td>
<td>8.2</td>
</tr>
<tr>
<td>11</td>
<td>0.25</td>
<td>8.0</td>
</tr>
<tr>
<td>12</td>
<td>0.1</td>
<td>7.5</td>
</tr>
<tr>
<td>13</td>
<td>0.1</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Salt solution: containing copper sulphate (100μM) and magnesium chloride (100mM); 
Enzyme solution: 1ml mushroom tyrosinase (2000-4000 units per mg), and 
Substrate solution: 48mg dihydroxyphenylalanine (DOPA)/100ml.

The enzyme and DOPA solutions were prepared immediately before use as they are light sensitive.

The inhibition of tyrosinase-catalysed oxidation of DOPA by dicarboxylic acids was followed spectrophotometrically 
by monitoring dopachrome formation at a wavelength of 492nm. The reaction was performed in 96-well microtitre plates 
with the addition of 30μl inhibitor (or buffer for the control), 50μl buffer and 20μl salt solution. DOPA (50μl) was added 
to start the reaction and each plate shaken for 30 seconds. Absorbance readings were taken after 10 minutes using 
a microtitre plate reader (Titertek Multiscan).

Results of the tyrosinase inhibition assay showed that, like azelaic acid, the unsaturated medium chain dioic acids 
were found to be effective tyrosinase inhibitors resulting in at least 50% inhibition of enzyme activity when present at 
10mM concentration. This is surprising because azelaic acid is a saturated dioic acid and therefore has markedly 
different properties. Thus azelaic acid is a crystalline solid at room temperature whilst C₈’C₁₀ unsaturated dioic acids 
are low melting-point oily substances.

It is possible that the enzyme thioredoxin reductase is a more significant enzyme than tyrosinase with respect to 
dioic acid-mediated inhibition of skin pigmentation. Recent research (described by Fitton & Goa in Drugs 41 (5), 780-798 
(1991) has shown that azelaic acid inhibits thioredoxin reductase. In the light of the disclosure in this specification the 
skilled worker would therefore expect unsaturated dioic acids to be inhibitors of this enzyme as well.

Example IV - Inhibition of Melanin Production

In a further assay to complement Example III, the effects of unsaturated dioic acids on in vitro melanocyte cultures 
were investigated.

Pigment producing cells derived from a mammalian melanoma were grown in culture by standard methods. Preferred 
cell lines are B16 (disclosed in EP 0 338 104) or S-91 (e.g. ATCC CCL 51.3, clone M-3) cells, but other lines or 
primary mouse or human melanocytes can be used.

Melanoma cells were grown in a complete cell culture medium (such as that described in EP 0 308 919) to approxi-
mately 1/3 confluence. The composition to be tested was then added to the culture medium.

The cells were cultured for a further period of 4 days and the amount of melanin produced was assayed by meas-
uring the absorbance at 540 nm of the total melanin extracted from the culture medium and from the harvested cells.

The method described above was used to assess the ability of compositions comprising unsaturated dioic acids 
(C₁₂’C₁₄ fraction, C₈’C₁₀ fraction, or mixed acids), at 0.1mM or 1.0mM, to reduce the amount of melanin produced by 
melanocyte cultures, relative to a negative control culture. Kojic acid (a substance used as a skin lightening agent)
was used as a positive control. The results are shown in Figure 4, which is a bar chart showing the percentage reduction in melanin in the treated cultures compared to the untreated control.

It was found that the various dicarboxylic acid fractions had substantially similar properties in this respect.

**Example V - Determination of antimicrobial activity**

The Minimum Inhibitory Concentration (MIC) of each of various unsaturated dicarboxylic acid mixtures was determined in the presence and absence of 10% Intralipid (Kabi Pharmacia, Inc.) using the agar dilution technique for susceptibility of 32 strains of *Propionibacterium acnes* and of 32 strains of various genera of aerobic bacteria. The method was as set out below.

A 5% stock solution for each agent was prepared by adding 10 grams of the dicarboxylic acid material to 200 milliliters of double strength Tryptic Soy Broth (TSB), (Baltimore Biological Laboratories). The pH of each solution was adjusted to 7.0 ± 0.2 with sodium hydroxide.

For each organic acid two sets of 200 ml capacity bottle/flasks were numbered 1 to 9. To each bottle was added 50 cc of double strength TSB. From the 5% stock solution, 50 cc of TSB were transferred to bottles #1 and #2. Serial transfers of 50 cc are made from bottle #2 through to bottle #8. Bottle #9 of each set contained only 50 cc of double strength TSB, without any dicarboxylic acid. To all 18 bottles were added 2 grams of granulated agar (BBL).

All bottles were autoclaved an 121°C, 15 psi for 15 minutes and then held at 50°C in a water bath.

To one set of bottles #1-9 were added 50 cc of hot, sterile water. The bottles were swirled to mix the contents and 25 cc was poured into each of four petri dishes and allowed to solidify. To the second set of bottles were added 50 cc of Intralipid (pre-warmed to 50°C). The contents were then mixed and pour as above.

Standard inocula of the test organisms were prepared by matching the bacterial suspension in 0.85% PSS (physiological saline solution) to a 0.5 McFarland Standard and diluting ten-fold to yield 10⁷ CFU (colony forming units). The inocula were loaded into 32 wells of a steers replicator. The multi-prong inoculator delivers 0.001 to 0.002 cc resulting in a final inoculum of 10⁴ CFU per spot.

Plates were inoculated from the lowest to highest concentration (to reduce the effects of "carry-over" of the inoculum), and allowed to dry. The plates were then inverted and incubated at 35°C for 24 hours. Plates inoculated with *Propionibacterium acnes* strains were incubate under anaerobic conditions for seven days at 35°C.

The agent-free control plates (#9) were examined at the end of the incubation for viability and signs of contamination. End-point MIC values were determined by observing the plate of lowest concentration of agent that inhibited visible micro-organism growth.

The results are summarised in Table III, which shows the MIC for medium chain unsaturated dicarboxylic acids (*Mixed dicarboxylic Acids*, i.e. C₉-C₁₄ mixed dicarboxylic acids), a C₁₂ enriched fraction, and for the C₁₈:₁ mono-unsaturated compound, compared with Azelaic acid, for a range of microorganisms. The data represent the results of experiments which were generally conducted on several different strains of each species (e.g. *P. acnes* strains ATCC 6919 and 29399 [ATCC stands for American Type Culture Collection]; *Staph. aureus* strains ATCC 25923, 35556 and 29213; *Staph. epidermidis* ATCC 35984, 31432 and 14490; *Micrococcus sedentarius* ATCC 27574; *M. luteus* ATCC 27141, 9341, and 15957; *Brevibacterium epidermidis* ATCC 35514; *Corynebacterium minutissimum* ATCC 23347, 23348 and 23349).

The presence or absence of "Intralipid" had no significant effect. A slight difference was observed only for the C₁₈:₁ mono-unsaturated compound, where there was a suggestion that Intralipid increased the MIC for *Staph. aureus* and decreased the MIC for *P. acnes* and *M. luteus*. 
<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Azelaic acid</th>
<th>Mixed diic acids ex Olive oil</th>
<th>C&lt;sub&gt;4&lt;/sub&gt; enriched mono unsat diic acids ex Olive oil</th>
<th>Mixed diic acids ex Sunflower oil</th>
<th>C&lt;sub&gt;14&lt;/sub&gt; diic acid ex Oleic acid</th>
<th>C&lt;sub&gt;14&lt;/sub&gt; diic acid ex &quot;Intrallipid&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionibacterium acnes</td>
<td>ATCC 6919</td>
<td>1.25</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.04</td>
<td>0.02</td>
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<tr>
<td>Propionibacterium acnes</td>
<td>ATCC 29399</td>
<td>1.25</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.04</td>
<td>0.02</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 25923</td>
<td>2.5</td>
<td>0.62</td>
<td>1.25</td>
<td>1.25</td>
<td>0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 35556</td>
<td>2.5</td>
<td>0.62</td>
<td>1.25</td>
<td>1.25</td>
<td>0.07</td>
<td>0.31</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 29213</td>
<td>2.5</td>
<td>1.25</td>
<td>0.62</td>
<td>2.5</td>
<td>0.15</td>
<td>0.31</td>
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<tr>
<td>Staphylococcus epidermidis</td>
<td>ATCC 35894</td>
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<td>0.62</td>
<td>2.5</td>
<td>0.31</td>
<td>0.31</td>
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<tr>
<td>Staphylococcus epidermidis</td>
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<td>&gt;2.5</td>
<td>1.25</td>
<td>0.62</td>
<td>2.5</td>
<td>0.31</td>
<td>0.31</td>
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<tr>
<td>Micrococcus sedentarius</td>
<td>ATCC 27574</td>
<td>2.5</td>
<td>0.15</td>
<td>0.62</td>
<td>0.62</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>ATCC 27141</td>
<td>2.5</td>
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<td>0.62</td>
<td>0.62</td>
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</tr>
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<td>Micrococcus luteus</td>
<td>ATCC 9341</td>
<td>&gt;2.5</td>
<td>1.25</td>
<td>0.62</td>
<td>0.62</td>
<td>0.15</td>
<td>0.07</td>
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<tr>
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<td>1.25</td>
<td>0.62</td>
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<td>0.07</td>
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<tr>
<td>Brevibacterium epidermidis</td>
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<td>&gt;2.5</td>
<td>0.62</td>
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<td>0.62</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td>Brevibacterium epidermidis</td>
<td>NCDO 2285</td>
<td>&gt;2.5</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
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<tr>
<td>Corynebacterium minutissimum</td>
<td>ATCC 23347</td>
<td>&gt;2.5</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.15</td>
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<tr>
<td>Corynebacterium minutissimum</td>
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<td>&gt;2.5</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Corynebacterium minutissimum</td>
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<td>&gt;2.5</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.15</td>
<td>0.15</td>
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<td>Pseudomonas aeruginosa</td>
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<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
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<td>Escherichia coli</td>
<td>ATCC 25222</td>
<td>2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
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<td>&gt;2.5</td>
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<tr>
<td>Candida albicans</td>
<td>ATCC 18804</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
</tr>
</tbody>
</table>
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In particular the C₁₈ compound exhibit d anti-microbial activity many times greater than that for azelaic acid.

A similar experiment was performed, using the same method, to compare the degree of inhibitory activity (for P. acnes) of azelaic acid, the C₁₈:₁ dioic acid (obtained from a substrate comprising oleic acid), a mixture of C₁₈:₁ fatty (mono-carboxylic) acid, the corresponding C₁₈:₁ and C₁₈:₂ dioic acids (obtained from a substrate comprising linoleic acid), and the C₁₈:₁ dioic acid (obtained from a substrate comprising palmitoleic acid). The results are shown below in Table IV. The MICs for azelaic acid and the C₁₈:₁ dioic acid were essentially as before, confirming the previous results. The corresponding fatty acid had very little activity. Thus, the C₁₈:₁ dioic acid then has almost 100x the activity of azelaic acid, although the equivalent fatty acid (oleic acid) is less active than azelaic acid.

In experiment 4, the degree of inhibition of the azelaic acid control, and therefore of the test samples also, was slightly less than that observed in previous experiments.

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
<th>Expt. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azelaic acid (control)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>C₁₈:₁ Fatty acid (control)</td>
<td>nd</td>
<td>nd</td>
<td>&gt;5</td>
<td>nd</td>
</tr>
<tr>
<td>C₁₈:₁ dioic acid ex Oleic acid</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>C₁₈:₂/₁₈:₁ dioic acid ex 60% Linoleic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.08</td>
</tr>
<tr>
<td>C₁₈:₁ dioic acid ex Palmitoleic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.16</td>
</tr>
</tbody>
</table>

In experiment 4 the degree of inhibition of the azelaic acid control, and therefore the test samples also, was slightly less than previously observed. The most probable reason for this was the increased incubation period of 18 days as against 7 days for the earlier work.

Claims

1. A pharmaceutical or cosmetic composition comprising an unsaturated dioic acid or a derivative of an unsaturated dioic acid, the derivative comprising 15 or more atoms in the main hydrocarbon chain.

2. A composition according to claim 1, wherein the unsaturated dioic acid contains from 8 to 22 carbon atoms (inclusive) and/or the derivative is a derivative of an unsaturated dioic acid containing from 15 to 22 carbon atoms (inclusive) in the main hydrocarbon chain.

3. A composition according to claim 1 or 2, comprising one or more unsaturated C₁₈ or C₁₈ dioic acids and/or a derivative thereof.

4. A composition according to claim 1, 2 or 3, having antimicrobial activity.

5. A composition according to any one of claims 1 to 4, being active against Propionibacterium acnes and Staphylococcus aureus.

6. A composition according to any one of claims 1 to 5, having skin-lightening activity.

7. A composition according to any one of claims 1 to 6, wherein the dioic acid derivative is an alcohol, a substituted or unsubstituted amide, a salt, a mono- or diester or compound.

8. A composition according to any one of claims 1 to 7, suitable for topical to the skin.

9. A composition according to any one of claims 1 to 8, wherein the composition comprises from 0.001% to 20% by weight of unsaturated dioic acid and/or derivative thereof.
10. A composition according to any one of claims 1 to 9, wherein the composition comprises from 0.01% to 1% by weight of unsaturated dioic acid and/or derivative thereof.

11. A composition according to any one of the preceding claims, having a pH in the range 6.8-7.2.

12. A method of treating human skin for cosmetic purposes, comprising use of an effective amount of a composition in accordance with any one of claims 1 to 11.

13. A cosmetic method of treating human skin in accordance with claim 12, for the purpose of treating a condition caused, maintained or exacerbated by *Propionibacterium acnes* and/or *Staphylococcus aureus*.

14. A cosmetic method of treating human skin in accordance with claim 12 or 13, for the treatment of one or more of the following: acne; wrinkles; dermatosis; hyper-pigmentary dermatosis; eczema; rosacea; lentigo; seborrhoea; impetigo; dandruff; and body malodour.

15. A method of lightening skin, comprising the use of an unsaturated dioic acid or a derivative thereof.

16. An unsaturated dioic acid, or a derivative of an unsaturated dioic acid, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain, for use as an active therapeutic or cosmetic substance.

17. An unsaturated dioic acid, or a derivative thereof, for use as an anti-microbial or as a skin-lightening agent.

18. A C_{16} or C_{18} unsaturated dioic acid or a derivative thereof, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain, for use as an active therapeutic or cosmetic substance.

19. A method of making a therapeutic or cosmetic composition for topical application to the skin, comprising mixing an effective amount of an unsaturated dioic acid or a derivative thereof with a dermatologically acceptable cosmetic or pharmaceutical carrier, the derivative comprising 15 or more carbon atoms in the main hydrocarbon chain.

20. A method according to claim 19, wherein the unsaturated dioic acid or derivative thereof is a C_{16} or C_{18} unsaturated dioic acid or derivative.


22. A method according to claim 21, comprising use of *Candida cloacae* strain 5GLA12.

23. A method according to claim 21 or 22, wherein the substrate comprises a C_{16}-C_{22} unsaturated compound.

24. A method according to any one of claims 21, 22 or 23, wherein the substrate comprises oleic, linoleic, linolenic or arachidonic acids.

25. A method according to any one of claims 21 to 24, wherein the substrate comprises a triglyceride.

26. A method according to any one of claims 21 to 25, wherein the substrate comprises olive oil, sunflower oil or castor oil.

27. A method according to any one of claims 21 to 26, wherein the yeast is not grown under conditions of nitrogen-limitation.

28. A method according to any one of claims 21 to 27, wherein alteration of pH affects the dioic acid product profile of the process.

Patentansprüche

1. Pharmazeutische oder kosmetische Zusammensetzung, umfassend eine ungesättigte Dicarbonsäure und/oder ein Derivat einer ungesättigten Dicarbonsäure, wobei das Derivat 15 oder mehr Kohlenstoffatome in der Kohlen-
Zusammensetzung nach Anspruch 1, worin die ungesättigte Dicarbonsäure 8 bis (einschließlich) 22 Kohlenstoffatome enthalten und/oder das Derivat ein Derivat einer ungesättigten Dicarbonsäure, enthaltend 15 bis (einschließlich) 22 Kohlenwasserstoffatome in der Kohlenwasserstoffhauptkette, ist.

Zusammensetzung nach Anspruch 1 oder 2, umfassend eine oder mehrere ungesättigte C_{16}- oder C_{18}-Dicarbonsäuren und/ oder ein Derivat davon.

Zusammensetzung nach Anspruch 1, 2 oder 3, die antimikrobielle Wirksamkeit aufweist.

Zusammensetzung nach einem der Ansprüche 1 bis 4, die gegen Propionibacterium acnes und Staphylococcus aureus wirksam ist.

Zusammensetzung nach einem der Ansprüche 1 bis 5, die eine hautklärende Wirksamkeit aufweist.

Zusammensetzung nach einem der Ansprüche 1 bis 6, worin das Dicarbonsäurederivat ein Alkohol, ein substituiertes oder unsubstituiertes Amid, ein Salz, ein Mono- oder Diester oder eine Mercaptoverbinding ist.

Zusammensetzung nach einem der Ansprüche 1 bis 7, die zum topischen Anwenden auf der Haut geeignet ist.

Zusammensetzung nach einem der Ansprüche 1 bis 8, worin die Zusammensetzung 0,001 % bis 20 Gew.- % ungesättigte Dicarbonsäure und/oder Derivat davon umfaßt.

Zusammensetzung nach einem der Ansprüche 1 bis 9, worin die Zusammensetzung 0,01 bis 1 Gew.- % ungesättigte Dicarbonsäure und/oder Derivat davon umfaßt.

Zusammensetzung nach einem der vorhergehenden Ansprüche mit einem pH-Wert in dem Bereich von 6,8 bis 7,2.

Verfahren zur Behandlung von menschlicher Haut zu kosmetischen Zwecken, umfassend das Verwenden einer wirksamen Menge einer Zusammensetzung nach einem der Ansprüche 1 bis 11.

Kosmetisches Verfahren zur Behandlung menschlicher Haut nach Anspruch 12 zum Zwecke der Behandlung eines Leidens, das von Propionibacterium acnes und/oder Staphylococcus aureus bedingt, aufrechterhalten oder verschlimmert wird.

Kosmetisches Verfahren zur Behandlung menschlicher Haut nach Anspruch 12 oder 13 zur Behandlung von einem oder mehreren aus folgendem: Akne, Falten, Dermatose, hyper-pigmentäre Dermatose, Ekzem, Rosacea, Lentigo, Seborrhoea, Impetigo, Dandruff und unangenehmem Körperguson.

Verfahren zum Klären der Haut, umfassend das Verwenden einer ungesättigten Dicarbonsäure oder eines Derivates davon.

Ungesättigte Dicarbonsäure oder Derivat einer ungesättigten Dicarbonsäure, wobei das Derivat 15 oder mehr Kohlenstoffatome in die Kohlenwasserstoffhauptkette umfaßt, zur Verwendung als therapeutischer oder kosmetischer Wirkstoff.

Ungesättigte Dicarbonsäure oder Derivat davon zur Verwendung als ein antimikrobielles oder als ein hautklärndes Mittel.

Ungesättigte C_{16}- oder C_{18}-Dicarbonsäure oder Derivat davon, wobei das Derivat 15 oder mehr Kohlenstoffatome in der Kohlenwasserstoffhauptkette umfaßt, zur Verwendung als therapeutischer oder kosmetischer Wirkstoff.

Verfahren zur Herstellung einer therapeutischen oder kosmetischen Zusammensetzung zum topischen Anwenden auf der Haut, umfassend das Mischen einer wirksamen Menge einer ungesättigten Dicarbonsäure oder eines Derivates davon mit einem dermatologisch akzeptablen kosmetischen oder pharmazeutischen Träger, wobei das Derivat 15 oder mehr Kohlenstoffatome in der Kohlenwasserstoffhauptkette umfaßt.
20. Verfahren nach Anspruch 19, wobei die ungesättigte Dicarbonsäure oder das Derivat davon eine ungesättigte C_{16} oder C_{18} Dicarbonsäure oder ein Derivat ist.


22. Verfahren nach Anspruch 21, umfassend das Verwenden von *Candida* *albicans*, Stamm 5GLA12.

23. Verfahren nach Anspruch 21 oder 22, wobei das Substrat eine ungesättigte C_{16} bis C_{22} Verbindung umfaßt.


25. Verfahren nach einem der Ansprüche 21 bis 24, wobei das Substrat ein Triglycerid umfaßt.


27. Verfahren nach einem der Ansprüche 21 bis 26, wobei die Hefe nicht unter stickstofflimitierenden Bedingungen gezüchtet wird.


**Revendications**

1. Composition pharmaceutique ou cosmétique comprenant un acide dioïque insaturé et/ou un dérivé d’un acide dioïque insaturé, le dérivé comprenant 15 atomes de carbone ou plus dans la chaîne hydrocarbonée principale.

2. Composition selon la revendication 1, dans laquelle l’acide dioïque insaturé contient de 8 à 22 atomes de carbone (limites comprises) et/ou le dérivé est un dérivé d’un acide dioïque insaturé contenant de 15 à 22 atomes de carbone (limites comprises) dans la chaîne hydrocarbonée principale.

3. Composition selon la revendication 1 ou 2, comprenant un ou plusieurs acides dioïques insaturés en C_{16} ou C_{18} et/ou un de leurs dérivés.

4. Composition selon la revendication 1, 2 ou 3, ayant une activité antimicrobiennne.

5. Composition selon l’une quelconque des revendications 1 à 4, qui est active contre *Propionibacterium acnes* et *Staphylococcus aureus*.

6. Composition selon l’une quelconque des revendications 1 à 5, ayant une activité d’éclaircissement de la peau.

7. Composition selon l’une quelconque des revendications 1 à 6, dans laquelle le dérivé d’acide dioïque est un alcool, un amide substitué ou non substitué, un sel, un mono- ou un diester, ou un composé mercapto.

8. Composition selon l’une quelconque des revendications 1 à 7, convenant à une application topique sur la peau.

9. Composition selon l’une quelconque des revendications 1 à 8, dans laquelle la composition comprend de 0,001 à 20 % en poids d’un acide dioïque insaturé et/ou d’un de ses dérivés.

10. Composition selon l’une quelconque des revendications 1 à 9, dans laquelle la composition comprend de 0,01 à 1 % en poids d’un acide dioïque insaturé et/ou d’un de ses dérivés.

11. Composition selon l’une quelconque des revendications précédentes, ayant un pH compris entre 3,8 et 7,2.

12. Procédé pour traiter la peau humaine à des fins cosmétiques, qui comprend l’utilisation d’une quantité efficace
d’une composition selon l’une quelconque des revendications 1 à 11.

13. Procédé cosmétique pour traiter la peau humaine selon la revendication 12, aux fins de traiter une affection provoquée, entretenue ou exacerbée par *Propionibacterium acnes* et/ou *Staphylococcus aureus*.

14. Procédé cosmétique de traitement de la peau humaine selon les revendications 12 ou 13, pour le traitement d’une ou plusieurs des affections suivantes : acné, rides, dermatose, dermatose hyperpigmentaire, eczéma, acné rosacée, lentigo, séborrhée, impétigo, pellicules et mauvaises odeurs corporelles.

15. Procédé d’éclaircissement de la peau, comprenant l’utilisation d’un acide dioïque insaturé ou d’un de ses dérivés.

16. Acide dioïque insaturé, ou dérivé d’un acide dioïque insaturé, le dérivé comprenant 15 atomes de carbone ou plus dans la chaîne hydrocarbonée principale, par utilisation en tant que principe actif thérapeutique ou cosmétique.

17. Acide dioïque insaturé, ou l’un de ses dérivés, pour utilisation en tant qu’agent antimicrobien ou agent d’éclaircissement de la peau.

18. Acide dioïque insaturé en C₁₆ ou C₁₈ ou l’un de ses dérivés, le dérivé comprenant 15 atomes de carbone ou plus dans la chaîne hydrocarbonée principale, pour utilisation en tant que principe actif thérapeutique ou cosmétique.

19. Procédé de préparation d’une composition thérapeutique ou cosmétique pour application topique sur la peau, qui consiste à mélanger une quantité efficace d’un acide dioïque insaturé ou d’un de ses dérivés à un excipient cosmétique ou pharmaceutique acceptable d’un point de vue dermatologique, le dérivé comprenant 15 atomes de carbone ou plus dans la chaîne hydrocarbonée principale.

20. Procédé selon la revendication 19, dans lequel l’acide dioïque insaturé ou son dérivé est un acide dioïque insaturé en C₁₆ ou C₁₈ ou l’un de ses dérivés.

21. Procédé de préparation d’un acide dioïque insaturé, qui comprend une β-oxydation limitée, avec raccourcissement de chaîne, d’un substrat insaturé, par utilisation d’une levure qui prolifère dans un milieu de croissance.

22. Procédé selon la revendication 21, qui comprend l’utilisation de la souche de *Candida cloacae* 5GLA12.

23. Procédé selon la revendication 21 ou 22, dans lequel le substrat comprend un composé insaturé en C₁₆-C₂₂.

24. Procédé selon l’une quelconque des revendications 21, 22 ou 23, dans lequel le substrat comprend les acides oléiques, linoléiques, linoléniques ou arachidoniques.

25. Procédé selon l’une quelconque des revendications 21 à 24, dans lequel le substrat comprend un triglycéride.


27. Procédé selon l’une quelconque des revendications 21 à 26, dans lequel la levure ne prolifère pas dans des conditions de limitation d’azote.

28. Procédé selon l’une quelconque des revendications 21 à 27, dans lequel une modification du pH affecte le profil des acides dioïques produits par le procédé.
FIGURE 3

pH DECREASED FROM 7.5 TO 7.1

DIoIC ACID (g/l)

TIME (days)

--- C16

--- C14

--- C12

--- C10

--- C8

--- TOTAL C8-C16
EFFECT OF SUNFLOWER OIL DERIVED MEDIUM CHAIN UNSATURATED DIOIC ACIDS ON MELANIN PRODUCTION BY MELANOMA CELLS

% REDUCTION IN MELANIN

SUNFLOWER OIL MCU DCA's

Figure 4: Kojic acid is the control.