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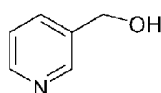
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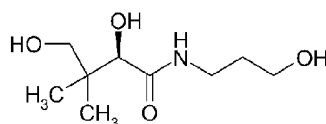
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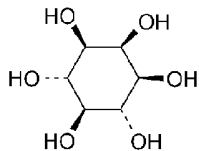
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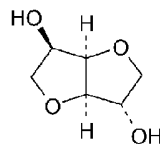
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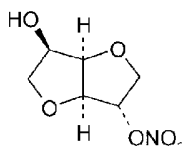
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(57) Abstract: The present invention relates to a docosahexaenoic acid ester with an alcohol chosen among the group-B vitamins or provitamins, advantageously comprised by: nicotinylic alcohol of the following formula (I), panthenol of the following formula (II), and inositol of the following formula (III) or with isosorbide of the following formula (IV) or isosorbide mononitrate of the following formula (V). It also relates to a method of preparation of same, a pharmaceutical composition comprising same and the use of same in the treatment or prevention of cardiovascular disease, in particular auricular fibrillation.

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DHA ESTERS AND USE THEREOF IN TREATMENT AND PREVENTION OF
CARDIOVASCULAR DISEASE

5 The present invention relates to docosahexaenoic acid
(DHA) esters with alcohols chosen among the group-B vitamins
or provitamins such as nicotinyl alcohol (B3), panthenol
(B5) or inositol (B7), or isosorbide or isosorbide
mononitrate, and in particular pyridin-3-ylmethyl
10 docosahexaeneoate, and the use of same as a drug in the
treatment and prevention of cardiovascular diseases.

 The omega-3 polyunsaturated fatty acids, in particular
EPA and DHA advantageously purified and concentrated in the
form of ethyl ester, are known for their potential use in
15 the treatment of certain cardiovascular diseases and in the
modulation of corresponding risk factors. In particular,
they are known in the treatment of hyperlipidemia,
hypercholesterolemia and hypertension. Clinical trials
carried out with formulations containing a high
20 concentration of EPA and DHA ethyl ester on patients having
suffered a myocardial infarction showed their effectiveness
in reducing mortality and, in particular, sudden death.
These results were attributed in part to a stabilizing
effect on the cell membranes of ventricular cardiomyocytes,
25 which prevent the appearance of malignant arrhythmia in the
presence of ischemic myocytes, as seen in patients following
an infarction or in experimental models which reproduce such
conditions.

 In addition, it is also known according to patent
30 application WO 2004/047835 that DHA and EPA ethyl esters can
be used to prevent auricular fibrillation. However,
surprisingly, the inventors of the present application have
discovered that DHA and EPA do not have the same effect on

auricular fibrillation: DHA has a much greater effect on
auricular fibrillation than does EPA. Thus, it is more
advantageous to use DHA alone than a mixture of DHA and EPA
in the treatment of auricular fibrillation and, undoubtedly,
5 in the treatment of most cardiovascular disease.

The group-B vitamins covers hydrosoluble molecules
belonging to very different chemical classes, but all having
as a main function the capability to control enzymatic
10 activities anywhere in the metabolism. These vitamins are
named: thiamine (B1), riboflavin (B2), niacin (B3),
panthothenic acid (B5), pyridoxine (B6), biotin (B8), folic
acid (B9) and cyanocobalamin(B12).

15 The group-B vitamins and provitamins have advantages
related to their functions. In particular, nicotiny alcohol
is the alcohol derived from nicotinic acid (vitamin B3). It
is rapidly converted into nicotinic acid in the human body.

Nicotinic acid, also called niacin, is a water-soluble
20 group-B vitamin that can be synthesized from tryptophan.
However, the effective therapeutic doses for purposes of
lowering cholesterol and lipids are higher than the
quantities synthesized by the body. Thus, an oral supplement
proves to be essential in targeting the lowering of
25 cholesterol and/or triglycerides.

In terms of action mechanism, it is suspected that
nicotinic acid inhibits the release of free fatty acids from
adipose tissue, leading to a decrease in the fatty acid
supply to the liver. Since fewer fatty acids are esterified
30 into triglycerides, fewer will be incorporated in low-
density lipoproteins (LDL), thus reducing LDL cholesterol
levels. It has also been noted that nicotinic acid increases
HDL cholesterol levels appreciably, most likely by

inhibition of the catabolism of this form of HDL cholesterol.

In particular, nicotinic acid has a strong peripheral vasodilator effect. Thus, the intravenous injection of
5 nicotinyl alcohol after its conversion into nicotinic acid leads to vasodilatation favorable to a drop in arterial pressure.

Nicotinic acid is widely used in therapies for lowering cholesterol and lipids.

10 It has also been shown that nicotinic acid can be combined with HMG-CoA reductase inhibitors, such as statins, for example, in cases in which the lowering of cholesterol by these HMG-CoA reductase inhibitors does not prove to be adequate. Such a combination can be beneficial when the
15 benefits from the effects of each compound are sought, in particular lowering LDL cholesterol with statins and raising HDL cholesterol with nicotinic acid. In addition, nicotinic acid is suitable for the treatment of mixed dyslipidemia and is thus capable of influencing both cholesterol and
20 triglyceride levels.

Panthenol is the alcohol derivative of pantothenic acid, more commonly known as vitamin B5. In the body, panthenol is transformed into pantothenic acid. Pantothenic acid then becomes a significant part of the compound
25 coenzyme A, which is of particular interest in cellular metabolism. Indeed, it takes part in the metabolism of lipids, carbohydrates and proteins. Panthenol also participates in the formation of acetylcholine and adrenal steroids. It also acts in the detoxification of foreign
30 bodies and in resistance to infection.

Inositol (vitamin B7) mobilizes fats by preventing their accumulation. It also has an anxiolytic effect, it stimulates the nervous system and the liver and it decreases

blood cholesterol level. It is implicated in an increase in serotonin activity, control of intracellular calcium concentration, maintenance of cell membrane potential and cytoskeleton assembly.

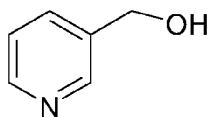
5 Isosorbide, in particular isosorbide mononitrate, is a powerful peripheral vasodilator.

Surprisingly, the inventors have discovered that docosahexaenoic acid (DHA) esters with alcohols chosen among group-B vitamins or provitamins such as nicotinyl alcohol (B3), panthenol (B5) and inositol (B7), or with isosorbide
10 or isosorbide mononitrate, in particular pyridin-3-ylmethyl docosahexaeneoate (docosahexaenoic acid (DHA) ester with nicotinyl alcohol), also have significant activity with respect to cardiovascular disease.

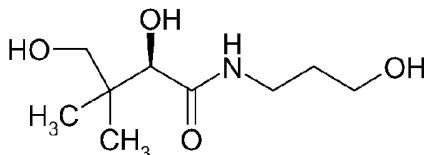
15 Thus, the present invention relates to a docosahexaenoic acid ester with an alcohol chosen among the group-B vitamins or provitamins, advantageously comprised by:

- nicotinyl alcohol of the following formula:

20



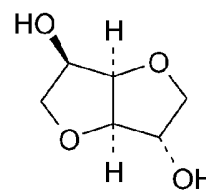
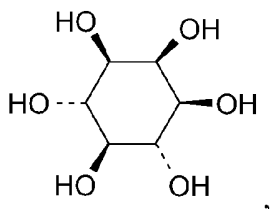
- panthenol of the following formula:



and

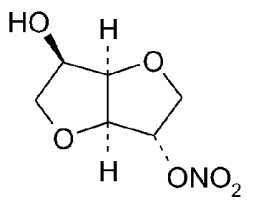
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- inositol of the following formula:



or with isosorbide of the following formula:

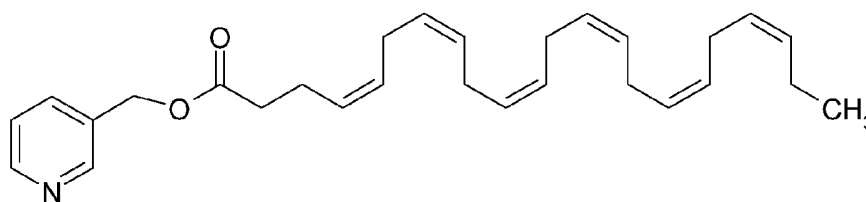
or isosorbide mononitrate of the following formula:



5

Advantageously the ester according to the present invention is pyridin-3-ylmethyl docosahexaeneoate of following general formula (1):

10



(1)

The present invention also relates to a method of preparation of the docosahexaenoic acid ester according to the present invention, in particular the pyridin-3-ylmethyl docosahexaeneoate (1) according to the present invention, by transesterification of docosahexaenoic acid ethyl ester with

15

an alcohol chosen among the group comprised of nicotinyl alcohol, panthenol, isosorbide, isosorbide mononitrate and inositol, advantageously with nicotinyl alcohol.

Transesterification can be carried out by methods well-known to those persons skilled in the art.

Advantageously, transesterification according to the present invention is carried out in the presence of a catalyst. Advantageously, such a catalyst is an alkaline-metal carbonate or alkaline-earth carbonate, advantageously K_2CO_3 . Advantageously, the molar ratio of alkaline-metal carbonate or alkaline-earth carbonate to DHA ethyl ester is in the range of 1/1 to 6/1. Advantageously, the molar ratio of alcohol to DHA ethyl ester is in the range of 1/1 to 6/1, even more advantageously the molar ratio of nicotinyl alcohol to DHA ethyl ester is in the range of 1/1 to 6/1. Advantageously, the transesterification reaction is carried out in a solvent, advantageously chosen among dioxane or THF; advantageously THF is chosen. Advantageously, THF is degassed by nitrogen bubbling. Even more advantageously, the reaction mixture is heated under reflux, advantageously for at least 14 hours.

In another specific embodiment of the invention, the catalyst of the transesterification method according to the present invention is a lipase, advantageously a *Candida antarctica* lipase. In particular, the lipase is in an immobilized form. Advantageously, the lipase is Novozyme[®] sold by Novo Nordisk. Advantageously, the reaction takes place in a medium without solvent, or in a solvent such as 2-methyl-2-butanol or acetonitrile, advantageously in a medium without solvent in the case of nicotinyl alcohol and in a solvent in the case of panthenol. Advantageously, in the case of inositol, the solvent used is an ionic polar solvent such as 1-butyl-3-methylimidazolium BF₄ or 1-butyl-

3-methylimidazolium $C(CN)_2$. Advantageously, the reaction takes place at a temperature higher than room temperature, advantageously at 60 °C.

Advantageously, ethanol is eliminated during the
5 reaction, advantageously under a vacuum or by nitrogen bubbling, even more advantageously by nitrogen bubbling. In this way the conversion rate is increased, the reaction is accelerated and the parasitic hydrolysis reaction is eliminated.

10 Advantageously, the molar ratio of alcohol to DHA ethyl ester is between 1 and 5, advantageously between 1.5 and 4.5.

Advantageously, the reaction is carried out for between 1 hour and 100 hours, advantageously between 1 hour and 72
15 hours, advantageously between 1 hour and 48 hours, even more advantageously between 1 hour and 3 hours.

In another specific embodiment of the method according to the present invention, the transesterification reaction takes place in an anhydrous solvent, in a non-anhydrous
20 solvent in the presence of a water trap such as, for example, lithium chloride, $MgCl_2$ or silica gel, or without solvent in a dry atmosphere. In this way the parasitic hydrolysis reaction is eliminated.

Advantageously, the transesterification reaction takes
25 place with pure docosahexaenoic acid ethyl ester (at least 95% pure, available commercially or purified by methods well-known to those persons skilled in the art from a mixture of ethyl ester fatty acids) or with a mixture containing at least 70% molar DHA ethyl ester. In the case
30 in which the DHA ethyl ester used is a mixture, it is advisable to purify the ester obtained following the transesterification reaction.

The present invention also relates to a pharmaceutical composition comprising the docosahexaenoic acid ester according to the present invention, in particular the pyridin-3-ylmethyl docosahexaeneoate according to the present invention, and at least one pharmaceutically-acceptable excipient.

The pharmaceutical compositions according to the present invention can be formulated for administration in mammals, including man. Dosing varies according to the treatment and to the disease in question. These compositions are prepared in such a way as to be administered by oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal route. In this case, the active ingredient can be administered in unit-dose forms or in a mixture with conventional pharmaceutical vehicles to animals or to humans. Suitable unit-dose administration forms include oral-route forms such as tablets, gelatin capsules, powders, granules and oral solutions or suspensions, sublingual and oral administration forms, subcutaneous, topical, intramuscular, intravenous, intranasal or intraocular administration forms and rectal administration forms.

When a solid composition is prepared in tablet form, the primary active ingredient is mixed with a pharmaceutical vehicle such as gelatin, starch, lactose, magnesium stearate, talc, gum arabic, silica or analogues. Tablets can be coated with sucrose or other suitable materials or they can be treated in such a way that they have delayed or extended activity and that they continuously release a predetermined quantity of the active ingredient.

A gelatin capsule preparation is obtained by mixing the active ingredient with a diluent and then pouring the mixture obtained into soft or hard gelatin capsules.

A preparation in syrup or elixir form can contain the active ingredient in conjunction with a sweetener, an antiseptic, as well as a flavoring agent and a suitable coloring agent.

5 Powders or granules that can be dispersed in water can contain the active ingredient in a mixture with dispersion agents, wetting agents or suspension agents, as well as with taste correctors or sweeteners.

Suppositories, which are prepared with binders that
10 melt at rectal temperature, such as cocoa butter or polyethylene glycol, for example, are used for rectal administration.

Of use in parenteral (intravenous, intramuscular, etc.), intranasal or intraocular administration are aqueous
15 suspensions, isotonic saline solutions or sterile injectable solutions containing pharmacologically-compatible dispersion agents and/or wetting agents.

The active ingredient can be also formulated in the form of microcapsules, optionally with one or more
20 additives.

Advantageously, the pharmaceutical composition according to the present invention is for administration by oral or intravenous route, advantageously by intravenous route in the case of treatment postinfarction.

25 The pharmaceutical composition according to the present invention can include other active ingredients that give rise to a complementary or possibly synergistic effect. Advantageously, the pharmaceutical composition does not include EPA ester.

30 The present invention also relates to the docosahexaenoic acid ester according to the present invention, in particular the pyridin-3-ylmethyl docosahexaeneoate according to the present invention, or the

pharmaceutical composition according to the present invention, for use as a drug.

The present invention also relates to the docosahexaenoic acid ester according to the present invention, in particular the pyridin-3-ylmethyl docosahexaeneoate according to the present invention, or the pharmaceutical composition according to the present invention, for use as a drug for the prevention and/or treatment of cardiovascular disease, advantageously related to heart rhythm (preferably rhythm disorders or conduction disorders), preferably chosen among auricular and/or ventricular arrhythmia, tachycardia and/or fibrillation; for the prevention and/or treatment of diseases represented by defects in electric conduction in myocardial cells; for the prevention and/or treatment of multiple risk factors for cardiovascular disease, advantageously chosen among hypertriglyceridemia, hypercholesterolemia, hypertension, hyperlipidemia, dyslipidemia, advantageously mixed dyslipidemia, and/or factor VII hyperactivity in blood coagulation; for the treatment and/or primary or secondary prevention of cardiovascular disease derived rhythm disorders, such as auricular and/or ventricular arrhythmia, tachycardia, fibrillation and/or electrical conduction defects induced by myocardial infarction, advantageously sudden death; and/or for treatment postinfarction.

Rhythm disorders comprise in particular sinoatrial node troubles such as sinus tachycardia; atrial arrhythmia such as atrial extrasystoles, regular atrial tachycardia or auricular fibrillation; junctional tachycardia such as paroxysmal junctional tachycardia or Wolff-Parkinson-White syndrome; or ventricular arrhythmia such as premature ventricular contraction, ventricular tachycardia or ventricular fibrillation.

Conduction disorders comprise in particular bradycardia.

Finally, the present invention relates to the docosahexaenoic acid ester according to the present
5 invention, in particular the pyridin-3-ylmethyl docosahexaeneoate according to the present invention, or the pharmaceutical composition according to the present invention, for use as a drug for the prevention and/or treatment of auricular fibrillation.

10 Without being bound by theory, it appears that the docosahexaenoic acid ester according to the present invention, in particular the pyridin-3-ylmethyl docosahexaeneoate according to the present invention, releases in the body alcohol and DHA, in particular
15 nicotinyl alcohol and DHA in the case of pyridin-3-ylmethyl docosahexaeneoate, via esterase activity. Thus, the docosahexaenoic acid ester according to the present invention appears to have the same activity as a mixture of DHA and alcohol. Thus, if this alcohol is a group-B vitamin
20 or provitamin, the docosahexaenoic acid ester according to the present invention will have the same effect as a mixture of DHA and a group-B vitamin or provitamin. It also appears that, in the case of pyridin-3-ylmethyl docosahexaeneoate, nicotinyl alcohol is transformed in the body into nicotinic
25 acid. Thus, the pyridin-3-ylmethyl docosahexaeneoate according to the present invention appears to have the same activity as a mixture of DHA and nicotinic acid. The advantage of the vasodilator effect of nicotinic acid is the most satisfactory distribution of DHA in the periphery, in
30 particular in the case of intravenous injection of pyridin-3-ylmethyl docosahexaeneoate, after conversion of nicotinyl alcohol into nicotinic acid.

Throughout the description and claims of the specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

5

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

10

The invention will be better understood in reference to the figure and to the examples which follow.

Figure 1 represents DHA-EE consumption percentage as a function of time for examples 3.1 (open tube), 3.2 (under vacuum) and 3.3 (under nitrogen bubbling) during the transesterification reaction in the presence of 200 mg of Novozyme® at 60 °C with an alcohol to ester ratio of 3.

The following are given as non-limiting examples.

10 **Example 1: Synthesis of pyridin-3-ylmethyl docosahexaeneoate using K₂CO₃**

1 g (2.8 mmol) of ethyl docosahexaeneoate (purity higher than 95%; supplied by Interchim) is placed in 5 ml of THF degassed by nitrogen bubbling in the presence of 1.53 g (11 mmol) of ground K₂CO₃ and 1.06 ml (10.9 mmol) of nicotinyl alcohol (purity higher than 98%; provided by Acros). The reaction mixture is heated under reflux for 7 h and then 0.76 g (5.5 mmol) of K₂CO₃ is added and heating is continued for 7 h.

20 After cooling, the reaction mixture is taken up in water and then extracted with ethyl acetate. The organic phases are dried on MgSO₄, filtered and then concentrated to dryness. The residue obtained is purified by silica flash chromatography (CH₂Cl₂ → 90/10 CH₂Cl₂/ethyl acetate gradient for 15 min). A clear oil is isolated (0.84g, yield 71%).

25 Silica gel TLC 60 F 254 Merck, 90/10 CH₂Cl₂/AcOEt, Rf=0.35.

30 **Example 2: Synthesis of pyridin-3-ylmethyl docosahexaeneoate using a lipase**

All reactions are carried out in a discontinuous mixing reactor (magnetic mixing) at the optimal temperature for each enzyme.

The products used are:

- a mixture of ethyl esters enriched to 70% in DHA ethyl ester (DHA-EE) (sold by Croda Chemical Ltd.) hereafter referred to as "70% DHA-EE ester mixture;"
- 5 - Novozyme[®], immobilized form of *Candida antarctica* lipase sold by Novo Nordisk;
- nicotinyl alcohol.

The reaction mixture is either:

- a medium without solvent which uses only the substrates; or
- 10 - an organic medium using various solvents.

The solvents used in this organic medium are:

- 2-methyl-2-butanol (2M2B), a moderately polar solvent that allows the joint solubilization of hydrophobic compounds such as polyunsaturated fatty acid esters and hydrophilic compounds such as nicotinyl alcohol;
- 15 or
- acetonitrile, for the same reasons as for 2M2B.

The reaction conditions are summarized in following table 1:

Table 1: Reaction conditions tested for the transesterification of 70% DHA-EE esters with nicotinyl alcohol.

Alcohol	Medium	[70% DHA-EE esters] (M)	[Alcohol]	Total volume (ml)	Alcohol/ester molar ratio
Nicotinyl alcohol	Organic (2M2B and acetonitrile)	0.43	0.64	12	1.5
	Solvent-free	1.5	4.5	3.5	3

Each condition was incubated with 200 mg of Novozyme[®] at 60 °C. Reactions in 2M2B carried out in open air (under a fume hood) were tested at 60 °C with 200 mg of Novozyme[®].

5 Regular 500 µl samples are taken until the reactions are complete. The reaction process is quenched by centrifugation for 5 minutes at 13,000 rpm, allowing withdrawal of the immobilized enzyme from the medium. All samples are stored at 4 °C until analyzed.

10 Control reactions without enzyme and control reactions without co-substrate (nicotinyl alcohol) were run in parallel.

Analyses are performed by using two HPLC methods (using an Agilent 1100 series apparatus) according to the following
15 parameters:

Method 1

- Zorbax SB-C18 column (4.6 mm X 25 cm)
- Temperature: 40 °C
- 20 - Flow rate: 1 ml/min
- Eluent: 0.02% methanol/acetic acid
- Detection: refractometry
- Run duration: 15 minutes

Method 2

- 25 - Zorbax SB-C18 column (4.6 mm X 25 cm)
- Temperature: 40 °C
- Flow rate: 3 ml/min
- Eluent: 50/50 acetonitrile/acetone
- Detection: refractometry
- 30 - Run duration: 15 minutes

The samples taken during the various reactions are diluted beforehand to a concentration less than 100 mM in a

0.02% methanol/acetic acid mixture in the case of method 1 and in acetone in the case of method 2.

Results and discussion:

5 Two species appear during the transesterification reaction. The first is eluted at 4.15 minutes and corresponds to the ester hydrolysis product and the second is eluted at 4.85 minutes under analysis conditions. The latter compound corresponds to the product of
10 transesterification between the 70% DHA-EE esters and nicotiny alcohol. Here, only one product is expected, as the nicotiny alcohol has only a single primary hydroxyl.

The conversion percentages obtained under the various reaction conditions are given in following table 2:

15

Table 2: Conversion percentage obtained during the transesterification of 70% DHA-EE ethyl esters with nicotiny alcohol (: in this case, the tube is left open to allow evaporation of the ethanol produced during the
20 reaction).*

Reaction conditions	Conversion percentages of DHA-EE into DHA-nicotiny alcohol
Acetonitrile	31% in 72 h
2M2B	47% in 48 h
2M2B in open air*	60% in 118 h
Solvent-free	11% in 72 h
Solvent-free in open air*	100% in 72 h

Conversion rates are higher when the reactions are carried out in open air; the ethanol produced evaporates

which shifts the equilibrium of the reaction towards the synthesis of DHA-nicotinyl alcohol. These transesterification reactions are accompanied by heavy blackening of the reaction mixture.

5 Hydrolysis products appear preferentially when 2M2B is used as a reaction solvent. However, a weak hydrolysis reaction is also present in a medium without solvent. Thus it appears that water is also present in the nicotinyl alcohol used or that ambient moisture causes this parasitic
10 reaction.

The feasibility of transesterification reactions of 70% DHA-EE esters with nicotinyl alcohol has been demonstrated and such reactions show advantageous conversion rates near to or greater than 90%, in particular when ethanol produced
15 during the reaction is eliminated from the reaction mixture. However, a parasitic hydrolysis reaction due to the presence of water in the solvents used and/or to ambient moisture interferes with these syntheses.

It thus appears of interest to try to avoid the
20 parasitic hydrolysis reaction observed. Completely anhydrous solvents could be used, for example. It is also possible to carry out these same reactions in the presence of a water trap (lithium chloride, $MgCl_2$ or silica gel, for example) to eliminate any possibility of hydrolysis.

25 For the nicotinyl alcohol-DHA ester synthesis reaction, ethanol produced during the reaction appears to be an element that limits the reactions. Its elimination shifts the equilibrium of the reaction towards the synthesis of the esters considered. Thus, it is advisable to optimize this
30 elimination, notably when carrying out syntheses under reduced pressure. This allows rapid evaporation of ethanol and thus an increase in reaction speeds.

Example 3: Synthesis of pyridin-3-ylmethyl docosaheptaenoate using a lipase; transesterification optimization; evaporation of ethanol produced during the reaction and elimination of oxidative browning.

5 A synthesis reaction similar to that of example 2 was carried out using the same starting products (nicotiny alcohol, 70% DHA-EE ester mixture, Novozyme®) in a medium without solvent at 60 °C in the presence of 200 mg of Novozyme® in an alcohol to ester ratio of 3. The reactor
10 used is the same as that of example 2 and the analysis methods are the same.

Example 3.1:

The only difference compared to example 2 is that the
15 reaction was carried out in an open receptacle (open tube).

Results (figure 1):

The transesterification reaction is "slow," taking nearly 80 hours in total. Oxidative browning is present. "Strong" parasitic hydrolysis is present.

20

Example 3.2:

The only difference compared to example 2 is that the reaction was carried out under vacuum.

Results (figure 1):

25 There is acceleration of the reaction compared to example 3.1 but it remains "slow," taking nearly 48 hours in total.

Additionally, oxidative browning and parasitic hydrolysis persist.

30

Example 3.3:

The only difference compared to example 2 is that the reaction was carried out under nitrogen bubbling.

Results (figure 1):

There is very significant acceleration of the reaction, which becomes total in less than 3 hours because of instantaneous elimination of the ethanol produced during the reaction and an improved mixture.

The absence of oxidative browning is noted.

Parasitic hydrolysis is strongly decreased.

Example 4: Synthesis of DHA ester with panthenol using a lipase

The experimental and analytic conditions are the same as in example 2 except for the following differences:

Reaction conditions are summarized in following table 3:

Table 3: Reaction conditions tested for the transesterification of 70% DHA-EE esters with panthenol.

Alcohol	Medium	[70% DHA-EE esters] (M)	[Alcohol]	Total volume (ml)	Alcohol/ester molar ratio
Panthenol	Organic (2M2B and acetonitrile)	0.43	1.28	12	3

Results and discussion:

Two species are eluted at 3.9 minutes and 4.14 minutes under analysis conditions. Panthenol has two primary alcohols. Thus the production of several products (three maximum) could be envisaged. However, for the control without co-substrate (panthenol), the peak at 4.14 minutes appears. Said peak would thus correspond to ethyl ester hydrolysis related to the presence of water in the solvent

used. This reaction is observed only in the presence of enzyme.

Consequently, only the first peak corresponds to panthenol-DHA ester synthesis.

5 The conversion percentages obtained under the various reaction conditions are summarized in following table 4:

Table 4: Conversion percentage obtained during the transesterification of 70% DHA-EE ethyl esters with panthenol (: in this case, the tube is left open to allow*
10 *evaporation of the ethanol produced during the reaction).*

Reaction conditions	Conversion percentages of DHA-EE into DHA-panthenol
Acetonitrile	68% in 136 h
2M2B	76% in 115 h
2M2B in open air*	88% in 96 h

It appears that the conversion percentage of 70% DHA-EE
15 esters increases when the reaction is carried out in open air. Indeed, under this condition, the ethanol produced during the reaction evaporates. The equilibrium of the reaction is thus shifted towards the synthesis of panthenol-DHA esters. Moreover, these conversion values are certainly
20 underestimated due to the joint evaporation of 2M2B solvent (medium concentration effect). These transesterification reactions are also accompanied by heavy blackening of the reaction mixture.

The feasibility of transesterification reactions of 70%
25 DHA-EE esters with panthenol has been demonstrated and such reactions show advantageous conversion rates near to or greater than 90%, in particular when ethanol produced during

the reaction is eliminated from the reaction mixture. However, a parasitic hydrolysis reaction due to the presence of water in the solvents used and/or to ambient moisture interferes with these syntheses.

5 It thus appears of interest to try to avoid the parasitic hydrolysis reaction observed. Completely anhydrous solvents could be used, for example. It is also possible to carry out these same reactions in the presence of a water trap (lithium chloride, $MgCl_2$ or silica gel, for example) to
10 eliminate any possibility of hydrolysis.

For the panthenol-DHA ester synthesis reaction, ethanol produced during the reaction appears to be an element that limits the reactions. Its elimination shifts the equilibrium of the reaction towards the synthesis of the esters
15 considered. Thus, it is advisable to optimize this elimination, notably when carrying out syntheses under reduced pressure. This allows rapid evaporation of ethanol and thus an increase in reaction speeds.

20 **Example 5: Comparative results of the action of EPA and DHA on ultrarapid potassium current and thus on auricular fibrillation.**

The cardiac action potential is the basic electrical unit of excitable cardiac cells and represents the activity
25 of several types of ion channels responsible for the various phases of the action potential. Different types of action potentials correspond to different cardiac regions, thus allowing sequential and coordinated activity in these regions. For this reason, Kv 1.5 potassium channels, coded
30 by the KCNA5 gene, are expressed only in auricle tissue and are responsible for the ultrarapid potassium current (I_{Kur}) which acts in the repolarization of the auricular action potential. This highly localized expression of Kv 1.5 is in

fact a target of choice in the treatment of auricular fibrillation, a pathology in which changes in auricular action potentials are observed.

Thus, the effects of DHA and EPA on I_{Kur} were studied. For this purpose, the human isoform of the Kv 1.5 channel (hKv 1.5) was transfected in a stable manner in HEK 293 (human embryonic kidney) cells and the current resulting from the activity of these channels was studied using a whole-cell patch-clamp technique.

10

Materials and methods

Maintenance of the cell line.

HEK 293-hKv 1.5 cells are grown under standard conditions (37 °C, incubator at 95% O₂ and 5% CO₂) in Falcon dishes up to 80% confluence. They are then removed and cultured in 35 mm petri dishes containing the following culture medium: DMEM (Invitrogen); 10% fetal bovine serum (Invitrogen); a mixture of 100 U/ml penicillin, 100 µg/ml streptomycin and 0.25 mg/ml glutamine (Invitrogen); and 1.25 mg/ml of Geneticin[®] as a selective antibiotic.

20

Electrophysiology.

I_{Kur} is studied using the whole-cell patch-clamp technique at ambient temperature (19-22 °C). The pipette medium contains: 125 mM K-aspartate, 20 mM KCl, 10 mM EGTA, 5 mM HEPES, 5 mM Mg-ATP, 1 mM MgCl₂, pH 7.3 (KOH). The extracellular medium contains: 140 mM NaCl, 20 mM HEPES, 5 mM D(+)-glucose, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, pH 7.4 (NaOH).

25

I_{Kur} is induced every 15 seconds by 300 ms, +60 mV depolarizing pulses from a -80 mV holding potential, followed by -50 mV repolarization. The amplitude of the current peak is established from the maximum current obtained during the first 100 ms of the depolarizing pulse.

30

The amplitude of the current at the end of the pulse is determined during the last 20 ms of the depolarizing pulse.

Reagents.

DHA and EPA are supplied by Sigma. Stock solutions
5 (10 mM) are prepared in ethanol and the final concentration of solvent is 0.25%.

Results:

Results are summarized in following table 5.

10

Table 5: Percent inhibition of I_{Kur} by DHA and EPA at various concentrations.

Concentration	DHA				
	Peak I_{Kur}		End of pulse I_{Kur}		n
	Mean	SEM	Mean	SEM	
1 μ M	8.2	6.3	10.1	5.8	5
3.2 μ M	10.9	6.9	14.5	6.5	5
5.6 μ M	15.4	4.8	33.7	7.8	6
10 μ M	22.6	4.0	78.0	4.2	6
25 μ M	58.1	13.6	86.5	3.4	5

Concentration	EPA				
	Peak I_{Kur}		End of pulse I_{Kur}		n
	Mean	SEM	Mean	SEM	
1 μ M	14.6	1.7	14.9	1.9	5
3.2 μ M	16.1	3.1	19.9	4.4	5
10 μ M	17.5	6.4	36.6	7.2	10
25 μ M	5.4	6.8	61.6	7.3	5

15

EPA slightly decreases peak I_{Kur} amplitude (maximum inhibition of $17.5 \pm 6.4\%$, $n=10$, $p < 0.05$ at $10 \mu\text{M}$) and end-of-pulse current amplitude ($61.6 \pm 7.3\%$, $n=5$, $p < 0.05$ at $25 \mu\text{M}$).

5 DHA inhibits peak I_{Kur} amplitude by a maximum of $58.1 \pm 13.6\%$ ($n=5$, $p < 0.005$) and that of end-of-pulse current amplitude by $86.5 \pm 3.4\%$ ($n=5$, $p < 0.005$) at $25 \mu\text{M}$.

Conclusion:

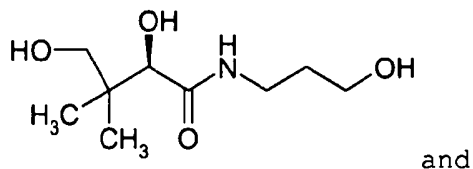
10 These results show that the application of DHA inhibits, more strongly than EPA and in a concentration-dependant manner, the ultrarapid potassium current (I_{Kur}) of human Kv 1.5 channels transfected in HEK 293 cells. DHA acts preferentially on end-of-pulse current, suggesting an effect on the inactivation of Kv 1.5 channels. Moreover, this
15 effect is accompanied by a decrease in peak I_{Kur} (contrary to that observed for EPA), potentiating I_{Kur} inhibition by DHA.

These effects on I_{Kur} indicate a beneficial action of DHA on auricular fibrillation.

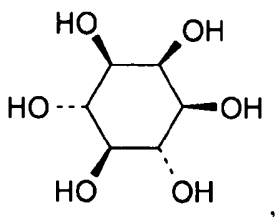
The claims defining the invention are as follows:

1. A docosahexaenoic acid ester with an alcohol chosen among the group consisting of:

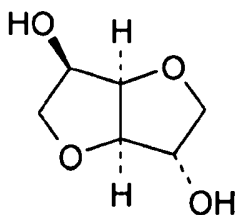
5 - panthenol of the following formula:



- inositol of the following formula:

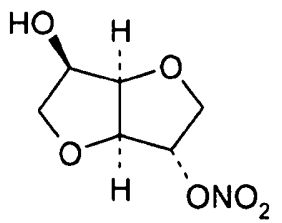


or with isosorbide of the following formula:



10

or isosorbide mononitrate of the following formula:



2. A docosahexaenoic ester with panthenol.

3. A method of preparation of docosahexaenoic acid ester according to claim 1 or claim 2 by transesterification of docosahexaenoic acid ethyl ester with the alcohol chosen among the group consisting of panthenol, isosorbide, isosorbide mononitrate and inositol.
4. A method according to claim 3, wherein said method is carried out in the presence of a catalyst.
5. A method according to claim 4, wherein the catalyst is a lipase, advantageously *Candida antarctica* lipase.
6. A method according to claim 5, wherein ethanol is eliminated during the reaction, advantageously by nitrogen bubbling.
7. A method according to claim 5 or claim 6, wherein the reaction takes place in an anhydrous solvent or without solvent in a dry atmosphere.
8. A pharmaceutical composition comprising docosahexaenoic acid ester according to claim 1 or claim 2 and at least one pharmaceutically-acceptable excipient.
9. Docosahexaenoic acid ester according to claim 1 or claim 2, or a pharmaceutical composition according to claim 8, for use as a drug.
10. Docosahexaenoic acid ester according to claim 1 or claim 2, or pyridine-3-ylmethyl docosahexaenoate, or a pharmaceutical composition according to claim 8, when used for the prevention and/or treatment of cardiovascular diseases related to heart

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5 rhythm, preferably chosen among auricular and/or ventricular arrhythmia, tachycardia and/or fibrillation; for the prevention and/or treatment of diseases represented by defects in electric conduction in myocardial cells; for the prevention and/or treatment of multiple risk factors for cardiovascular disease, chosen among hypertriglyceridemia, hypercholesterolemia, hypertension, hyperlipidemia, dyslipidemia, advantageously mixed dyslipidemia; for the treatment and/or primary or secondary prevention of cardiovascular disease derived from heart rhythm disorders, such as auricular and/or ventricular arrhythmia, tachycardia, fibrillation and/or electrical conduction defects induced by myocardial infarction, advantageously sudden death; and/or for treatment postinfarction.

15

11. Docosahexaenoic acid ester according to claim 1 or claim 2, or pyridine-3-ylmethyl docosahexaenoate, or a pharmaceutical composition according to claim 8, when used for the prevention and/or treatment of auricular fibrillation.

20

12. A method of treatment and/or prevention of cardiovascular diseases related to heart rhythm, preferably chosen among auricular and/or ventricular arrhythmia, tachycardia and/or fibrillation; prevention and/or treatment of diseases represented by defects in electric conduction in myocardial cells; prevention and/or treatment of multiple risk factors for cardiovascular disease, chosen among hypertriglyceridemia, hypercholesterolemia, hypertension, hyperlipidemia, dyslipidemia, advantageously mixed dyslipidemia, treatment and/or primary or secondary prevention of cardiovascular disease derived from heart rhythm disorders, such as auricular and/or ventricular arrhythmia, tachycardia, fibrillation and/or electrical conduction defects induced by myocardial infarction, advantageously sudden death; and/or treatment postinfarction;

25
30

comprising administering to a person in need thereof a therapeutically effective amount of a docosahexaenoic acid ester according to claim 1 or claim 2, or pyridine-3-ylmethyl docosahexaenoate, or a pharmaceutical composition according to claim 8.

13. A method of treatment and/or prevention of auricular fibrillation comprising administering to a person in need thereof, a therapeutically effective amount of a docosahexaenoic acid ester according claim 1 or claim 2, or pyridine-3-ylmethyl docosahexaenoate, or a pharmaceutical composition according to claim 8.

14. A docosahexaenoic acid ester prepared by the method of any one of claims 3 to 7.

15. A docosahexaenoic acid ester according to claim 1 or claim 2 substantially as hereinbefore described with reference to any one of the Examples and/or Figure.

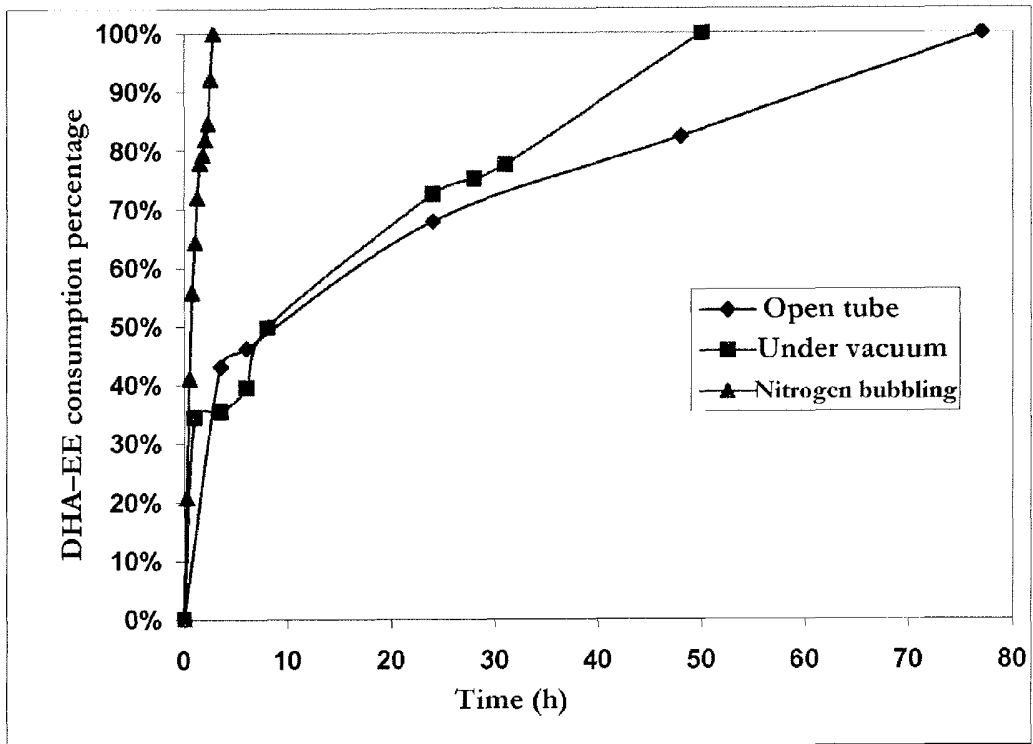


FIG. 1