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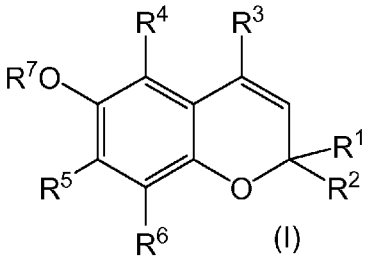
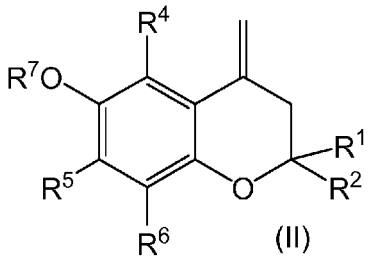
(54) **Title:** NOVEL USE OF SUBSTITUTED 2*H*-CHROMENS AND THEIR DERIVATIVES



(I)



(II)

(57) **Abstract:** The present invention is directed towards the use of 2*H*-chromens and their derivatives of formula (I) and/or of formula (II) wherein R^1 and R^2 are independently from each other H or CM_{1-11} -alkyl or $(CH_2)_n$— OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and R^7 is H or C_{1-6}-alkyl, as antioxidants in PUFA-containing edible oils such as marine oil, microbial oil, fungal oil, algal oil and PUFA-containing plant oil for human consumption. The present invention is further directed towards these PUFA- containing edible oils comprising at least one compound of formula (I) and/or at least one compound of formula (II). The present invention is further directed to a method of preserving the shelf life of PUFAs and/or their esters in an edible oil comprising the step of adding at least one compound of formula (I) and/or at least one compound of formula (II) to said edible oil, as well as to a method of limiting the amount of oxidation of PUFAs and/or their esters in an edible oil which is exposed to air, comprising adding at least one compound of formula (I) and/or at least one compound of formula (II) to said edible oil, preferably in an amount of said compound of formula (I) and/or said compound of formula (II) ranging from 10 to 500 ppm, preferably ranging from 30 to 300 ppm, more preferably ranging from 100 to 250 ppm, based on the total amount of said edible oil.

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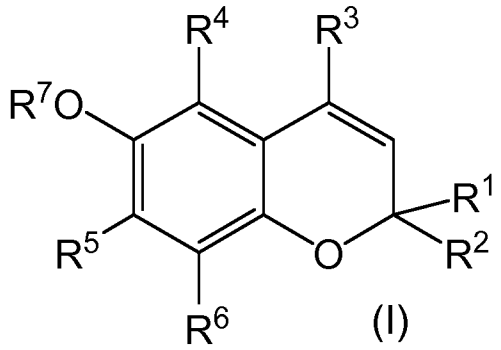
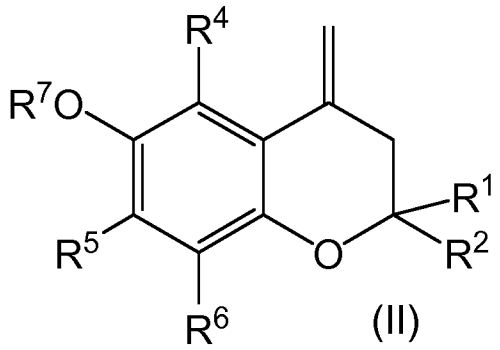
Novel use of substituted 2*H*-chromens and their derivatives

The present invention is directed to the use of a compound of formula (I) and/or a compound of formula (II) as antioxidant in oil,



(I)



(II)

wherein the oil contains polyunsaturated fatty acids and/or their esters, and wherein the oil is for human consumption, i.e. wherein the oil is edible, and wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and

wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and

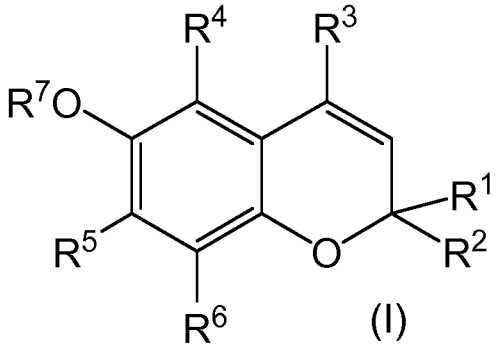
R^7 is H or C_{1-6}-alkyl.

Oils containing polyunsaturated fatty acids and/or their esters are gaining more and more attention, because of their beneficial health effects in humans.

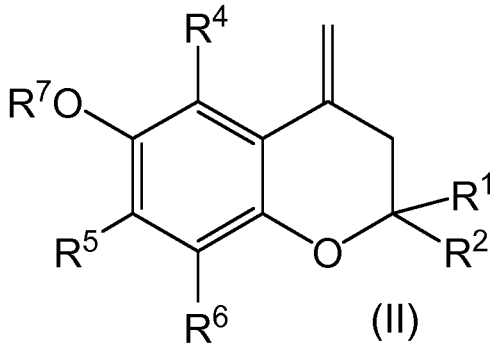
Since these oils are only of limited stability, because they are oxidized very easily, there is a need to provide efficient antioxidants for their stabilization.

Detailed description of the invention

This need is fulfilled by the present invention, which is directed to the use of at least one compound of formula (I) and/or at least one compound of formula (II) as antioxidant in oil,



(I)



(II)

wherein the oil contains polyunsaturated fatty acids and/or their esters, and wherein the oil is for human consumption, i.e. the oil is edible, and

wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and

wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and

R^7 is H or C_{1-6}-alkyl;

and with the preferences for the substituents R^1 to R^7 as given below.

Compounds of formulae (I) and (II)

"alkyl" and "alkoxy" in the context of the present invention encompass linear alkyl and branched alkyl, and linear alkoxy and branched alkoxy, respectively.

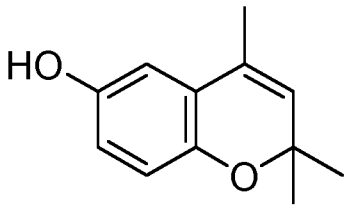
If one of R^1 and R^2 is an alkyl with more than 4 C-atoms or if one of R^1 and R^2 is a $(CH_2)_n$—OH group with more than 4 C-atoms, the other one is preferably H.

Preferably R^1 and R^2 are independently from each other H or C_{1-4}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 4, R^3, R^4, R^6 and R^7 are independently from each other H or C_{1-4}-alkyl, and R^5 is H or C_{1-4}-alkyl or C_{1-4}-alkoxy.

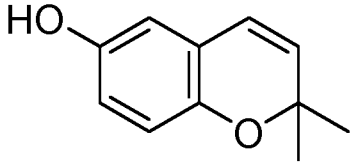
More preferably R^1 and R^2 are independently from each other H or C_{1-2}-alkyl or $(CH_2)_n$—OH with n being 1 or 2, R^3, R^4, R^6 and R^7 are independently from each other H or C_{1-2}-alkyl, and R^5 is H or C_{1-2}-alkyl or C_{1-2}-alkoxy.

Even more preferably R^1 and R^2 are independently from each other H or methyl or (CH_2)—OH, R^3, R^4, R^6 and R^7 are independently from each other H or methyl or ethyl, and R^5 is H or methyl or methoxy.

Especially preferred are the following compounds of formulae (1) to (7) (see also **Fig. 1**), whereby compounds of formulae (1) to (5) are preferred:



(1)



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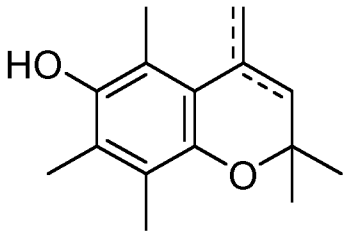

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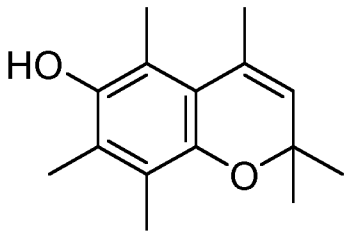

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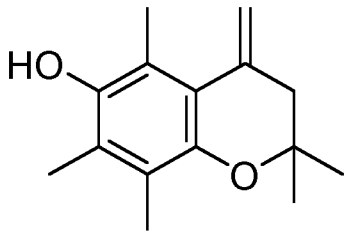

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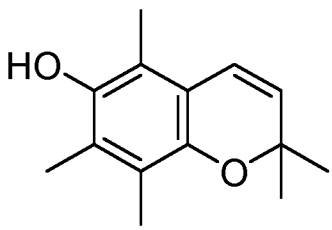

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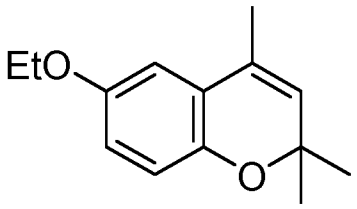
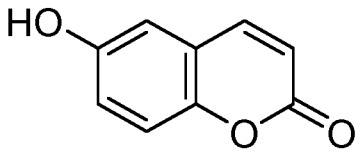


(5)

whereby "Et" = ethyl



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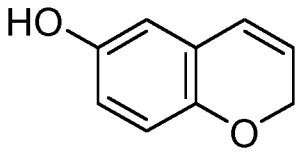


(7)

Use as antioxidants

The compounds of the present invention are efficient as antioxidants in PUFA-containing oils for human consumption.

"PUFA(s)" means polyunsaturated fatty acid(s) such as docosahexaenoic acid ("DHA") and/or eicosapentaenoic acid ("EPA") and/or docosapentaenoic acid ("DPA") and/or oleic acid and/or stearidonic acid and/or linoleic acid and/or alpha-linolenic acid ("ALA") and/or gamma-linolenic acid and/or arachidonic acid ("ARA") and/or the esters of all of them, whereby the term "esters" encompasses monoglycerides, diglycerides and triglycerides as well as C_{1-6}-alkyl esters such as especially the methyl esters and the ethyl esters, whereby the triglycerides are often dominant.

DHA, EPA, ALA and stearidonic acid are omega-3 fatty acids, whereas linoleic acid, gamma-linolenic acid and ARA are omega-6 fatty acids.

The term "DPA" encompasses two isomers, the omega-3 fatty acid clupanodonic acid (7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid) and the omega-6 fatty acid osbond acid (4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid).

In accordance with the invention, the polyunsaturated fatty acid (PUFA) is preferably DHA and/or EPA and/or DPA and/or any ester thereof, more preferably the polyunsaturated fatty acid (PUFA) is preferably DHA and/or EPA and/or any ester thereof.

Examples of PUFA-containing oils for human consumption are

- marine oil, such as preferably fish oil,
- microbial biomass containing polyunsaturated fatty acids and/or their esters ("**microbial oil**"), preferably containing high amounts of docosahexaenoic acid ("DHA") and/or eicosapentaenoic acid ("EPA") and/or docosapentaenoic acid ("DPA") and/or their esters, and
- oil containing high amounts of PUFAs and/or their esters, preferably containing high amounts of docosahexaenoic acid ("DHA") and/or eicosapentaenoic acid ("EPA") and/or docosapentaenoic acid ("DPA") and/or their esters, extracted from microbial biomass, such as fungae ("**fungal oil**") or algae ("**algal oil**"), and
- plant oil with relatively high amounts of PUFAs and/or their esters, ("**PUFA-containing plant oil**"), such as e.g. canola seed oil, linseed/flaxseed oil, hempseed oil, pumpkin seed oil, evening primrose oil, borage seed oil, blackcurrent seed oil, sallow thorn/sea buckthorn oil, chia seed oil, argan oil and walnut oil.

Marine oils, microbial oils and algal oils are especially preferred.

Further objects of the present invention

Thus, in addition, the present invention is

(1) directed to the use of at least one compound of formula (I) and/or at least one compound of formula (II) with the preferences as given above as antioxidant(s) in marine oils, microbial oils, oils containing high amounts of

PUFAs and/or their esters extracted from microbial biomass and plant oils with relatively high amounts of PUFAs and/or their esters for human consumption; as well as

(2) directed to these PUFA-containing oils for human consumption comprising at least one compound of formula (I) and/or at least one compound of formula (II) with the preferences as given above.

Furthermore, the present invention is directed to

(3) an edible oil comprising PUFAs and/or their esters and at least one compound of formula (I) and/or at least one compound of formula (II);

(4) a method of preserving the shelf life of PUFAs and/or their esters in an edible oil comprising the step of adding at least one compound of formula (I) and/or at least one compound of formula (II) to said edible oil, preferably in an amount of said compound of formula (I) and/or said compound of formula (II) ranging from 10 to 500 ppm, preferably ranging from 30 to 300 ppm, more preferably ranging from 100 to 250 ppm, based on the total amount of the edible oil;

(5) a method of limiting the amount of oxidation of PUFAs and/or their esters in an edible oil which is exposed to air, comprising adding at least one compound of formula (I) and/or at least one compound of formula (II) to said edible oil, preferably in an amount of said compound of formula (I) and/or said compound of formula (II) ranging from 10 to 500 ppm, preferably ranging from 30 to 300 ppm, more preferably ranging from 100 to 250 ppm, based on the total amount of the edible oil.

For all these objects (1) to (5) of the present invention the preferences with respect to the compound of formula (I) and the compound of formula (II) and the PUFA-containing oil for human consumption, i.e. the PUFA-containing edible oil, as given above and below apply.

Further antioxidants

The compounds of formulae (I) and/or (II) can be used in combination with one or more other antioxidants as described below.

In an embodiment of the present invention the edible PUFA-containing oils of the present invention comprising at least one compound of formula (I) and/or at least one compound of formula (II) additionally comprise ascorbyl palmitate.

Instead of ascorbyl palmitate other esters of ascorbic acid such as the esters of ascorbic acid with linear C_{12-20} alkanols, preferably the esters of ascorbic acid with linear C_{14-18} alkanols, may also be used, so that further embodiments of the present invention are directed to edible PUFA-containing oils comprising at least one compound of formula (I) and/or at least one compound of formula (II) that additionally comprise esters of ascorbic acid with linear C_{12-20} alkanols, preferably esters of ascorbic acid with linear C_{14-18} alkanols, more preferably ascorbyl palmitate.

The edible PUFA-containing oils of the present invention comprising at least one compound of formula (I) and/or at least one compound of formula (II) may also comprise additionally alpha-tocopherol and/or gamma-tocopherol, whereby an ester of ascorbic acid with a linear C_{12-20} alkanol with the preferences as given above may additionally be present.

The PUFA-containing oils themselves are described in more detail below.

PUFA-containing oils

In the context of the present invention the term "PUFA-containing oil" encompasses

- marine oil, such as especially fish oil,
- microbial biomass containing polyunsaturated fatty acids ("PUFAs"), especially docosahexaenoic acid ("DHA") and/or eicosapentaenoic acid ("EPA") and/or docosapentaenoic acid ("DPA") and/or their esters ("**microbial oil**");
- oil containing high amounts of PUFAs, especially containing high amounts of DHA and/or EPA and/or DPA and/or their esters extracted

from microbial biomass as e.g., fungi ("fungal oil") or algae ("algal oil");

- Plant oil with high amounts of PUFAs and/or their esters ("**PUFA-containing plant oil**"), such as e.g. canola seed oil, linseed/flaxseed oil, hempseed oil, pumpkin seed oil, evening primrose oil, borage seed oil, blackcurrent seed oil, sallow thorn/sea buckthorn oil, chia seed oil, argan oil and walnut oil.

The term "DHA" does not only encompass the acid but also derivatives thereof such as monoglycerides, diglycerides and triglycerides as well as C_{1-6}-alkyl esters such as the methyl and ethyl esters. The same applies for "EPA" and "DPA" and all the other PUFAs.

Fish oil and algal oil are commonly used for human consumption. Instead of fish oil and algal oil also the other PUFA-containing oils named above may be used for human consumption, i.e.:

- microbial biomass containing PUFAs ("**microbial oil**")
- oil containing high amounts of PUFAs extracted from microbial biomass, such as especially fungal oil, and
- plant oil with high amounts of PUFAs.

The above-mentioned PUFA-containing oils may not only be used as alternative of fish oil and algal oil, but also in addition.

Details of these PUFA-containing oils for human consumption are given below.

Marine oil

Examples of suitable marine oils include, but are not limited to, Atlantic fish oil, Pacific fish oil, or Mediterranean fish oil, or any mixture or combination thereof.

In more specific examples, a suitable fish oil can be, but is not limited to, pollack oil, bonito oil, pilchard oil, tilapia oil, tuna oil, sea bass oil, halibut

oil, spearfish oil, barracuda oil, cod oil, menhaden oil, sardine oil, anchovy oil, capelin oil, herring oil, mackerel oil, salmonid oil, tuna oil, and shark oil, including any mixture or combination thereof.

Other marine oils suitable for use herein include, but are not limited to, squid oil, cuttle fish oil, octopus oil, krill oil, seal oil, whale oil, and the like, including any mixture or combination thereof.

For stabilizing marine oil an amount of at least one compound of formula (I) and/or at least one compound of formula (II) ranging from 10 to 500 ppm, preferably ranging from 30 to 300 ppm, more preferably ranging from 100 to 250 ppm, based on the total amount of the marine oil, is usually sufficient. The same applies for the other PUFA-containing oils such as microbial oil, algal oil, fungal oil and PUFA-containing plant oil.

A commercially available example of marine oil is the fish oil "MEG-3" (Bleached 30S TG Fish oil) from DSM Nutritional Products, LLC (US) whose specification and composition is shown in **Tables I and II** below:

Table I

ANALYSIS	SPECIFICATIONS
Colour	Max. 6 Gardner Colour
Free Fatty Acid (as % Oleic)	Max. 0.4%
p-Anisidine Value	Max. 12 (at time of release)
Peroxide Value	Max. 3 milli equivalents/kg (at time of release)
% Moisture	Max. 0.05%
Cold Test	Remains clear at 0°C for 3 hours
Cholesterol	Report Actual
TOTOX ((2 x Peroxide Value) + (*p*-Anisidine Value))	Max. 20

The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of oil. Traditionally this is expressed in units of milliequivalents or meq/kg.

Winterization is part of the processing of fish oil, and it is performed to remove solid fat in the oil. The "cold test" is performed to check if any solid fat is present and precipitated in the oil when cooled to 0°C within a specific period of time. In this fish oil (Product Code: FG30TG), any such precipitation is checked for 3 hours at 0°C.

Table II

Fatty Acid Profile	
EPA (A%)	Min. 18
EPA mg/g (as TG)	Min. 170
DHA (A%)	Min. 12
DHA mg/g (as TG)	Min. 110
EPA + DHA (A%)	Min. 30
Total Omega 3 (A%)	Min. 34

"TG" = triglyceride;
"A%" = "area %" = area percentage by GC based on 24 peak analysis (meaning the 24 highest peaks have been analyzed)

Oil containing high amounts of PUFAs, especially containing high amounts of DHA and/or EPA and/or DPA and/or their esters, extracted from microbial biomass as e.g., fungi ("fungal oil") or algae ("algal oil")

Algal oil

"Algal oil" is an oil containing high amounts of DHA and/or EPA and/or DPA and/or their esters extracted from algae as microbial source/biomass.

An example of algal oil is the commercially available "Algal oil containing EPA+DPA" from DSM Nutritional Products, LLC (US) whose composition is shown in the **Table III** below:

Table III

Fatty Acid Profile	
DHA + EPA content, mg/g oil	587 mg/g
DHA content, mg/g oil	401 mg/g
EPA content, mg/g oil	186 mg/g
TOTOX ((2 x Peroxide Value) + (*p*-Anisidine Value))	5
Free Fatty Acid	0.6%
Moisture	< 0.05%

A further example of a crude oil containing high amounts of DHA and/or EPA extracted from microbial sources as e.g., algae, is the oil extracted from Algae *Schizochytrium* Biomass, whose specification is given in the following **Table IV.**

Table IV

Specification	**Aqua (Base Product)**
DHA + EPA, mg/g oil	**minimal 500 mg/g**
DHA content, mg/g oil	**minimal 250 mg/g (at least 25% -> 40%)**
EPA content, mg/g oil	**minimal 100 mg/g (at least 10% -> 25%)**
Minimal ratio EPA:DHA	**1:4**
Maximal ratio EPA:DHA	**1:1**
TOTOX ((2 x Peroxide Value) + (*p*-Anisidine Value))	maximum 35
Free fatty acid	maximal 5%
Moisture	maximal 0.75%
DPA n-3 (omega-3 docosapentaenoic acid), %	< 6
Arachidonic Acid, %	< 2
Stearic, %	< 2.5
Palmitic, %	< 30
Shelf life	6 months at 25°C
Total Fat	Record
Crude Fat	> 92%

Microbial biomass containing polyunsaturated fatty acids ("PUFAs"), especially docosahexaenoic acid and/or eicosapentaenoic acid and/or docosapentaenoic acid ("DPA") and/or their esters

The biomass preferably comprises cells which produce PUFAs heterotrophically. According to the invention, the cells are preferably selected from algae, fungi, particularly yeasts, bacteria, or protists. The cells are more preferably microbial algae or fungi.

Suitable cells of oil-producing yeasts are, in particular, strains of *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon* and *Lipomyces*.

Oil produced by a microorganism or obtained from a microbial cell is referred to as “microbial oil”. Oil produced by algae and/or fungi is referred to as an algal and/or a fungal oil, respectively.

As used herein, a "microorganism" refers to organisms such as algae, bacteria, fungi, protist, yeast, and combinations thereof, e.g., unicellular organisms. A microorganism includes but is not limited to, golden algae (e.g., microorganisms of the kingdom *Stramenopiles*); green algae; diatoms; dinoflagellates (e.g., microorganisms of the order *Dinophyceae* including members of the genus *Crypthecodinium* such as, for example, *Crypthecodinium cohnii* or *C. cohnii*); microalgae of the order *Thraustochytriales*; yeast (*Ascomycetes* or *Basidiomycetes*); and fungi of the genera *Mucor*, *Mortierella*, including but not limited to *Mortierella alpina* and *Mortierella sect. schmuckeri*, and *Pythium*, including but not limited to *Pythium insidiosum*.

In one embodiment, the microorganisms of the kingdom *Stramenopiles* may in particular be selected from the following groups of microorganisms: *Hamatores*, *Proteromonads*, *Opalines*, *Developayella*, *Diplophrys*, *Labrinthulids*, *Thraustochytrids*, *Biosecids*, *Oomycetes*, *Hypochytridiomycetes*, *Commation*, *Reticulosphaera*, *Pelagomonas*, *Pelagococcus*, *Ollicola*, *Aureococcus*, *Parmales*, *Diatoms*, *Xanthophytes*, *Phaeophytes* (brown algae), *Eustigmatophytes*, *Raphidophytes*, *Synurids*, *Axodines* (including *Rhizochromulinales*, *Pedinellales*, *Dictyochales*),

Chrysomeridales, Sarcinochrysidales, Hydrurales, Hibberdiales, and Chromulinales.

In one embodiment, the microorganisms are from the genus *Mortierella*, genus *Crypthecodinium*, genus *Thraustochytrium*, and mixtures thereof. In a further embodiment, the microorganisms are from *Crypthecodinium Cohnii*. In a further embodiment, the microorganisms are from *Mortierella alpina*. In a still further embodiment, the microorganisms are from *Schizochytrium sp*. In yet an even further embodiment, the microorganisms are selected from *Crypthecodinium Cohnii, Mortierella alpina, Schizochytrium sp.*, and mixtures thereof.
In a still further embodiment, the microorganisms include, but are not limited to, microorganisms belonging to the genus *Mortierella*, genus *Conidiobolus*, genus *Pythium*, genus *Phytophthora*, genus *Penicillium*, genus *Cladosporium*, genus *Mucor*, genus *Fusarium*, genus *Aspergillus*, genus *Rhodotorula*, genus *Entomophthora*, genus *Echinosporangium*, and genus *Saprolegnia*.

In an even further embodiment, the microorganisms are from microalgae of the order *Thraustochytriales*, which includes, but is not limited to, the genera *Thraustochytrium* (species include *arudimentale, aureum, benthicola, globosum, kinnei, motivum, multirudimentale, pachydermum, proliferum, roseum, striatum*); the genera *Schizochytrium* (species include *aggregatum, limnaceum, mangrovei, minutum, octosporum*); the genera *Ulkenia* (species include *amoeboidea, kerguelensis, minuta, profunda, radiate, sailens, sarkariana, schizochytrops, visurgensis, yorkensis*); the genera *Aurantiacochytrium*; the genera *Oblongichytrium*; the genera *Sicyoidochytium*; the genera *Parientichytrium*; the genera *Botryochytrium*; and combinations thereof. Species described within *Ulkenia* will be considered to be members of the genus *Schizochytrium*. In another embodiment, the microorganisms are from the order *Thraustochytriales*. In yet another embodiment, the microorganisms are from *Thraustochytrium*.

In still a further embodiment, the microorganisms are from *Schizochytrium sp*.

In certain embodiments, the oil can comprise a marine oil. Examples of suitable marine oils are the ones as given above.

The biomass according to the invention preferably comprises cells, and preferably consists essentially of such cells, of the taxon *Labyrinthulomycetes* (*Labyrinthulea*, net slime fungi, slime nets), in particular, those from the family of *Thraustochytriaceae*. The family of the *Thraustochytriaceae* (*Thraustochytrids*) includes the genera *Althomia*, *Aplanochytrium*, *Aurantiochytrium*, *Botryochytrium*, *Elnia*, *Japonochytrium*, *Oblongichytrium*, *Parietichytrium*, *Schizochytrium*, *Sicyoidochytrium*, *Thraustochytrium*, and *Ulkenia*. The biomass particularly preferably comprises cells from the genera *Aurantiochytrium*, *Oblongichytrium*, *Schizochytrium*, or *Thraustochytrium*, more preferably from the genus *Schizochytrium*.

In accordance with the invention, the polyunsaturated fatty acid (PUFA) is preferably DHA and/or EPA and/or their esters as defined above.

The cells present in the biomass are preferably distinguished by the fact that they contain at least 20 weight-%, preferably at least 30 weight-%, in particular at least 35 weight-%, of PUFAs, in each case based on cell dry matter.

In a very preferred embodiment of the current invention, cells, in particular a *Schizochytrium* strain, is employed which produces a significant amount of EPA and DHA, simultaneously, wherein DHA is preferably produced in an amount of at least 20 weight-%, preferably in an amount of at least 30 weight-%, in particular in an amount of 30 to 50 weight-%, and EPA is produced in an amount of at least 5 weight-%, preferably in an amount of at

least 10 weight-%, in particular in an amount of 10 to 20 weight-% (in relation to the total amount of lipid as contained in the cells, respectively).

Preferred species of microorganisms of the genus *Schizochytrium*, which produce EPA and DHA simultaneously in significant amounts, as mentioned before, are deposited under ATCC Accession No. PTA-10208, PTA-10209, PTA-10210, or PTA-10211, PTA-10212, PTA-10213, PTA-10214, PTA-10215.

DHA and EPA producing *Schizochytrium* strains can be obtained by consecutive mutagenesis followed by suitable selection of mutant strains which demonstrate superior EPA and DHA production and a specific EPA:DHA ratio. Any chemical or nonchemical (e.g. ultraviolet (UV) radiation) agent capable of inducing genetic change to the yeast cell can be used as the mutagen. These agents can be used alone or in combination with one another, and the chemical agents can be used neat or with a solvent.

Methods for producing the biomass, in particular, a biomass which comprises cells containing lipids, in particular PUFAs, particularly of the order *Thraustochytriales*, are described in detail in the prior art (see e.g. WO 91/07498, WO 94/08467, WO 97/37032, WO 97/36996, WO 01/54510). As a rule, the production takes place by cells being cultured in a fermenter in the presence of a carbon source and a nitrogen source, along with a number of additional substances like minerals that allow growth of the microorganisms and production of the PUFAs. In this context, biomass densities of more than 100 grams per litre and production rates of more than 0.5 gram of lipid per litre per hour may be attained. The process is preferably carried out in what is known as a fed-batch process, i.e. the carbon and nitrogen sources are fed in incrementally during the fermentation. When the desired biomass has been obtained, lipid production may be induced by various measures, for example by limiting the nitrogen source, the carbon source or the oxygen content or combinations of these.

In a preferred embodiment of the current invention, the cells are grown until they reach a biomass density of at least 80 or 100 g/l, more preferably at least 120 or 140 g/l, in particular at least 160 or 180 g/l (calculated as dry-matter content). Such processes are for example disclosed in US 7,732,170.

Preferably, the cells are fermented in a medium with low salinity, in particular, so as to avoid corrosion. This can be achieved by using chlorine-free sodium salts as the sodium source instead of sodium chloride, such as, for example, sodium sulphate, sodium carbonate, sodium hydrogen carbonate or soda ash. Preferably, chloride is used in the fermentation in amounts of less than 3 g/l, in particular, less than 500 mg/l, especially preferably less than 100 mg/l.

PUFA-containing plant oils: Plant oils with relatively high amounts of PUFAs, especially with high amounts of DHA and/or EPA such as e.g., canola seed oil

The plant cells may, in particular, be selected from cells of the families *Brassicaceae*, *Elaeagnaceae* and *Fabaceae*. The cells of the family *Brassicaceae* may be selected from the genus *Brassica*, in particular, from oilseed rape, turnip rape and Indian mustard; the cells of the family *Elaeagnaceae* may be selected from the genus *Elaeagnus*, in particular, from the species *Oleae europaea*; the cells of the family *Fabaceae* may be selected from the genus *Glycine*, in particular, from the species *Glycine* max.

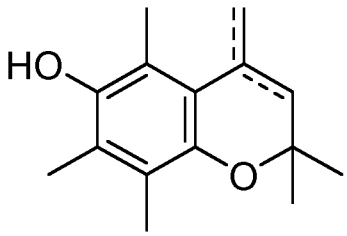
Examples:

- Canola seed oil with a content of DHA of at least 9% by weight, of at least 12% by weight, of at least 15% by weight, or of at least 20% by weight, based on the total weight of the canola seed oil;
- Canola seed oil with a content of EPA of at least 9% by weight, of at least 12% by weight, of at least 15% by weight, or of at least 20% by weight, based on the total weight of the canola seed oil.

Examples of PUFA-containing plant oils containing high amounts of other PUFAs than EPA and/or DHA and/or DPA and/or their esters are linseed/flaxseed oil, hempseed oil, pumpkin seed oil, evening primrose oil, borage seed oil, blackcurrent seed oil, sallow thorn/sea buckthorn oil, chia seed oil, argan oil and walnut oil.

Novel compounds

The compounds of formulae (3) and (5) are novel. Therefore, the present invention is also directed to them and their synthesis. Compound of formula (3) is a mixture of compounds of formulae (3a) and (3b).

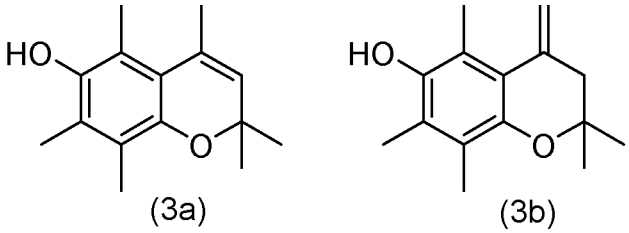


(3)

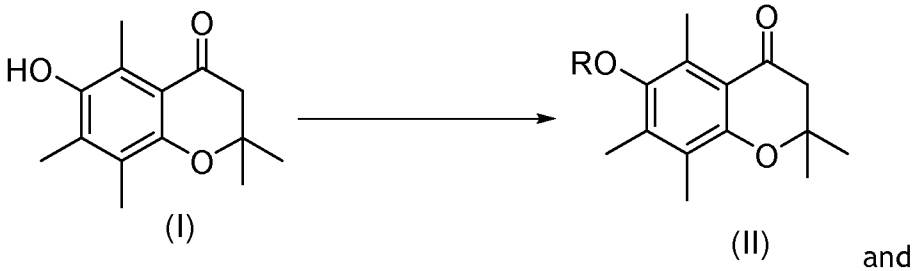
The synthesis of compound of formula (3) is shown in **Fig. 2**. Compound of formula (3) is a mixture of compounds of formulae (3a) and (3b), preferably in the molar ratio of 82:18.

The starting material for compound of formula (3), 6-hydroxy-2,2,5,7,8-pentamethylchroman-4-one, may be prepared according to US 2006/193797, Example 1.

The present invention is also directed to a process for the manufacture of a mixture of compounds of formulae (3a) and (3b) comprising the following steps:

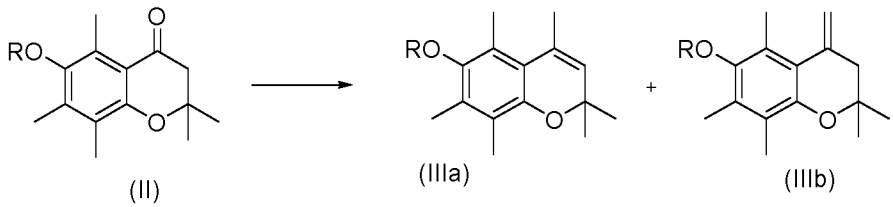


a) optional protection of the hydroxy group of the compound of formula (I) to obtain the compound of formula (II),



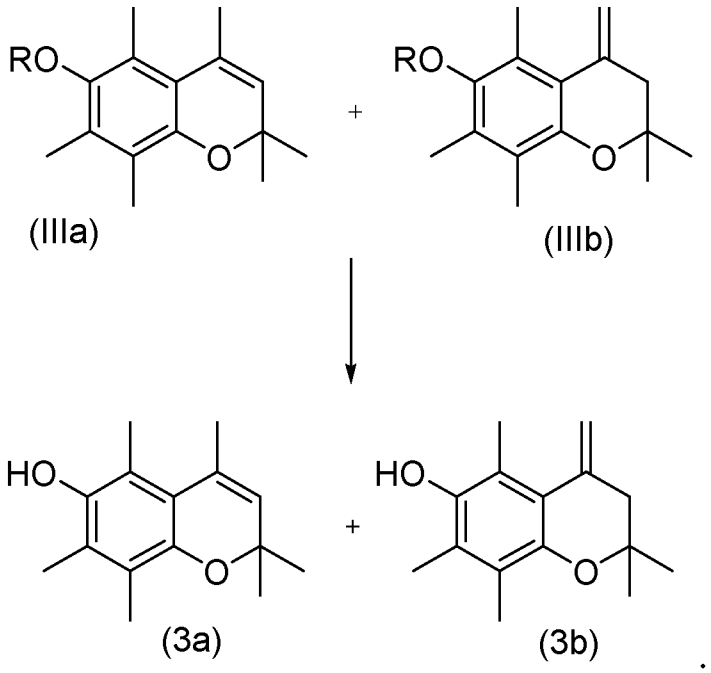
and

b) methyl-Grignard addition to the compound of formula (II) and water elimination to obtain a mixture of compounds of formulae (IIIa) and (IIIb); or methyl-Grignard addition to the compound of formula (I) and water elimination to obtain a mixture of compounds of formulae (3a) and (3b), respectively;



and

c) optional deprotection of the compounds of formulae (IIIa) and (IIIb) to a mixture of compounds of formulae (3a) and (3b)



Step b):

The methyl-Grignard may be added to the compound of formula (II) (preferred) or to the compound of formula (I) (less preferred). In the latter case (methyl-Grignard addition to the compound of formula (I) double the amount of the methyl-Grignard reagent has to be used.

If the methyl-Grignard is added to the compound of formula (I) a mixture of compounds of formulae (IIIa) and (IIIb), wherein R = H is obtained in step b), i.e. a mixture of compounds of formula (3a) and (3b). In this case, step c) is not carried out.

Step a):

The hydroxy group of the compound of formula (I) is a phenolic group which may be protected as ether, acetal or ester, preferably as ether or acetal, according to state-of-the-art methods.

Examples of such phenol protecting groups are methyl ethers; benzyl ethers; silyl ethers, such as e.g. trimethylsilyl, tert-butyldimethylsilyl, triisopropylsilyl and triethylsilyl ethers; tetrahydropyran ethers (acetals); an

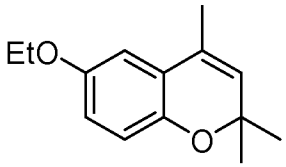
acetal formed with isopropylene methyl ether (2-methoxy-2-propanyl acetal) and pivaloyl esters.

Step c):

The protected phenolic group ("OR" in the compound of formula (II)) may be deprotected easily, i.e. by state-of-the-art methods, to the phenolic group again.

The synthesis of compound of formula (5) is shown in **Fig. 3**. The starting material for compound of formula (5), 6-hydroxy-2,2-dimethylchroman-4-one (compound of formula (IV)), may be prepared according to C. L. Lucas, B. Lygo, A. J. Blake, W. Lewis, C. J. Moody. Regioselectivity of the Claisen Rearrangement in meta-Allyloxy Aryl Ketones: An Experimental and Computational Study, and Application in the Synthesis of (R)-(−)-Pestalotheol D. *Chem. Eur. J.* **2011**, *17*, 1972–1978.

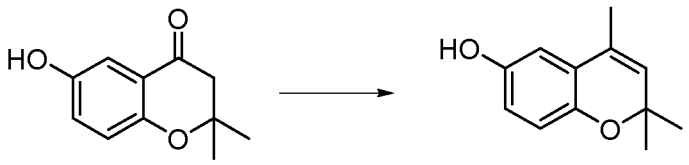
A further embodiment of the present invention is a process for the manufacture of compound of formula (5) comprising the following steps:



(5)

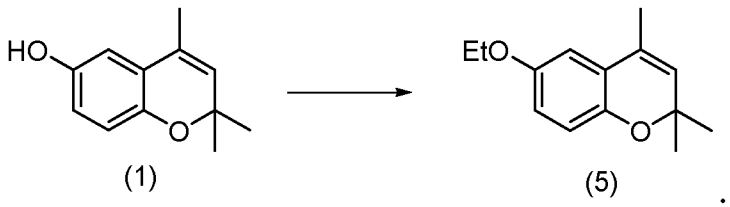
whereby "Et" is ethyl,

i) methyl-Grignard addition to compound of formula (IV) and water elimination to obtain the compound of formula (1),



(IV) (1) and

ii) etherification of compound of formula (1) to obtain the compound of formula (5)



(1) (5)

Step ii)

The etherification of compound of formula (1) to obtain the compound of formula (5) is either achieved by reaction with an ethyl halide (preferably chloride or bromide) or a carbonate such as diethyl carbonate or dimethyl carbonate.

Preferred embodiments of the present invention

Fish oil/algal oil

The Protection Factors of compound of formula (1) in fish oil could be improved by the addition of ascorbyl palmitate ("AP") (see **Table 5** in the experimental part) indicating the possibility of combining AP to all the compounds of formulae (1) to (7) to improve the oxidative stability of matrices containing high amounts of unsaturated fatty acids such as fish oil.

Polymers are combination of complex compounds generated at the end of the oxidation cascade of unsaturated fatty acids and, they indicate the levels of overall oxidation of the matrix. The generation of such polymers in fish oil containing these novel antioxidant compounds of formulae (1) to (7) could be reduced considerably when AP was added as a synergistically acting compound (see **Table 6** in the experimental part).

The invention is now further illustrated in the following non-limiting examples.

Examples

Examples

Examples 1-6: Syntheses of compounds of formulae (1) to (5) and (7)

Compound of formula (6), 6-hydroxycoumarin, is commercially available, e.g. from Aldrich, catalog# 642665.

The following abbreviations have been used:

min	minute(s)
h	hour(s)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DMF	dimethylformamide
MeOH	methanol
THF	tetrahydrofuran

Example 1: Synthesis of compound of formula (1) (2,2,4-trimethyl-2H-chromen-6-ol)

See **Fig. 4**:

Step 1: acetone, pyrrolidine, acetonitrile;

Step 2: 1) MeMgCl, THF, 2) HCl, pTsOH.

For step 1, a procedure by Moody was followed (C. L. Lucas, B. Lygo, A. J. Blake, W. Lewis, C. J. Moody. Regioselectivity of the Claisen Rearrangement in meta-Allyloxy Aryl Ketones: An Experimental and Computational Study, and Application in the Synthesis of (R)-(−)-Pestalotheol D. *Chem. Eur. J.* **2011**, *17*, 1972–1978).

Step 2: A 750 mL 4-necked sulfonation flask equipped with mechanical stirrer, thermometer, 250 mL addition funnel, argon inlet and reflux condenser was inertized with argon and then charged with MeMgCl (3.0 M

solution in THF, 128 mL, 384 mmol, 2.4 mol equiv.) at 20°C. Subsequently, a solution of 6-hydroxy-2,2-dimethylchroman-4-one (33.9 g, 160 mmol) in dry THF (160 mL) was charged into the addition funnel. The solution was then added over 35 min, keeping the inner temperature below 20°C. The reaction was then heated to reflux for 3 h. Additional MeMgCl (3.0 M solution in THF, 32 mL, 96 mmol, 0.6 mol equiv.) was added. The reaction was heated to reflux for another 1.75 h, then cooled to room temperature and stirred overnight. Subsequently, the mixture was slowly quenched by addition of HCl (4 M in water, 132 mL, 528 mmol, 3.3 mol equiv.) over 30 min, keeping the inner temperature below 30°C. p-Toluenesulfonic acid monohydrate (0.30 g, 1.6 mmol, 1 mol%) was added and the mixture was heated to reflux for 1 h. The reaction was cooled to room temperature and diluted with EtOAc (150 mL). After phase separation, the aqueous phase was extracted with EtOAc (150 mL) and the combined organic phases were washed with water (2x 100 mL) and then concentrated in vacuo to furnish 32.8 g of crude product as dark oil. The crude product was then purified by column chromatography. The crude product was diluted with EtOAc/toluene/hexanes 15:15:70 (w/w) and then charged onto a silica gel column; eluent EtOAc/toluene/hexanes 15:15:70 (w/w). The combined product fractions were concentrated and subsequently dissolved in EtOH (100 mL) and then filtered. The solution was concentrated to dryness, furnishing 2,2,4-trimethyl-2H-chromen-6-ol as brownish crystals (27 g, 92 wt% by qNMR, 76% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 6 H), 1.97 (d, *J* = 1.5 Hz, 3 H), 4.64 (s, 1 H, OH), 5.45 (q, *J* = 1.4 Hz, 1 H), 6.58-6.63 (m, 1 H), 6.65-6.72 (m, 2 H) ppm.

Example 2: Synthesis of compound of formula (2) (2,2-dimethyl-2H-chromen-6-ol)

See **Fig. 5**:

Step 1: $CuCl_2$, DBU (= 1,8-diazabicyclo[5.4.0]undec-7-ene), CH_3CN;

Step 2: PPh_3AuNTf_2, DCM

Step 1: A 500 mL 4-necked flask with magnetic stirrer, thermometer, and argon supply was charged with hydroquinone (22.1 g, 200 mmol, 1.0 mol equiv.) and dissolved in acetonitrile (200 mL). The solution was cooled to 0-4°C (ice-bath) and DBU (66.5 g, 440 mmol, 2.2 mol equiv.) and copper(II) chloride (0.080 g, 0.596 mmol, 0.3 mol%) were added. Then, 3-chloro-3-methylbut-1-yne (21 g, 200 mmol, 1.0 mol equiv.) was added dropwise within 20 min. After stirring at 0-4°C for 1.5 h the beige-brown reaction was quenched by slow addition to a vigorous stirring mixture of HCl (100 mL, 25% in water) and ice (100 g). Ethyl acetate (300 mL) was added and the water phase was extracted with ethyl acetate (200 mL). The combined organic phases were washed with HCl (100 mL, 1N in water), sat. aq. $NaHCO_3$ (100 mL) and finally brine (100 mL, 10% NaCl in water), dried over sodium sulfate, filtered and concentrated in vacuo (40 °C/150-20 mbar). The residue was purified by column chromatography: The sample was diluted with little eluent and charged onto a silica gel column; eluent gradient heptane/EtOAc 90:10 to 80:20 (w/w). The pure fractions were combined, concentrated in vacuo (40°C/200-10 mbar) and dried under high vacuum at 40°C, furnishing 6.46 g 4-((2-methylbut-3-yn-2-yl)oxy)phenol as colorless crystals (22% yield).

Step 2: An oven-dried 250 mL flask with magnetic stirrer, thermometer and argon supply was charged with 4-((2-methylbut-3-yn-2-yl)oxy)phenol (6.4 g, 34.4 mmol, 1.0 mol equiv.) was dissolved under argon atmosphere in dry DCM (100 mL) and cooled to 0°C. $Ph_3PAuNTf_2$ (0.046 g, 0.062 mmol, 0.2 mol%) was added and the ice bath was removed. After 100 min, another portion $Ph_3PAuNTf_2$ (0.25 g, 0.337 mmol, 1.0 mol%) was added. After 4 h, the dark solution was concentrated in vacuo (40°C/500-20 mbar). The dark residue was purified by column chromatography: The sample was diluted with little eluent and charged onto a silica gel column; eluent gradient heptane/EtOAc 95:5 to 70:30 (w/w). The pure fractions were combined, concentrated in vacuo (40°C/200-10 mbar) and dried under high vacuum at 40°C, furnishing 2.85 g 2,2-Dimethyl-2*H*-chromen-6-ol as off-white crystals (45% yield, mp 89-90°C). ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 6 H), 4.66 (s, 1

H, OH), 5.64 (d, *J* = 9.8 Hz, 1 H), 6.26 (d, *J* = 9.8 Hz, 1 H), 6.50 (d, *J* = 2.8 Hz, 1 H), 6.59 (dd, *J* = 8.7, 2.8 Hz, 1 H), 6.67 (d, *J* = 8.3 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.6 (2 C), 75.8 (1 C), 112.8 (1 C), 115.4 (1 C), 116.9 (1 C), 122.1 (2 C), 131.9 (1 C), 146.7 (1 C), 149.4 (1 C) ppm.

Example 3: Synthesis of compound of formula (3) (2,2,4,5,7,8-hexamethyl-2H-chromen-6-ol)

See **Fig. 2**:

Step 1: Bn-Br, K_2CO_3, DMF;

Step 2: 1) MeMgCl, THF; 2) aq. HCl, pTsOH;

Step 3: BCl_3, DCM.

The starting material, 6-hydroxy-2,2,5,7,8-pentamethylchroman-4-one, was prepared as published in US 2006/193797, Example 1.

Step 1: A 3-necked 250 mL round-bottom flask equipped with thermometer, reflux condenser, argon inlet, magnetic stirring and oil bath was charged with 6-hydroxy-2,2,5,7,8-pentamethylchroman-4-one (14.94 g, 63.8 mmol, 1.0 mol equiv.), K_2CO_3 (17.63 g, 127.6 mmol, 2.0 mol equiv.) and dissolved in DMF (65 mL). The apparatus was then inertized with argon. Subsequently, benzyl bromide (12.0 g, 70.2 mmol, 1.1 mol equiv.) was added and the reaction was stirred for 1 h at room temperature, then 1 h at 40°C. The mixture was cooled to room temperature and quenched slowly with water (150 mL). EtOAc (150 mL) was added. The aqueous phase was extracted with EtOAc (100 mL). The combined organic phases were washed with water (2x 50 mL), then filtered and concentrated in vacuo, furnishing the crude product as brown solid. The crude product was recrystallized by dissolving in toluene (19 g) at 55°C and slow addition of n-hexane (77 g) at 50-55°C. The solution was allowed to cool to room temperature, upon which crystals formed spontaneously at 20°C. The mixture was cooled to 0°C for 1 h. The crystals were isolated by filtration; the filter cake was washed with the mother liquor and then with little cold n-hexane. The crystals were dried in vacuo at 60°C for 18 h, furnishing 6-(benzyloxy)-2,2,5,7,8-pentamethylchroman-4-one as colorless solid (14.7 g, 45.3 mmol, mp 89-

90°C). A second crop of crystals was obtained by dissolving the concentrated mother liquor in toluene (4.5 g) and addition of n-hexane (19 g): 3.03 g of 6-(benzyloxy)-2,2,5,7,8-pentamethylchroman-4-one crystals could be obtained (~9.3 mmol, mp 88-89°C); combined yield: 85%.

Step 2: A 3-necked 250 mL round-bottom flask equipped with thermometer, reflux condenser, argon inlet, magnetic stirring and oil bath was charged with 6-(benzyloxy)-2,2,5,7,8-pentamethylchroman-4-one (16.9 g, 52.0 mmol, 1.0 mol equiv.) and dissolved in dry THF (104 mL). The apparatus was then inertized with argon. Subsequently, MeMgCl (3 M in THF, 26 mL, 78 mmol, 1.5 mol equiv.) was added dropwise within 45 min at 15-20°C. The reaction was then stirred for 4 h at room temperature. HPLC analysis indicated ~55 area% starting material and ~35 area% addition product. No further conversion was observed; probably due to steric constrains around the ketone moiety. The mixture was cooled to 0°C and HCl (4 M in water, 29 mL, 116 mmol, 2.2 mol equiv.) was added slowly over 20 min at 0-10°C. p-Toluenesulfonic acid (0.33 g, 1.7 mmol, 3 mol%) was added and the mixture was heated to reflux for 30 min. After cooling, water (100 mL) and Et_2O (100 mL) were added. The aqueous phase was extracted with Et_2O (2x 100 mL) and the combined organic phases were washed with water (2x 50 mL). The organic phases were concentrated in vacuo (60°C/26 mbar). The residue was re-dissolved in toluene (25 mL) and EtOH (25 mL) and concentrated in vacuo (60°C /23 mbar), furnishing the crude product as yellow oil that crystallized on standing (18.24 g,). The crude product was purified by column chromatography (eluent toluene/hexane 50:50 (w/w) until complete elution of product, then toluene and finally toluene/EtOAc 95:5 (w/w) to recover starting material). Product fractions were combined, filtered and concentrated in vacuo, furnishing 6-(benzyloxy)-2,2,4,5,7,8-hexamethyl-2H-chromene in an 82:18 mixture with its olefin-isomer as colorless solid (5.46 g, 16.9 mmol, 82:18 ratio of olefin-isomers by HPLC, 33% yield). Fractions containing starting material were combined, filtered and concentrated to furnish 12.8 g of starting material, which still contained some toluene, even after prolonged drying. ^1H NMR (major isomer, 300 MHz,

$CDCl_3$) δ 1.35 (s, 6 H), 2.14 (s, 3 H), 2.18 (d, *J* = 1.5 Hz, 3 H), 2.23 (s, 3 H), 2.41 (s, 3 H), 4.73 (s, 2 H), 5.49 (q, *J* = 1.5 Hz, 1 H), 7.31-7.46 (m, 3 H), 7.46-7.55 (m, 2 H) ppm.

Step 3: A 3-necked 250 mL round-bottom flask equipped with thermometer, reflux condenser, argon inlet, magnetic stirring and dry ice bath was charged with 6-(benzyloxy)-2,2,4,5,7,8-hexamethyl-2H-chromene (5.90 g, 18.3 mmol, 82:18 mixture of olefin isomers, 1.0 mol equiv.) and dissolved in dichloromethane (100 mL). The apparatus was then inertized with argon. Subsequently, the solution was cooled to -40°C and BCl_3 (1 M in DCM, 39.5 mmol, 2.1 mol equiv.) was added dropwise over 35 min. The mixture was stirred for 4 h at -40°C and then warmed to -20°C. Brine (11 mL) was added dropwise over 9 min, keeping the temperature between -20 and -10°C. At 0°C, water (40 mL) was added. A suspension formed, which was filtered and the cake was washed with DCM (50 mL). The aqueous phase of the filtrate (2 phases) was separated and extracted with DCM (50 mL). The combined organic phases were then washed with water (3x 50 mL) and concentrated in vacuo (60°C/20 mbar), furnishing the crude product as yellow oil. The crude product was then purified by column chromatography on silica gel, eluting with hexanes/EtOAc 93:7 (w/w). The product fractions contained varying ratios of product and olefin-isomer. They were combined and concentrated in vacuo, furnishing PM-chromenol and its isomer as yellow oil (3.64 g, 86% yield, mp 66-67°C). ^{1}H NMR (major isomer, 300 MHz, $CDCl_3$) δ 1.35 (s, 6 H), 2.16 (s, 3 H), 2.17 (d, *J* = 1.5 Hz, 3 H), 2.19 (s, 3 H), 2.35 (s, 3 H), 4.30 (s, 1 H), 5.50 (q, *J* = 1.3 Hz, 1 H) ppm.

Example 4: Synthesis of compound of formula (4) (2,2,5,7,8-pentamethyl-2H-chromen-6-ol)

See **Fig. 6:**

Step 1: 1) $NaBH_4$, MeOH, THF; 2) HCl, pTsOH.

A 3-necked 250 mL round-bottom flask equipped with thermometer, reflux condenser, argon inlet, magnetic stirring and oil bath was charged with 6-

hydroxy-2,2,5,7,8-pentamethylchroman-4-one (9.36 g, 40.0 mmol, 1.0 mol equiv.) and dissolved in THF (40 mL). The apparatus was then inertized with argon. Subsequently, $NaBH_4$ (1.51 g, 1.0 mol equiv.) was added followed by cautious addition of MeOH (3.25 mL, 2.0 mol equiv.) at room temperature. Gas evolution and exothermicity was observed. After 5 h, additional $NaBH_4$ (0.76 g, 0.5 mol equiv.) and MeOH (1.6 mL, 1.0 mol equiv.) were added. After 18 h, additional $NaBH_4$ (0.76 g, 0.5 mol equiv.) and MeOH (1.6 mL, 1.0 mol equiv.) were added. 2 h later, HCl (4 M in water, 40 mL, 160 mmol, 4 mol equiv.) was added slowly under slight cooling with a water bath. p-Toluenesulfonic acid (0.76 g, 4.0 mmol, 10 mol%) was added and heated to reflux for 1.5 h. The reaction was cooled to room temperature and quenched with water (100 mL). The aqueous phase was extracted with EtOAc (2x 100 mL) and the combined organic phases were washed with water (2x 50 mL). The organic phase was concentrated in vacuo, re-dissolved in EtOH (100 mL), filtered and concentrated in vacuo (60°C/20 mbar), furnishing the crude product as yellow oil. The crude product was recrystallized in toluene (9 g) and diluted with n-hexane (18 g). The solution was cooled to 0°C for 1 h and then filtered. The cake was washed with the mother liquor and subsequently with n-hexane (10 mL). After drying (45°C/20 mbar, 2 h), the product was obtained as colorless solid (3.3 g, mp 75-77°C). A second crop of crystals was obtained from the concentrated mother liquor (3.7 g); combined yield: 83%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 6 H), 2.14 (s, 3 H), 2.17 (s, 3 H), 2.19 (s, 3 H), 4.22 (s, 1 H), 5.65 (d, *J* = 10.0 Hz, 1 H), 6.52 (d, *J* = 10.0 Hz, 1 H) ppm.

Example 5: Synthesis of compound of formula (5) (6-ethoxy-2,2,4-trimethyl-2H-chromene)

See **Fig. 3**, 2nd step.

A 3-necked 250 mL round-bottom flask equipped with thermometer, reflux condenser, argon inlet, magnetic stirring and oil bath was charged with crude 2,2,4-trimethyl-2H-chromen-6-ol (13.0 g, 74 area% purity by HPLC, ~50.6 mmol, 1.0 mol equiv.) and dissolved in DMSO (35 mL) and THF (70 mL)

forming a yellow solution. The apparatus was inertized with argon. Subsequently, ethyl iodide (11 mL, 136.8 mmol, 2.7 mol equiv.) and K_2CO_3 (18.9 g, 136.8 mmol, 2.7 mol equiv.) were added. The suspension was then heated to 50°C for 24 h and monitored by HPLC. After cooling to room temperature, water (100 mL) was added and diluted with diethyl ether (100 mL). The aqueous phase was extracted with diethyl ether (100 mL). The combined organic phases were washed with water (2x 50 mL), concentrated in vacuo (50°C/70 mbar), and further dried at 50°C/4 mbar. The crude product was purified by column chromatography on silica gel, eluting with hexanes/iPr_2O 96:4 (w/w). Product fractions were combined and concentrated in vacuo to give a yellow oil, which was re-dissolved in EtOH (100 mL), filtered and then concentrated in vacuo. The residue was again dissolved in EtOH (100 mL) and concentrated in vacuo (60°C/20 mbar), furnishing EtO-TM-chromenol as yellowish oil (11.0 g, 91 wt% by qNMR, 45.9 mmol, 91% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (m, 6 H), superimposed by 1.40 (t, *J* = 7.0 Hz, 3 H), 1.99 (d, *J* = 1.5 Hz, 3 H), 4.00 (q, *J* = 7.0 Hz, 2 H), 5.45 (q, *J* = 1.3 Hz, 1 H), 6.65-6.77 (m, 3 H) ppm.

Example 6: Synthesis of compound of formula (7) (2H-chromen-6-ol)

See **Fig. 7**:

Step 1: $Ph_3PAuNTf_2$, DCM.

The starting material, 4-(prop-2-yn-1-yloxy)phenol, was prepared as described in J. C. Jaen, L. D. Wise, T. G. Heffner, T. A. Pugsley, L. T. Meltzer. Dopamine autoreceptor agonists as potential antipsychotics. 2. (Aminoalkoxy)-4*H*-1-benzopyran-4-ones. *J. Med. Chem.* **1991**, *34*, 248-256.

A 250 mL round-bottom flask equipped with magnetic stirrer and argon supply was charged with 6.5 g 4-(prop-2-yn-1-yloxy)phenol (43.8 mmol, 1.0 mol equiv.) under argon, dissolved in dry DCM (100 mL) and cooled under stirring to 0-4 C. Subsequently, $Ph_3PAuNTf_2$ (0.058 g, 0.079 mmol, 0.18 mol%) was added and the reaction was stirred for 40 min at 0-4 C (cooled with an ice bath). After 2 h the dark reaction solution was concentrated in

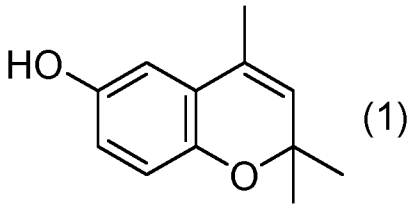
vacuo (40°C/200-10 mbar). The dark residue was purified by column chromatography: The sample was diluted with little EtOAc and charged onto a silica gel column; eluent gradient heptane/EtOAc 90:10 to 75:25 (w/w). The pure product fractions were combined, concentrated in vacuo (40°C/200-10 mbar) and dried under high vacuum at 40°C, furnishing 4.8 g 2*H*-chromen-6-ol as an oil (31.8 mmol, purity 98.2% by qNMR, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72-4.79 (m, 2 H), superimposed by 4.76 (br s, 1H, OH), 5.82 (dt, *J* = 9.8, 3.6 Hz, 1 H), 6.37 (dt, *J* = 9.9, 1.6 Hz, 1 H), 6.49 (d, *J* = 3.0 Hz, 1 H), 6.55-6.63 (m, 1 H), 6.63-6.71 (m, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.4 (1 C), 113.1 (1 C), 115.4 (1 C), 116.3 (1 C), 123.1 (1 C), 123.3 (1 C), 124.4 (1 C), 147.8 (1 C), 149.8 (1 C) ppm.

Example 7: Antioxidant activities in fish oil and algal oil

Compound of formula (1) has been tested. The blank oil, i.e. oil without any antioxidant, and oil containing "MNT" have been used as benchmark. Any compound better in antioxidant activity than the blank oil indicates that it has antioxidant activity. The comparison with MNT gives an indication about the amount of the antioxidant effect, relative to the activity of MNT.

"MNT" are mixed natural tocopherols commercially available as e.g. "Tocomix 70 IP" from AOM (Buenos Aires, Argentina). Tocomix 70 IP comprises d-alpha-tocopherol, d-beta-tocopherols, d-gamma-tocopherols and d-delta-tocopherol, whereby the total amount of tocopherols is at least 70.0 weight-% and the amount of non-alpha tocopherols is at least 56.0 weight-%.

Compound of formula (1) was evaluated primarily for its oxidative stability by the Oil Stability Index measurements.



(1)

Two different levels of compound of formula (1) (0.5 and 2 mg/g) were used in 5 g of natural fish oil (Product Code: FG30TG) and used in the Oil Stability Instrument at 80°C with the air flow rate of 40 psi. The solubility of different amounts and types of antioxidants used in OSI was checked before and after the application. Crude algal oil contained about 1.6mg/g of mixed natural tocopherols (MNT) prior to use in these experiments whereas fish oil did not contain any antioxidants. Compound of formula (1) was used in the Oxidative Stability instruments under the same conditions used for fish oil evaluations. Also, the synergistic effect of compound of formula (1) with ascorbyl palmitate (AP) was determined using the OSI values. The polymers generated at the end of the experiment were determined by LC.

Results:

The solubility of the amounts of compound of formula (1) used in the oxidative stability study are shown in **Table 1**.

Table 1: Solubility of compound of formula (1) in fish oil

Compound	Appearance	Amount (mg/g)	Solubility in fish oil	
			Room temp.	80°C
compound of formula (1)	Yellow powder	0.5	Soluble	Soluble
		2.0	Soluble	Soluble
		2.0	Soluble	Soluble

The Oil Stability Index for compound of formula (1) at 500 and 2000 ppm levels, in comparison with the same amounts of MNT, is shown in **Table 2**.

Table 2: Oxidative stability of FG30TG fish oil with compound of formula (1) (SD = standard deviation)

	OSI (h)	SD
Blank (FG30TG)	4.70	0.1
0.5 mg/g of compound of formula (1)	5.95	0.2
2 mg/g of compound of formula (1)	6.73	1.0
0.5 mg/g of MNT	6.93	0.2
2 mg/g of MNT	7.925	0.1

The Protection Factors of compound of formula (1) in fish oil is shown in **Table 3**.

Table 3: Protection Factors of compound of formula (1) in FG30TG fish oil

	Protection Factor (%)
0.5 mg/g of compound of formula (1)	25.26
2 mg/g of compound of formula (1)	41.58
0.5 mg/g of MNT	45.79
2 mg/g of MNT	66.84

The improvement of the oxidative stability of the oil soluble compound of formula (1) when combined with AP is shown in **Table 4**.

Table 4: Improvement of the effect of compound of formula (1) in FG30TG fish oil using AP (SD = standard deviation)

	OSI (h)	SD
Blank (FG30TG)	4.63	0.0
2 mg/g of compound of formula (1)	5.70	0.4
2 mg/g of compound of formula (1) + 0.5 mg/g of AP	7.60	0.1
2 mg/g of MNT	8.03	0.9
2 mg/g of MNT + 0.5 mg/g of AP	15.25	1.5

Improvements of the Protection Factors of the oil soluble compound of formula (1) with AP in fish oil is shown in **Table 5**.

The Protection Factors of compound of formula (1) as well as MNT in fish oil could be improved by the addition of AP (see **Table 5**) indicating the possibility of combining AP to all the compounds of formulae (1) to (7) to

improve the oxidative stability of matrices containing high amounts of unsaturated fatty acids such as fish oil.

Table 5: Improvement of the Protection Factors of compound of formula (1) with AP in FG30TG fish oil

	Protection Factor (%)
2 mg/g of compound of formula (1)	23.1
2 mg/g of compound of formula (1) + 0.5 mg/g of AP	64.1
2 mg/g of MNT	73.3
2 mg/g of MNT + 0.5 mg/g of AP	229.4

Polymers generated at the end of the stabilization experiment of fish oil with compound of formula (1) and AP are shown in **Table 6**.

Table 6: Reduction of polymers in FG30TG oil with a compound (AP) synergistic to compound of formula (1) (SD = standard deviation)

	Polymers (%)	**SD**
Blank (FG30TG)	43.97	3.7
2 mg/g of compound of formula (1)	34.67	3.4
2 mg/g of compound of formula (1) + 0.5 mg/g of AP	32.65	1.0
2 mg/g of MNT	33.87	1.1
2 mg/g of MNT + 0.5 mg/g AP	12.72	2.6

Polymers are combination of complex compounds generated at the end of the oxidation cascade of unsaturated fatty acids and, they indicate the levels of overall oxidation of the matrix. The generation of such polymers in fish oil containing compound of formula (1) could be reduced considerably when AP was added as a synergistic compound (**Table 6**).

Tables 7, 8 and **9** show the PV (peroxide value), *p*-AV (*p*-anisidine value) and CD (**c**onjugated **d**ienoic acid in %) of the fish oil samples stabilized with compound of formula (1). For the storage stability study compound of formula (1) was used in fish oil at only 2 mg/g level. Compared to the same level of MNT, compound of formula (1) showed much higher PVs than those

of MNT (**Table 7**). MNT had the lowest PV values. There was no considerable variation in *p*-AV and CD (**Tables 8-9**) during the storage.

Table 7: Variation of PV with compound of formula (1) in FG30TG

	Initial	**4 days**	**6 days**	**8 days**	**11 days**	**13 days**	**17 days**
Blank(FG30TG)	0.9	1.6	2.2	2.7	5.6	7.8	11.9
2 mg/g of MNT	0.9	1.1	1.2	1.4	1.7	1.5	2.1
2 mg/g of compound of formula (1)	0.9	2	2.6	3.8	6.8	9.8	12.1

Table 8: Variation of *p*-AV with compound of formula (1) in FG30TG

	Initial	**4 days**	**6 days**	**8 days**	**11 days**	**13 days**	**17 days**
Blank(FG30TG)	9.9	9.8	9.9	10.5	10.9	11.2	11.9
2 mg/g of MNT	9.9	10.3	9.9	10.1	10	9.8	10
2 mg/g of compound of formula (1)	9.9	4.4	7.2	6.8	7.6	8.1	8.5

Table 9: Variation of CD with compound of formula (1) in FG30TG

	Initial	**4 days**	**6 days**	**8 days**	**11 days**	**13 days**	**15 days**
Blank(FG30TG)	0.7	0.7	0.6	0.7	0.7	0.8	0.7
2 mg/g of MNT	0.7	0.7	0.6	0.7	0.7	0.7	0.7
2 mg/g of compound of formula (1)	0.7	0.8	0.8	0.9	0.9	0.8	0.8

The Oil Stability Indices of compound of formula (1) in crude algal oil are shown in **Table 10**. This algal oil already contained about 1.6 mg/g of MNT. Addition of extra 2 mg/g of MNT to algal oil did not improve the OSI values either (**Table 10**).

Table 10: Oxidative stability of crude algal oil with compound of formula (1) (SD = standard deviation)

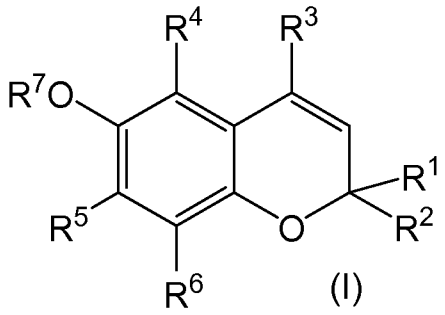
	OSI (h)	SD
Crude algal oil	14.85	0.4
0.5 mg/g of compound of formula (1)	16.60	0.6
2 mg/g of compound of formula (1)	18.55	1.0
0.5 mg/g of MNT	15.95	0.1
2 mg/g of MNT	16.35	0.2

Results

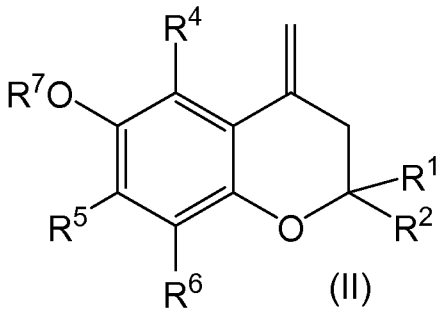
Compound of formula (1) shows antioxidant properties in fish oil and algal oil at different levels.

Claims

1. Use of a compound of formula (I) and/or a compound of formula (II) as antioxidant in oil,



(I)



(II)

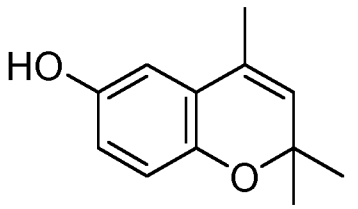
wherein the oil contains polyunsaturated fatty acids and/or their esters, and wherein the oil is for human consumption, and
wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and wherein
R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and
R^7 is H or C_{1-6}-alkyl.

2. The use according to claim 1, whereby in compound of formula (I) and/or in compound of formula (II) R^1 and R^2 are independently from each other H or C_{1-4}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 4, R^3, R^4, R^6 and R^7 are independently from each other H or C_{1-4}-alkyl, and R^5 is H or C_{1-4}-alkyl or C_{1-4}-alkoxy.

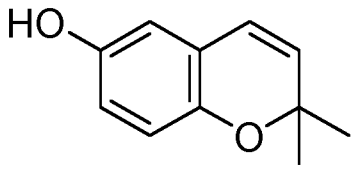
3. The use according to claim 1 or 2, whereby in compound of formula (I) and/or in compound of formula (II) R^1 and R^2 are independently from each other H or C_{1-2}-alkyl or $(CH_2)_n$—OH with n being 1 or 2, R^3, R^4, R^6 and R^7 are independently from each other H or C_{1-2}-alkyl, and R^5 is H or C_{1-2}-alkyl or C_{1-2}-alkoxy.

4. The use according to one or more of the preceding claims, whereby in compound of formula (I) and/or in compound of formula (II) R^1 and R^2 are independently from each other H or methyl or (CH_2)—OH, R^3, R^4, R^6 and R^7 are independently from each other H or methyl or ethyl, and R^5 is H or methyl or methoxy.

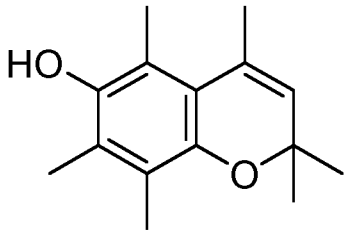
5. The use according to one or more of the preceding claims, whereby the compound of formulae (I) or (II) is one of the following compounds of formulae (1) to (7):



(1)



(2)



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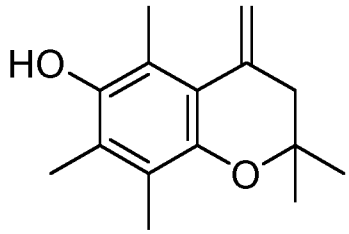
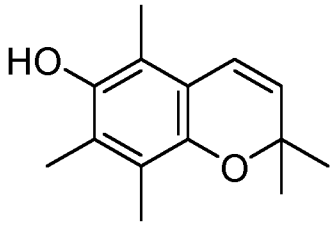


(3B)



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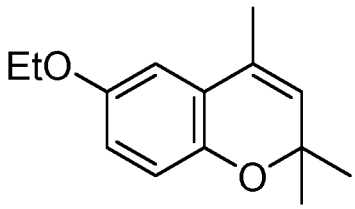
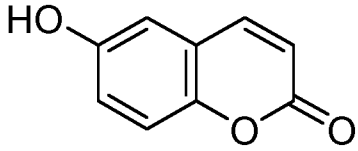


(5)



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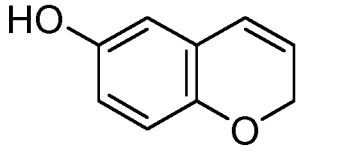
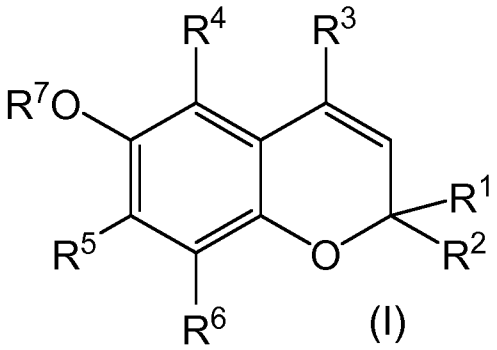
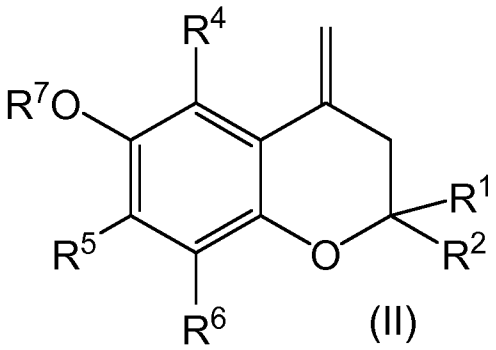


(7)

6. Oil containing polyunsaturated fatty acids (PUFAs) and/or their esters for human consumption comprising a compound of formula (I) and/or a compound of formula (II),



(I)



(II)

wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and

wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and

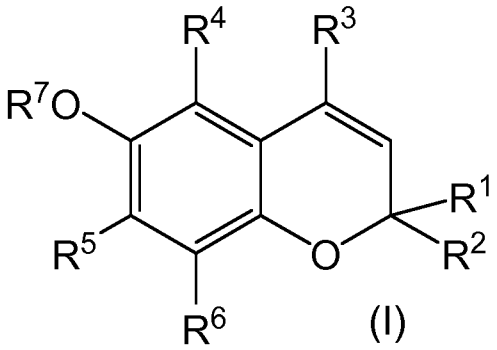
R^7 is H or C_{1-6}-alkyl.

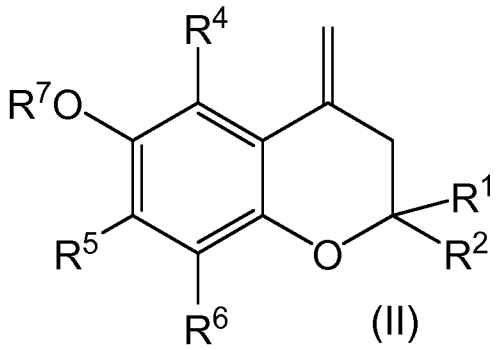
7. The oil containing polyunsaturated fatty acids (PUFAs) and/or their esters for human consumption according to claim 6, whereby the PUFA-containing oil is marine oil or microbial oil or fungal oil or algal oil or PUFA-containing plant oil, preferably whereby the PUFA-containing oil is marine oil or algal oil, more preferably whereby the PUFA-containing oil is algal oil.

8. The oil containing polyunsaturated fatty acids (PUFAs) and/or their esters for human consumption according to claim 6 and/or claim 7 additionally comprising esters of ascorbic acid with linear C_{12-20} alkanols, preferably esters of ascorbic acid with linear C_{14-18} alkanols, more preferably ascorbyl palmitate.

9. The oil containing polyunsaturated fatty acids (PUFAs) and/or their esters for human consumption according to any one or more of claims 6 to 8 additionally comprising alpha-tocopherol and/or gamma-tocopherol.

10. Marine oil for human consumption comprising at least one compound of formula (I) and/or at least one compound of formula (II),



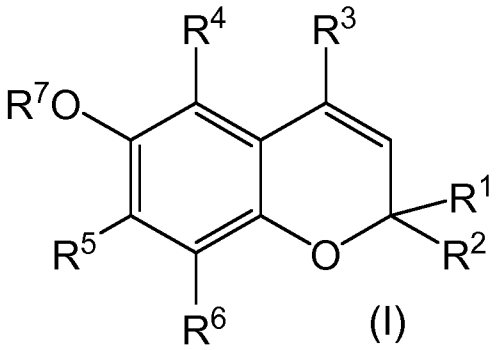


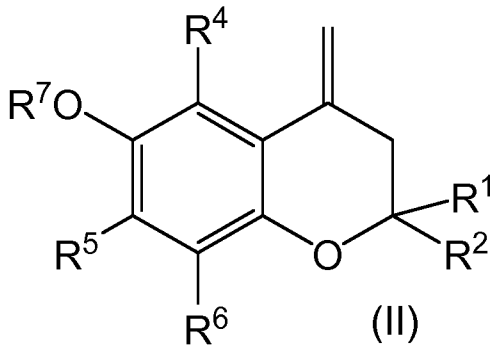
wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and
wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and
R^7 is H or C_{1-6}-alkyl.

11. The marine oil according to claim 10 additionally comprising esters of ascorbic acid with linear C_{12-20} alkanols, preferably esters of ascorbic acid with linear C_{14-18} alkanols, more preferably ascorbyl palmitate.

12. The marine oil according to claim 10 and/or claim 11 additionally comprising alpha-tocopherol and/or gamma-tocopherol.

13. Microbial oil for human consumption comprising a compound of formula (I) and/or a compound of formula (II),





wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and

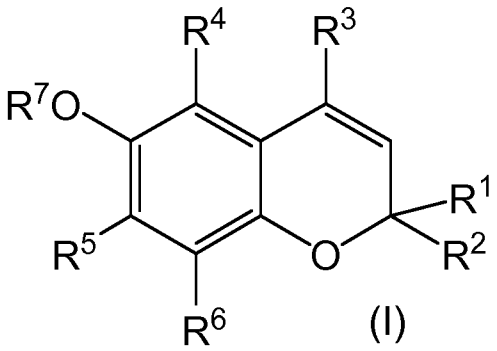
wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and

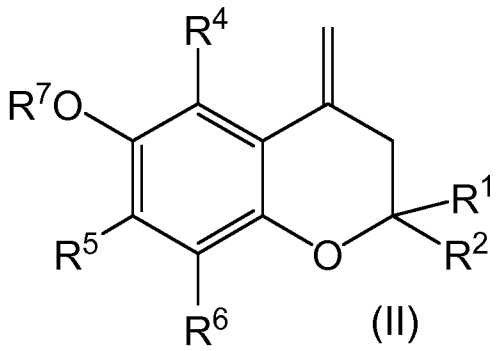
R^7 is H or C_{1-6}-alkyl.

14. The microbial oil according to claim 13 additionally comprising esters of ascorbic acid with linear C_{12-20} alkanols, preferably esters of ascorbic acid with linear C_{14-18} alkanols, more preferably ascorbyl palmitate.

15. The microbial oil according to claim 13 and/or claim 14 additionally comprising alpha-tocopherol and/or gamma-tocopherol.

16. Algal oil for human consumption comprising at least one compound of formula (I) and/or at least one compound of formula (II),



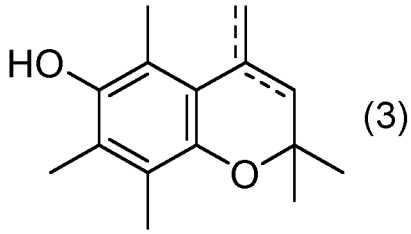


wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and
wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and
R^7 is H or C_{1-6}-alkyl.

17. The algal oil according to claim 16 additionally comprising esters of ascorbic acid with linear C_{12-20} alkanols, preferably esters of ascorbic acid with linear C_{14-18} alkanols, more preferably ascorbyl palmitate.

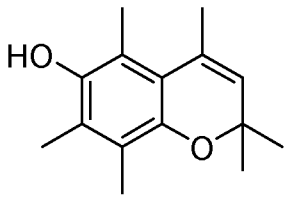
18. The algal oil according to claim 16 and/or claim 17 additionally comprising alpha-tocopherol and/or gamma-tocopherol.

19. 2,2,4,5,7,8-hexamethyl-2H-chromen-6-ol of formula (3).

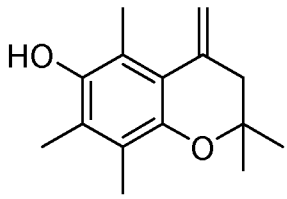


(3)

20. A process for the manufacture of a mixture of compounds of formulae (3a) and (3b) comprising the following steps:

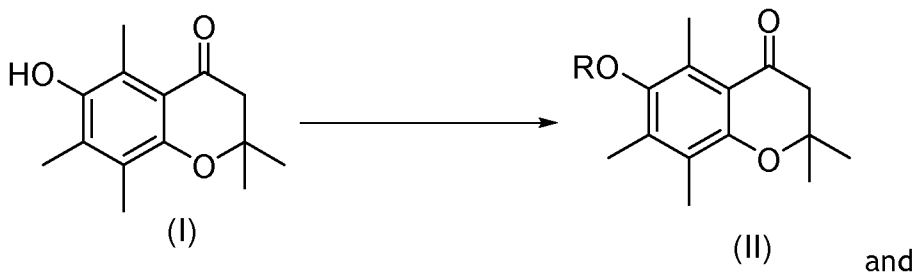


(3a)



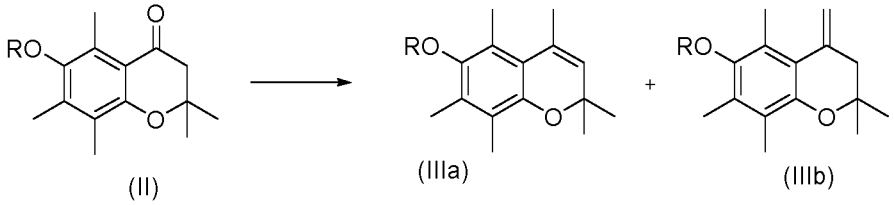
(3b)

a) optional protection of the hydroxy group of compound of formula (I) to obtain the compound of formula (II),



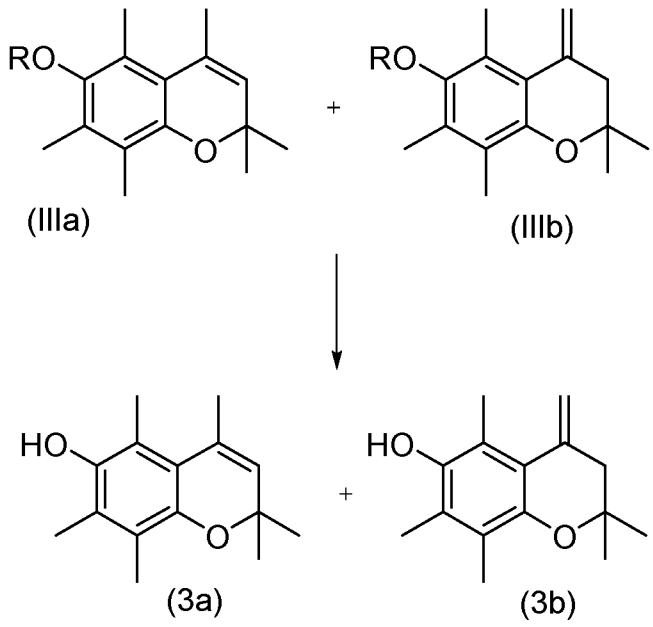
and

b) methyl-Grignard addition to the compound of formula (II) and water elimination to obtain a mixture of compounds of formulae (IIIa) and (IIIb); **or** methyl-Grignard addition to the compound of formula (I) and water elimination to obtain a mixture of compounds of formulae (3a) and (3b), respectively;

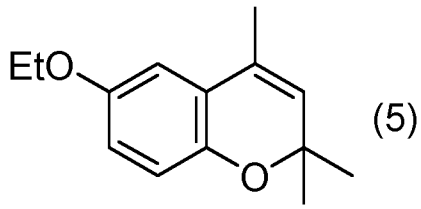


and

c) optional deprotection of the ether compounds of formulae (IIIa) and (IIIb) to a mixture of compounds of formulae (3a) and (3b)

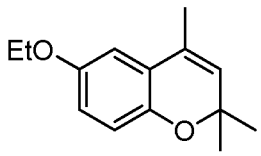


21. 6-ethoxy-2,2,4-trimethyl-2H-chromene of formula (5) with Et being ethyl.



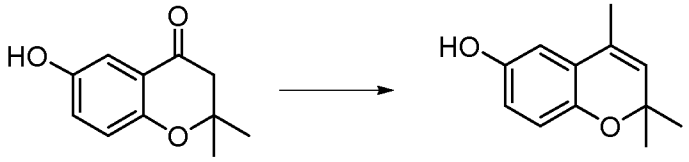
(5)

22. A process for the manufacture of compound of formula (5) with Et being ethyl comprising the following steps:



(5)

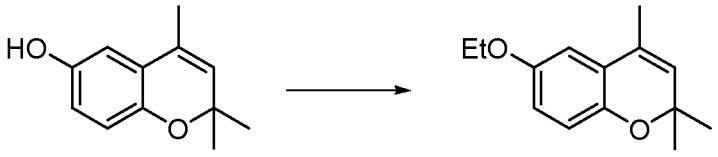
i) methyl-Grignard addition to compound of formula (IV) and water elimination to obtain the compound of formula (1),



(IV) (1)

and

ii) etherification of compound of formula (1) to obtain the compound of formula (5)

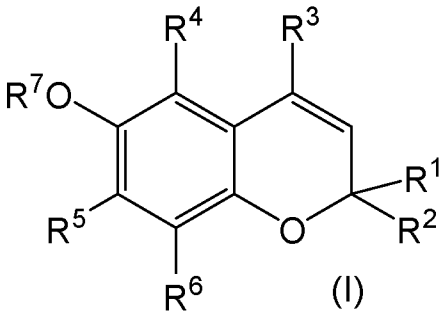


(1) (5)

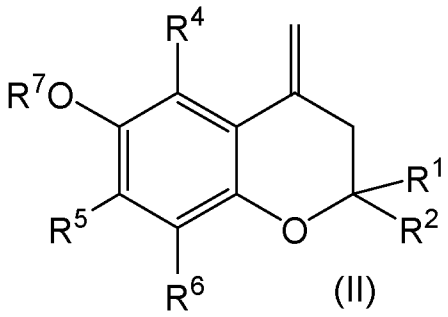
.

23. An edible oil comprising

at least one compound of formula (I) and/or at least one compound of formula (II),
and PUFAs and/or their esters,



(I)

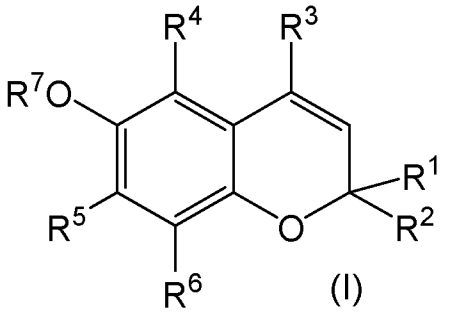


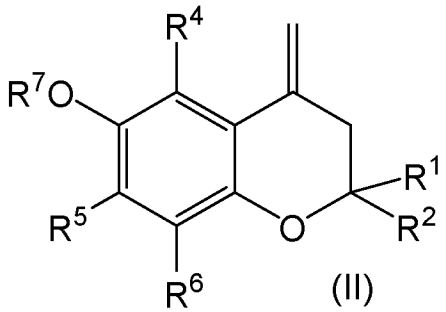
(II)

wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and
wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and
R^7 is H or C_{1-6}-alkyl.

24. The edible oil according to claim 23, whereby the edible oil is marine oil or microbial oil or fungal oil or algal oil or PUFA-containing plant oil, preferably whereby the edible oil is marine oil or algal oil, more preferably whereby the edible oil is algal oil.

25. A method of preserving the shelf life of PUFAs and/or their esters in an edible oil comprising the step of adding at least one compound of formula (I) and/or at least one compound of formula (II) to said edible oil,





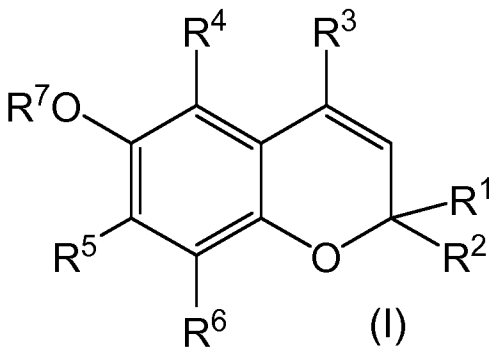
wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and

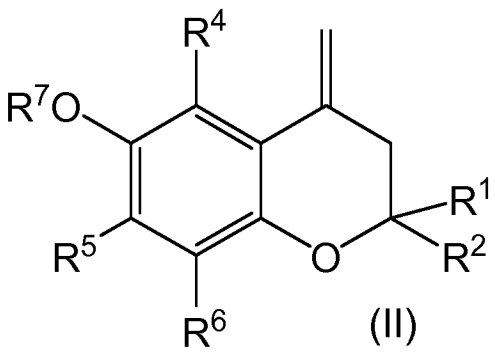
wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and

R^7 is H or C_{1-6}-alkyl.

26. The method according to claim 25, wherein the compound of formula (I) and/or the compound of formula (II) is/are added to said edible oil in an amount ranging from 10 to 500 ppm, preferably ranging from 30 to 300 ppm, more preferably ranging from 100 to 250 ppm, based on the total amount of said edible oil.

27. A method of limiting the amount of oxidation of PUFAs and/or their esters in an edible oil which is exposed to air, comprising adding at least one compound of formula (I) and/or at least one compound of formula (II) to said edible oil,





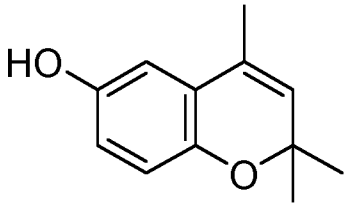
wherein R^1 and R^2 are independently from each other H or C_{1-11}-alkyl or $(CH_2)_n$—OH with n being an integer from 1 to 6 or R^1 and R^2 together represent a keto group, and

wherein R^3, R^4, R^5, and R^6 are independently from each other H or C_{1-6}-alkyl or C_{1-6}-alkoxy, and

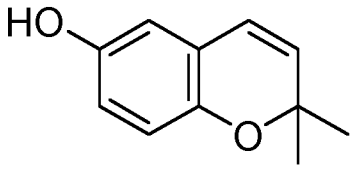
R^7 is H or C_{1-6}-alkyl.

28. The method according to claim 27, wherein the compound of formula (I) and/or the compound of formula (II) is/are added to said edible oil in an amount ranging from 10 to 500 ppm, preferably ranging from 30 to 300 ppm, more preferably ranging from 100 to 250 ppm, based on the total amount of said edible oil.

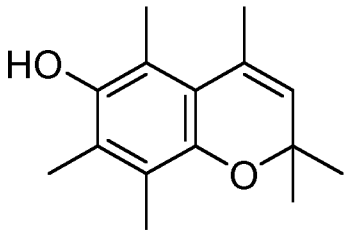
Fig. 1



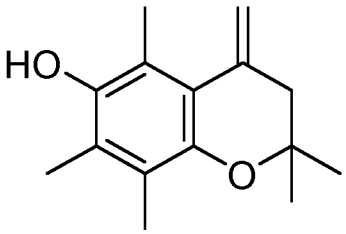
(1)



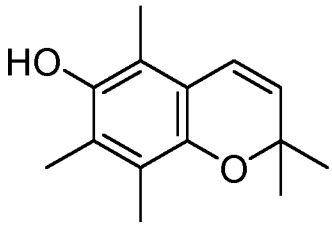
(2)



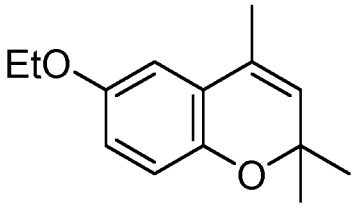
(3A)



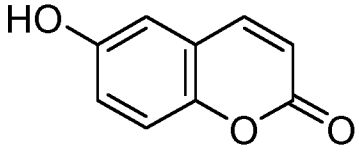
(3B)



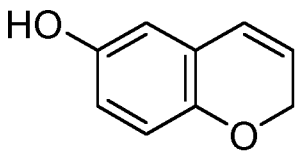
(4)



(5)



(6)



(7)

Fig. 2

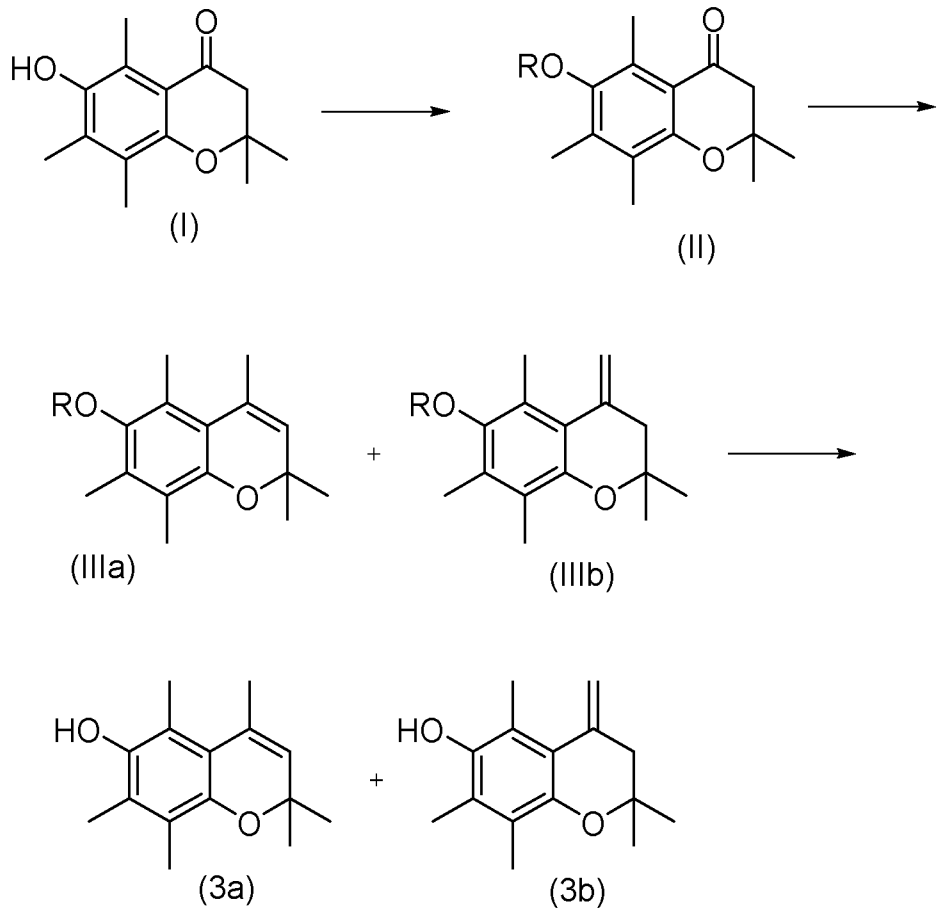

HO
O
O
(I)
RO
O
O
(II)
RO
O
(IIIa)
+
RO
O
(IIIb)
HO
O
(3a)
+
HO
O
(3b)

Fig. 3

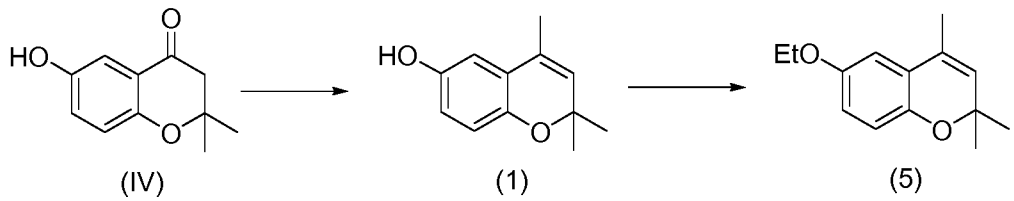
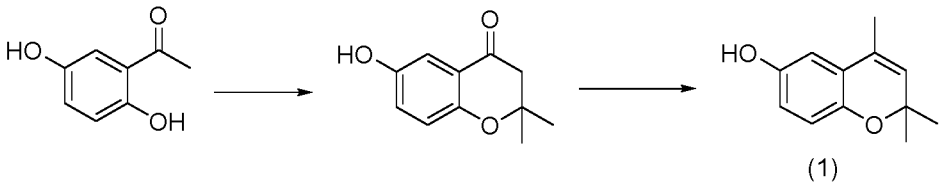
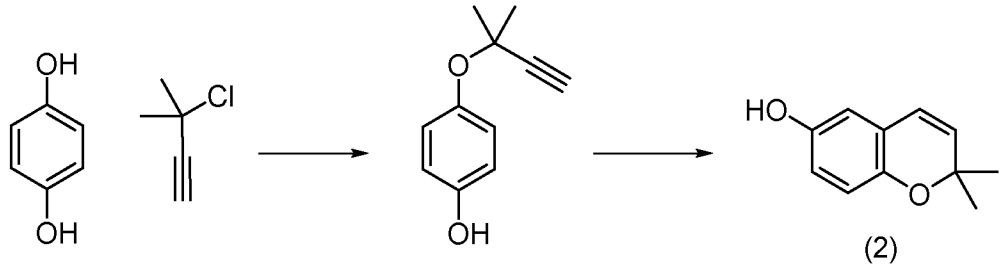

HO
O
O
(IV)
HO
O
(1)
EtO
O
(5)

Fig. 4



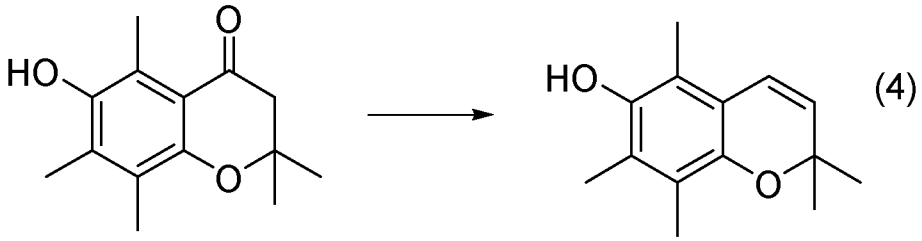
(1)

Fig. 5



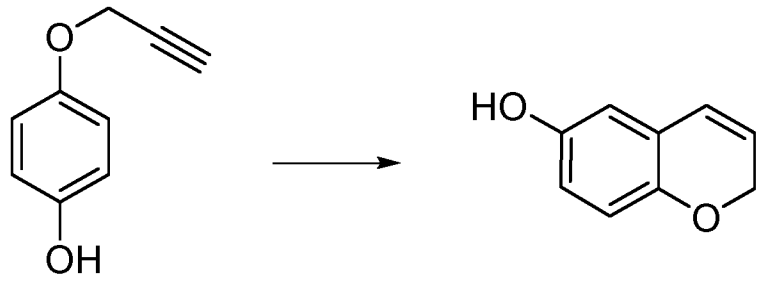
(2)

Fig. 6



(4)

Fig. 7



(7)