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(54) Title: STABILIZATION OF THE COMPOSITION OF A MIXTURE

(57) Abstract: Described is a method for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin, especially in extracts from plants of the genus Petasites, preferably Petasites hybridus, by the addition of at least one suitable stabilizer and/or the removal of at least one destabilizing substance, e.g. a fatty acid.



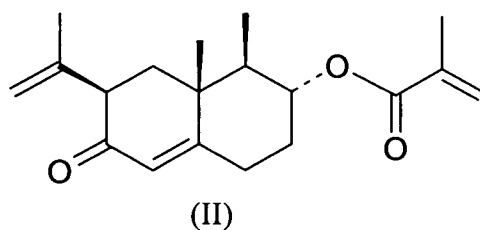
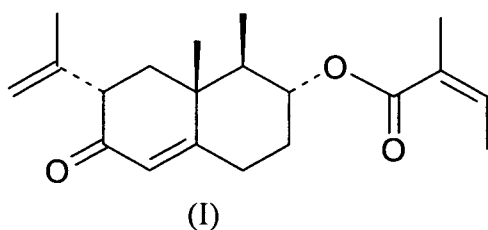
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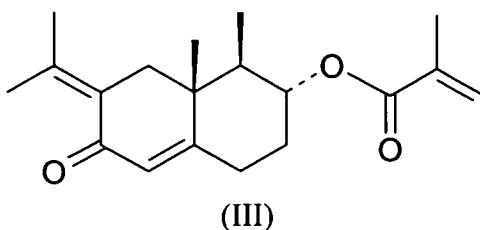
Stabilization of the composition of a mixture**FIELD OF THE INVENTION**

The invention relates to a method for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin, especially in extracts from plants of the genus *Petasites*, preferably *Petasites hybridus* (L.) Gaertner, Meyer & Scherb, by the addition of at least one suitable stabilizer and/or the removal of at least one destabilizing substance, and to stabilized extracts, and stabilized pharmaceutical formulations comprising said stabilized extracts or their active ingredients.

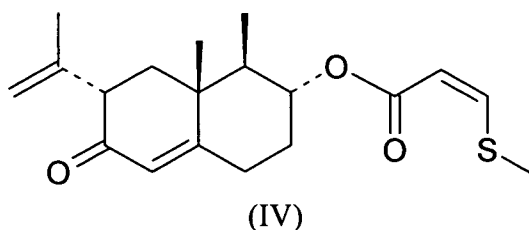
BACKGROUND OF THE INVENTION

Extracts from plants of the genus *Petasites*, like especially *Petasites hybridus* and *Petasites officinalis*, are well-known drugs for the treatment of various diseases (cf. e.g. EP0281656B1) including allergic and/or inflammatory diseases, e.g. seasonal allergic rhinitis (hay fever), perennial allergic rhinitis, atopic dermatitis, chronic obstructive pulmonary disease (COPD), asthma, and gastrointestinal diseases; and migraine. They are available as marketed drugs, e.g. under the trade name Tesalin® which is obtained by extraction with carbon dioxide (CO₂) in a subcritical state as described in European patent EP1023079B1. The main active ingredients of said drugs are petasin of the below formula I, neopetasin of the below formula II, and isopetasin of the below formula III.





In addition, said drugs comprise low amounts of sulfur-containing petasins (S-petasins), wherein the sulfur atom is incorporated in the ester moiety of the petasins, i.e. S-petasin of the below formula IV, neo-S-petasin and iso-S-petasin.



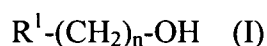
As a rough indication, freshly prepared extracts from *Petasites hybridus* contain petasin as main active ingredient besides about one third to one fourth - relative to the amount of petasin - of neopetasin and isopetasin, together with minor amounts of S-, neo-S-, and iso-S-petasins.

However, upon storage, especially at elevated temperatures, the amount of the active ingredients relative to each other does not remain constant, but changes in favour of isopetasin and iso-S-petasin due to an isomerisation reaction. Therefore, comprehensive studies were conducted to investigate how this isomerisation reaction can be prevented or, at least, slowed down to an acceptable extent. Efforts to slow the isomerisation reaction by protection of the petasin extracts as initially obtained (hereinafter designated as “native extracts”) through incorporation into cage-forming compounds, like cyclodextrins, failed. Studies concerning the effect of acids and bases did likewise not lead to useful insights. The addition of amines even led rapidly to complete isomerisation.

DESCRIPTION OF THE INVENTION

It has now been found during the course of said studies that the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin can be surprisingly inhibited, i.e. prevented or, at least, slowed down to a tolerable extent by addition of a sufficient amount of a suitable stabilizer, preferably a stabilizer selected from

a) a compound of formula I



wherein n is 0 or 1, R¹ may represent hydrogen when n is 1 only, or R¹ represents alkyl having up to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R¹ represents cycloalkyl, phenyl, or phenoxyethyl, each of which is unsubstituted or substituted in the cyclic moiety by C₁-C₇-alkyl or C₁-C₇-alkoxy,

b) a glycol of the formula HO-[CHR²-CH₂-O]_m-H, wherein R² is hydrogen or methyl and m is 1 to 500, or of the formula HO-[CH₂-CH₂-O]_x-[CH₂-CH(CH₃)-O]_y-[CH₂-CH₂-O]_z-H wherein x is 50-125, y is 20-70 and z is 50-125,

c) dibenzylether, and

d) hydrogenated plant oils, like hydrogenated castor oil (also called hydrogenated ricinus oil).

The mechanism by which the stabilization occurs is unknown at present. It can, however, be said that the extent of stabilization is linked to the amount of stabilizer relative to the amount of native extract, as has been shown in case of the stabilizer n-hexadecanol.

Usually, the best stabilization is achieved when the amount by weight of the stabilizer is 1 to 10fold, especially 2-8fold, preferably 3-6fold, e.g. about 4fold the amount by weight of the native extract. However, some stabilization effect, although usually not the best stabilization effect, occurs also when the amount by weight of the stabilizer is inferior, e.g. down to about one fourth, to the amount by weight of the native extract.

The invention relates to a method for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin by the addition of at least one suitable stabilizer, preferably a stabilizer described

herein above or below, and/or the complete or partial removal of at least one destabilizing substance, like preferably a fatty acid in free or bound form, especially in free form.

Among the fatty acids those in free form are considered to have the main destabilizing effect. However, it can not be excluded that free fatty acids are generated by hydrolysis of bound fatty acids.

The speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin in extracts from plants of the genus *Petasites* can not only be slowed down by the addition of at least one suitable stabilizer, but also by the removal of at least one destabilizing substance.

Usually, at least 50 %, especially more than 60, 70, 80 or 90 %, preferably more than 95 % and most preferably essentially all of the destabilizing substances are removed.

Fatty acids have been identified as destabilizing substances as shown in Examples 7 and 8 of the present application. In contrast thereto, acetic acid does not appear to have a significant destabilizing effect. Fatty acids are aliphatic monocarboxylic acids which are present, including the presence in a bound form, e.g. an esterified form, e.g. in the form of triglycerides or phospholipids, in an animal or vegetable fat, oil, or wax. Natural fatty acids commonly have a chain of four to 28, especially 8 to 28 carbons (usually unbranched and even numbered), which may be saturated or unsaturated. The native CO₂ extract from *Petasites hybridus* plants contains, in free and bound form taken together, linolenic acid [cis, cis, cis-CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₇COOH], linoleic acid [cis,cis-CH₃(CH₂)₄CH=CHCH₂CH=CH(CH₂)₇COOH], palmitic acid, oleic acid, stearic acid, arachidic acid (n-eicosanoic acid), about 11% of fatty acids having less than 16 carbon atoms, and about 1.4 % of fatty acids having more than 20 carbon atoms. The presence of fatty acids, like especially palmitic acid, in free form in the CO₂ extract from *Petasites hybridus* plants has been verified for the purposes of the present patent application by means of qualitative thin layer chromatography.

The evaluation of the effect of fatty acids in bound form, e.g. in the form of triglycerides, is currently under investigation. At present, it can neither be excluded that the fatty acids in

bound form also have a destabilizing effect nor that they serve over time as a source for the formation of additional fatty acids in free form once the free fatty acids have been removed. It can also not be excluded at present that under the conditions of neutralizing the free fatty acids, fatty acids in bound form, i.e. for example triglycerides, are saponified at least to some small extent.

Hence, the invention relates also to a method wherein at least one destabilizing substance is removed from an extract from plants of the genus *Petasites*, and to a process for removing from an extract from plants of the genus *Petasites* at least one substance promoting the isomerisation of petasin and neopetasin to isopetasin, by adding to said extract a suitable organic solvent which is immiscible or at most slightly miscible with water, like a suitable ether, e.g. diisopropylether, and a diluted aqueous solution of a suitable base, e.g. 0.1 molar aqueous sodium hydroxide solution, in an amount sufficient to neutralize the fatty acids present in said extract, stirring or shaking the obtained mixture, extracting the organic phase of said mixture with water, and isolating the purified extract from plants of the genus *Petasites* from said organic phase.

Alternatively, the fatty acids may be removed as follows: The native carbon dioxide extract is dissolved in a suitable solvent, e.g. an inert hydrophobic solvent, like especially a suitable hydrocarbon solvent, like a saturated aliphatic hydrocarbon solvent, like especially hexane, e.g. n-hexane. The obtained solution is passed through a column filled with a suitable anion exchanger, especially a slightly alkaline/weakly basic anion exchanger, like Lewatit® MP 62 (supplied by LANXESS Germany GmbH, Leverkusen, Germany). For example, one part by volume of native CO₂ extract is dissolved in 3 parts by volume of n-hexane. This solution is given on a column filled with Lewatit® MP 62. The ion exchanger removes all organic, but not anorganic acids.

The present invention relates also to a process for at least partial, but preferably essentially complete removal of all fatty acids from an extract from plants of the genus *Petasites*, especially *Petasites hybridus*, in a manner known known to a person skilled in the art, i.e. preferably by means of a suitable ion exchanger.

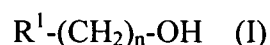
The present invention relates also to a process for removing from an extract from plants of the genus *Petasites* at least one fatty acid, i.e. preferably all fatty acids, promoting the isomerisation of petasin and neopetasin to isopetasin by passing a solution comprising said extract through a suitable weakly alkaline ion exchanger, or by neutralizing said fatty acid with a suitable base and extracting it with water. For example, the extract may be dissolved in a solvent which is immiscible or at most slightly miscible with water, like a suitable ether, e.g. diisopropylether. To the obtained solution a diluted aqueous solution of a suitable base, e.g. 0.1 molar aqueous sodium hydroxide solution, is added in an amount sufficient to neutralize the fatty acids present in said extract. After stirring or shaking the obtained mixture and extracting the organic phase of said mixture with water, the purified extract from plants of the genus *Petasites* is isolated from said organic phase.

The present invention relates also to an extract from plants of the genus *Petasites*, especially *Petasites hybridus*, which contains a stabilizer as defined herein and/or wherein the amount of fatty acids has been reduced below 50 % of the natural occurrence in the extract.

The present invention relates also to an extract from plants of the genus *Petasites*, especially *Petasites hybridus*, obtainable by the above purification process, to an extract from plants of the genus *Petasites* stabilized by a stabilizer, preferably a stabilizer as defined below, slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin, and to a method for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin, preferably in an extract from plants of the genus *Petasites*, by the addition of at least one suitable stabilizer and/or the removal of at least one destabilizing substance.

A suitable stabilizer is preferably selected from

a) a compound of formula I



wherein n is 0 or 1, R¹ may represent hydrogen when n is 1 only, or R¹ represents alkyl having up to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be

replaced by oxygen; or R^1 represents cycloalkyl, phenyl, or phenoxymethyl, each of which is unsubstituted or substituted in the cyclic moiety by C_1 - C_7 -alkyl or C_1 - C_7 -alkoxy,

b) a glycol of the formula $HO-[CHR^2-CH_2-O]_m-H$, wherein R^2 is hydrogen or methyl and m is 1 to 500, or of the formula $HO-[CH_2-CH_2-O]_x-[CH_2-CH(CH_3)-O]_y-[CH_2-CH_2-O]_z-H$ wherein x is 50-125, y is 20-70 and z is 50-125,

c) dibenzylether, and

d) hydrogenated plant oil, e.g. hydrogenated castor oil,

for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin.

Alkyl having up to 30 carbon atoms is straight-chain or branched alkyl having 1 to 30, preferably 1-22, especially 3-22 carbon atoms. Alkyl wherein one or more non-adjacent carbon atoms may be replaced by oxygen is e.g. ethoxyethyl.

A compound of the formula I wherein R^1 represents cycloalkyl is e.g. cyclohexanol.

A compound of the formula I wherein R^1 represents phenyl is e.g. phenol or benzylalcohol.

A compound of the formula I wherein R^1 represents phenoxymethyl is e.g. 2-phenoxyethanol.

A glycol of the formula $HO-[CHR^2-CH_2-O]_m-H$, wherein R^2 is hydrogen is e.g. polyethylene glycol wherein m is the number of ethylene oxide units. As an abbreviation for polyethylene glycols the term "PEG" is used in combination with a numerical value (e.g. PEG 4000) which, within the pharmaceutical industry, indicates the mean molecular weight. Unfortunately, the various pharmacopeias use different nomenclature for some PEG molecular weights. The molecular weights cited herein are in accordance with the European (2002) and US pharmacopeias. PEGs can be obtained for example from the company Clariant, Basle, Switzerland.

A glycol of the formula $HO-[CH_2-CH_2-O]_x-[CH_2-CH(CH_3)-O]_y-[CH_2-CH_2-O]_z-H$ wherein x is 50-125, y is 20-70 and z is 50-125, is a synthetic copolymer of ethyleneoxide and propyleneoxide, i.e. a polyoxyethylene-polyoxypropylene-triblock copolymer, e.g. Poloxamer 188 (Pluronic® F68) and Poloxamer 407.

The invention relates also to the use of an above-mentioned stabilizer for slowing down the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin, preferably in an extract from plants of the genus *Petasites*, especially *Petasites hybridus*.

The invention relates also to a pharmaceutical formulation comprising an effective dose of an extract from plants of the genus *Petasites*, especially from *Petasites hybridus*, together with an above-mentioned stabilizer and a pharmaceutical excipient, preferably to such a pharmaceutical formulation wherein at least one destabilizing substance, like especially a fatty acid, has been removed, e.g. as described above, from said extract from plants of the genus *Petasites*. Preferably, at least 50, 60, 70, 80, 90 or 95 % of the fatty acids, especially those present in free, i.e. non-bound, e.g. non-esterified, form are removed. Said formulation may be in liquid or especially in non-liquid form. The invention relates especially to such pharmaceutical formulation comprising propylene glycol and/or a hydrogenated plant oil, like hydrogenated ricinus oil.

The invention relates also to a pharmaceutical formulation comprising an effective dose of petasin, neopetasin, isopetasin or any mixture of those, an above-mentioned stabilizer and a pharmaceutical excipient. This kind of formulation is manufactured using pure petasin, pure neopetasin and/or pure isopetasin. Even in this case, the addition of an above-mentioned stabilizer may be meaningful, especially if the pharmaceutical excipient comprises fatty acids in free or bound form, e.g. fatty acid triglycerides.

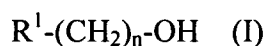
The invention relates also to a pharmaceutical formulation comprising petasin, neopetasin and/or isopetasin and a pharmaceutical excipient substantially free from free fatty acids.

The native extract obtained by extraction with carbon dioxide (CO₂) in a subcritical state contains about 25-35%, usually about 30% by weight of petasins. The native CO₂-extract is a very viscous oil. Therefore, in order to enable easier handling, substances like microcrystalline cellulose are added, preferably in a 3 to 4fold excess by weight

whereupon a powder is obtained. As has been shown in the Examples the effect of microcrystalline cellulose on the speed of isomerisation is negligibly small.

The invention relates especially to an above-mentioned pharmaceutical formulation wherein the stabilizer is selected from

a) a compound of formula I



wherein n is 1, R¹ represents alkyl having 2 to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R¹ represents cycloalkyl, phenyl, or phenoxyethyl, each of which is unsubstituted or substituted in the cyclic moiety by C₁-C₇-alkyl or C₁-C₇-alkoxy,

b) dibenzylether, and

c) a hydrogenated plant oil, like hydrogenated ricinus oil.

The present invention relates also to pharmaceutical formulations, especially non-liquid formulations, comprising 5-80 %, especially 10-70 %, preferably 15-60%, more preferably 20-50%, e.g. about 25 % by weight of a (optionally purified) Petasites extract together with an above-mentioned stabilizer and a pharmaceutical excipient. The pharmaceutical formulations are those for enteral or parenteral administration comprising especially the usual pharmaceutical excipients, and, if desired, a suitable cyclodextrin, e.g. β-cyclodextrin.

The daily dose of native extract to be administered to a human adult having about 70 kg of body weight is about 40-100 mg, e.g. about 55 mg.

DETAILED DESCRIPTION OF EXPERIMENTS

The following examples illustrate the invention.

General

The Petasites extract used in the following Examples may be prepared from Petasites hybridus as described in German Offenlegungsschrift DE19702168A1 or preferably by

extraction with carbon dioxide in a subcritical state as described in European patent EP1023079B1. The dry extract obtained from *Petasites hybridus* after evaporation of the carbon dioxide is designated herein as “native extract”. It contains about 30% by weight of petasins. In order to assess the effect of certain substances (designated herein as “stabilizers”) concerning their ability to hinder or slow the isomerisation to isopetasin said substances may be either added to the native extract (two component mixture) or to a mixture of native extract and microcrystalline cellulose (MCC; three component mixture). Usually, one part (“comparison part”) of said two or three component mixtures is frozen at -20 °C and serves for comparison purposes while the other part (“heated part”) is heated at 80 °C for 15 hours and, thereafter, analyzed by gas chromatography (GC) in order to determine the content of the various petasins contained therein expressed as percentage of the respective content (set as 100 %) in the above-mentioned comparison part which has been kept at -20 °C.

The gas chromatography (GC) is conducted using a Hewlett Packard 5890 Series 2 or Agilent 6890N instrument, equipped with a flame ionisation detector and a DB - 1 GC-column [i.e. a fused silica capillary GC column supplied by J&W Scientific (Agilent) comprising an amber-brown polyimide exterior coating which protects the tubing from breakage, the fused silica tubing and a stationary phase (consisting of dimethylpoly-siloxane, bonded and crosslinked) which is evenly coated (film thickness: 0.52 µm) onto the inner wall of the tubing], said column having a length of 25 m and an inside diameter of 0.32 mm, using hydrogen (purity 99.999 %) as carrier gas at a flow of 3 ml/minute at 180°C (pressure: about 70 kPa), and (-)- α -Santonin as internal standard. Periodic verification of the response is made with a control sample. Calculation of the content of the three petasins and the sum thereof is made automatically with the HP ChemStation (trade name) manufactured by Hewlett Packard.

Example 1: stabilization of petasins in “three component mixtures”

In the present Example the ratio by weight of native extract, microcrystalline cellulose and stabilizer is 1 : 3.75 : 3.75. The mixtures are prepared in a mortar, into which first MCC, then the stabilizer and finally the native extract are added, followed by mixing during 5 minutes. The thus obtained mixture is separated into two parts, one part (“comparison part”) of which is frozen at -20°C and serves for comparison purposes, while the second part is kept at 80°C during 15 hours.

After 15 hours at 80°C the above-mentioned three component mixtures are analyzed by gas chromatography (GC) in order to determine the content of neopetasin, petasin, and isopetasin contained therein expressed as percentage of the respective content (taken as 100 %) in the above-mentioned comparison part which has been kept at -20 °C. The results are given in the below table. As evident therefrom the sum of the 3 petasins sometimes exceeds 100% and, hence, reflects the size of the experimental error while values far below 100% are not only due to an experimental error, but reflect some degradation of the petasins having taken place under these conditions.

Table 1

Stabilizer	Percentage [%] of original content			
	Neo-petasin	Petasin	Isopetasin	Sum of these 3 petasins
None (native extract + MCC)	51.3	71.2	151.3	98.0
Methanol	99.8	101.4	107.1	102.7
Ethanol	99.8	102.4	107.1	103.3
Isopropanol	99.0	99.9	104.8	101.1
1-Pentanol	100.0	100.7	105.9	101.6
1-Dodecanol	90.1	92.8	104.1	96.3
1-Hexadecanol	90.9	94.9	112.6	97.5

Stabilizer	Percentage [%] of original content			
	Neo-petasin	Petasin	Isopetasin	Sum of these 3 petasins
1-Octadecanol	92.8	95.4	114.0	98.4
1-Docosanol	86.6	93.2	109.6	97.9
Cyclohexanol	101.4	101.1	107.8	102.4
Benzylalcohol	99.2	100.6	109.2	102.0
Phenol	96.1	97.9	106.1	99.9
Ethoxyethanol	99.8	100.7	106.0	101.9
2-Phenoxyethanol	97.2	99.2	106.4	100.2
Nonaethylene glycol	94.5	97.4	110.9	99.4
PEG 4000	87.5	92.5	104.1	95.8
PEG 6000	87.7	94.3	104.7	97.0
PEG 20000	85.8	90.2	101.3	93.4
Propylene glycol	91.2	95.5	110.8	99.0
Poloxamer 188*	95.5	98.6	115.0	100.8
Poloxamer 407**	93.6	94.8	115.1	98.0
Dibenzylether	81.2	90.0	125.5	98.2
Hydrogenated castor oil***	91.5	97.2	125.0	100.9

* Poloxamer 188 (Pluronic F68) is a synthetic copolymer of ethyleneoxide and propyleneoxide, i.e. a polyoxyethylene-polyoxypropylene-triblock copolymer. The approximate lengths of the two polyethylene glycol blocks is 75 repeat units while the approximate length of the propylene glycol block is 30 repeat units.

**Poloxamer 407 (also known by the BASF trade name Pluronic F127) is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol. The approximate lengths of the two

polyethylene glycol blocks is 101 repeat units while the approximate length of the propylene glycol block is 56 repeat units.

***Hydrogenated castor oil contains about 80 – 90 % of the triglyceride of ricinolic acid (a linear hydroxy fatty acid)

Example 2: stabilization of petasins in “two component mixtures”

The stabilizing effect of stearyl alcohol, i.e. n-octadecanol, and n-hexadecanol is determined analogously as described in Example 1, by combining the native extract with these stabilizers, but, in contrast to Example 1, without addition of microcrystalline cellulose (MCC), and with the ratio by weight of native extract and stabilizer being 1:7, i.e. the relative amount by weight of the stabilizer is doubled in comparison to Example 1 in order to “compensate” for the absence of MCC (twice 3.75 is 7). The results are evident from Table 2.

Table 2

Stabilizer	Percentage [%] of original content			
	Neo-petasin	Petasin	Isopetasin	Sum of all petasins
None (native extract without MCC)	51.3	71.2	151.3	98.0
stearyl alcohol	93.9	95.7	107.3	97.6
n-hexadecanol	95.6	96.9	106.2	98.4

Example 3: Varying the amount of the stabilizer

The stabilizing effect of n-hexadecanol is determined analogously as described in Example 1 with the following exception: Whereas in Example 1 the ratio by weight of native extract, microcrystalline cellulose (MCC) and stabilizer is 1 : 3.75 : 3.75, said ratio is varied in the present Example as follows:

Example 3a) 1 : 3.75 : 3.75

Example 3b) 1 : 4.69 : 2.81

Example 3c) 1 : 5.63 : 1.88

Example 3d) 1 : 6.56 : 0.94

Example 3e) 1 : 7.00 : 0.50

Example 3f) 1 : 7.25 : 0.25

Example 3g) 1 : 7.50 : 0.00

As evident from above, the ratio of native extract to stabilizer is varied stepwise very roughly from 1 : 4 to 1 : 0. In experiments 3a to 3g the content by weight of the native extract in the total three component mixtures is kept constant at about 11.8 %.

After 15 hours at 80°C the above-mentioned three component mixtures are analyzed by gas chromatography (GC) in order to determine the content of neopetasin, petasin, isopetasin and the sum of these 3 petasins contained therein expressed as percentage of the respective content (taken as 100%) in the above-mentioned comparison part which has been kept at - 20 °C. The results are given in the below table:

Table 3

Example	Percentage [%] of original content			
	Neo-petasin	Petasin	Isopetasin	Sum of these 3 petasins
3a	92.2	95.8	112.0	98.2
3b	90.8	96.2	115.4	98.8
3c	88.2	94.2	123.1	98.5
3d	82.1	91.3	137.0	98.2
3e	74.7	86.2	146.6	96.6
3f	68.2	82.7	162.2	96.5
3g (no stabilizer)	59.0	78.2	187.8	95.9

As evident from Table 3 the stabilization effect decreases with diminishing relative amounts of the stabilizer.

Example 4: Varying the amount of MCC

The stabilizing effect of n-hexadecanol is determined analogously as described in Example 1 with the following exception: Whereas in Example 1 the ratio by weight of native extract, microcrystalline cellulose (MCC) and stabilizer is 1 : 3.75 : 3.75, said ratio is varied in the present Example as follows:

Example 4a) 1 : 5.63 : 1.88

Example 4b) 1 : 2.79 : 1.88

Example 4c) 1 : 1.38 : 1.88

Example 4d) 1 : 0.53 : 1.88

As evident from above, roughly, in experiments 4a to 4d the ratio of native extract to stabilizer is kept constant at 1 : 1.88, whereas the ratio of native extract to MCC is increased stepwise about ten fold.

After 15 hours at 80°C the above-mentioned three component mixtures are analyzed by gas chromatography (GC) in order to determine the content of neopetasin, petasin, isopetasin and the sum of these three petasins contained therein expressed as percentage of the respective content in the above-mentioned comparison part (taken as 100 %) which has been kept at -20 °C. The results are given in the below table:

Table 4

Example	Percentage [%] of original content			
	Neo-petasin	Petasin	Isopetasin	Sum of these 3 petasins
4a	90.1	94.3	119.2	98.4
4b	91.4	94.3	122.2	99.1
4c	89.1	92.2	120.1	97.0
4d	88.8	93.6	122.5	98.3

As evident from Table 4 the effect of the variation of the ratio of native extract to MCC on the stabilization is rather negligible.

Example 5: stabilization of S-petasins in “three component mixtures”

The effect of stabilizers on S-petasins is determined analogously to Example 1. The results are evident from Table 5.

Table 5

Stabilizer	Percentage [%] of original content			
	Neo-S-petasin	S-Petasin	Iso-S-petasin	Sum of S-petasins
None (native extract without MCC)	64.8	84.4	163.2	102.2
Methanol	98.3	98.9	105.5	100.6
Ethanol	101.0	100.6	104.2	101.7
Isopropanol	94.7	97.2	100.6	97.5
1-Pentanol	100.2	100.5	102.3	100.9
1-Dodecanol	92.2	90.3	102.5	95.5
1-Hexadecanol	90.5	93.5	112.4	98.7
1-Octadecanol	93.4	94.1	101.5	96.4
1-Docosanol	92.2	90.3	102.5	95.5
Cyclohexanol	100.5	101.1	105.7	102.3
Benzylalcohol	95.4	97.4	102.8	98.5
Phenol	90.9	93.3	99.5	94.4
Ethoxyethanol	96.4	97.6	101.5	98.4
2-Phenoxyethanol	96.6	95.6	104.2	98.4
Nonaethyleneglycol	92.2	96.0	107.2	97.6
PEG 4000	90.3	92.3	98.0	94.0
PEG 6000	95.5	92.5	100.3	96.3

Propyleneglycol	93.3	93.1	102.7	96.0
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As evident from a comparison of Tables 1 and 5 the effect of the stabilizers on the stability of S-petasin and neo-S-petasin is roughly the same as on petasin and neopetasin.

Example 6: Same molar amount of various stabilizers

The stabilizing effect of various stabilizers is determined analogously as described in Example 1 with the following exception: Whereas in Example 1 the ratio by weight of native extract, microcrystalline cellulose (MCC) and stabilizer is 1 : 3.75 : 3.75, said ratio is used only in Example 6a, but varied in Examples 6b and 6c as follows:

Example 6b) 1 : 3.75 : 0.71

Example 6c) 1 : 3.75 : 1.67

Due to the different molecular weights of the stabilizers, the molar amount of the stabilizers is the same in Examples 6a to 6c.

Table 6

Example	Stabilizer	Percentage [%] of original content			
		Neopetasin	Petasin	Isopetasin	Sum of these 3 petasins
6a	1-Hexadecanol	94.8	96.5	110.7	98.6
6b	Ethanol	79.4	88.2	142.7	98.0
6c	Benzylalcohol	95.7	96.8	113.3	99.7

As evident by comparison with Table 1 (where the stabilizers are used in the same absolute amounts) the stabilizing effect of ethanol is considerably reduced when using the same molar amounts as the other stabilizers. However, this might, in part, be due to the fact that in Example 6 a relatively higher amount of the ethanol is evaporating within the closed vessel into the gaseous phase than for hexadecanol or benzylalcohol in Example 6 and also for ethanol in Example 1.

Example 7: purification of native extract (inter alia by removal of fatty acids)

The native extract from *Petasites hybridus* is split into three parts. The first part is kept at -20 °C and later serves for comparison purposes. The second part ("heated part") is heated at 80 °C for 15 hours and, thereafter analyzed by gas chromatography (GC) in order to determine the content of neopetasin, petasin, and isopetasin contained therein expressed as percentage of the respective content in the first part (taken as 100 %) which has been kept at -20 °C. To the third part consisting of 3.0 g of native extract are added 15.6 ml of 0.1 molar aqueous sodium hydroxide solution (i.e. an amount exactly sufficient to neutralize the free fatty acids present in the native extract) and 30 ml of diisopropylether. The obtained mixture is intensively agitated. Thereafter it is once shaken out, i.e. extracted, well with 30 ml of water followed by centrifugation for 10 minutes at 4000 rounds per minute in order to effect separation of the phases. The diisopropylether phase is washed five times with 30 ml of water. The diisopropylether is removed at 40°C and 180 mbar followed by blowing nitrogen through the purified extract for 20 minutes. A part of the purified extract (comparison part) is frozen, the other part ("heated part") is heated at 80 °C for 15 hours and, thereafter analyzed by gas chromatography (GC) in order to determine the content of neopetasin, petasin, and isopetasin contained therein expressed as percentage of the respective content in the above-mentioned comparison part which has been kept at -20 °C. The results are given in the below table:

Table 7

Kind of extract	Percentage [%] of original content			
	Neo-petasin	Petasin	Isopetasin	Sum of these 3 petasins
Native („first part")	100	100	100	100
Native („second part, heated")	60.6	77.8	195.3	99.2
Purified („comparison part")	100	100	100	100
Purified ("heated part")	90.1	94.4	118.0	98.5

As evident from Table 7 much more petasin (i.e. 94.4 %) is retained in the heated part of the purified extract than in the heated part („second part, heated”) of the native extract (77.8 %).

Alternatively, the fatty acids may be removed as follows:

One part by volume of native CO₂ extract is dissolved in 3 parts by volume of n-hexane. This solution is given on a glass column filled with an alkalescent ion exchanger, i.e. a slightly alkaline/weakly basic anion exchanger (Lewatit® MP 62; LANXESS Germany GmbH, Leverkusen, Germany). The ion exchanger removes all organic, but not anorganic acids.

Example 8: Effect of acids or bases on the stability of pure petasin

Pure petasin is isolated from the extract by chromatography over silica gel, followed by reverse phase chromatography over a C₁₈-column and repeated crystallization. 30.7 mg of the thus obtained pure petasin are dissolved in 20 ml of n-hexane. A part of the obtained solution (comparison part) is frozen, the other part (“heated part”) is heated at 80 °C for 15 hours and, thereafter analyzed by gas chromatography (GC) in order to determine the content of petasin contained therein expressed as percentage of the respective content in the above-mentioned comparison part which has been kept at -20 °C. The result is given in the below table (Example 8.1). Analogous experiments are carried out wherein the acids or the base mentioned in Table 8 are added to the petasin solution before heating to 80 °C

Table 8

Example	Components dissolved in 20 ml of n-hexane	Percentage [%] of original petasin content
8.1	30.7 mg of petasin	97 %
8.2	31.5 mg of petasin + 15.0 mg (59 µmol) of palmitic acid	63 %
8.3	18.6 mg of petasin + 3.5 mg (58 µmol) acetic acid (pH of the obtained solution: 3.8)	95 %

8.4	20.5 mg of petasin + addition of aqueous HCl solution (0.05 mol/litre) up to pH 3.5	106 %
8.5	23.1 mg of petasin + addition of aqueous NaOH solution (0.1 mol/l) up to pH 9.5	100 %

As evident from Table 8 the addition of palmitic acid negatively affects the stability of petasin, whereas acetic acid, hydrochloric acid and sodium hydroxide solution in the range of pH 3.5 to 9.5 do not appear to have a marked influence.

Example 9: Pharmaceutical tablet formulation

300 parts by weight of an extract of *Petasites hybridus* wherefrom the fatty acids have been removed analogously as described in Example 7, 300 parts by weight of Aerosil® 200V (pyrogenic silicon dioxide [amorphous fumed silica] of the company Degussa having a specific surface area of about 200 m²/g), and 300 parts by weight of microcrystalline cellulose are mixed in a Stephan mixer (i.e. a mixer supplied by the German company Stephan Machinery GmbH). Thereafter 240 parts by weight of Lutrol®F68 (Poloxamer 188; supplied by the German company BASF ; surface active compound; solubilizer; readily water soluble; block polymer of the type ABA, consisting of a central, hydrophobic block of polypropylene oxide, which is edged by two hydrophilic blocks of polyethylene oxide. Lutrol®F68 has an average molecular weight of about 8600. The polyoxyethylene units represent about 81% of the molecular weight whereas that one of polyoxypropylene stands for about 19 %.) and 360 parts by weight of Lutrol®F127 (Poloxamer 407; supplied by the German company BASF ; surface active compound; solubilizer; readily water soluble; block polymer of the type ABA, consisting of a central, hydrophobic block of polypropylene oxide, which is edged by two hydrophilic blocks of polyethylene oxide. Lutrol®F127 has an average molecular weight of about 12200. The polyoxyethylene units represent about 73% of the molecular weight whereas that one of polyoxypropylene stands

for about 27 %.) are melted at about 75°C and added in portions, i.e. portion-wise, under stirring to the obtained mixture. The resulting white granulate is sieved and pressed in a tableting machine to tablets each of which is weighing 500 mg.

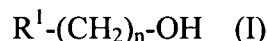
CLAIMS

1. Method for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin by the addition of at least one suitable stabilizer and/or the removal of at least one destabilizing substance.

2. Method for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin in extracts from plants of the genus Petasites by the addition of at least one suitable stabilizer and/or the removal of at least one destabilizing substance.

3. Method according to claim 1 or 2, wherein the stabilizer is selected from

a) a compound of formula I



wherein n is 0 or 1, R^1 may represent hydrogen when n is 1 only, or R^1 represents alkyl having up to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R^1 represents cycloalkyl, phenyl, or phenoxymethyl, each of which is unsubstituted or substituted in the cyclic moiety by C_1 - C_7 -alkyl or C_1 - C_7 -alkoxy,

b) a glycol of the formula $HO-[CH(R^2)-CH_2-O]_m-H$, wherein R^2 is hydrogen or methyl and m is 1 to 500, or of the formula $HO-[CH_2-CH_2-O]_x-[CH_2-CH(CH_3)-O]_y-[CH_2-CH_2-O]_z-H$ wherein x is 50-125, y is 20-70 and z is 50-125,

c) dibenzylether, and

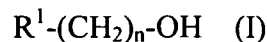
d) hydrogenated castor oil,

for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin.

4. Method according to any one of claims 1-3 wherein the destabilizing substance is a fatty acid in free and/or bound form.

5. Use of a stabilizer selected from

a) a compound of formula I



wherein n is 0 or 1, R^1 may represent hydrogen when n is 1 only, or R^1 represents alkyl having up to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R^1 represents cycloalkyl, phenyl, or phenoxymethyl, each of which is unsubstituted or substituted in the cyclic moiety by C_1 - C_7 -alkyl or C_1 - C_7 -alkoxy,

b) a glycol of the formula $HO-[CHR^2-CH_2-O]_m-H$, wherein R^2 is hydrogen or methyl and m is 1 to 500, or of the formula $HO-[CH_2-CH_2-O]_x-[CH_2-CH(CH_3)-O]_y-[CH_2-CH_2-O]_z-H$ wherein x is 50-125, y is 20-70 and z is 50-125,

c) dibenzylether, and

d) hydrogenated castor oil,

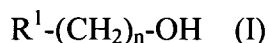
for slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin.

6. Pharmaceutical formulation comprising petasin, neopetasin and/or isopetasin and a pharmaceutical excipient substantially free from free fatty acids.

7. Pharmaceutical formulation comprising an extract from plants of the genus Petasites wherein the amount of fatty acids has been reduced below 50 % of the natural occurrence in the extract, and a pharmaceutical excipient.

8. Pharmaceutical formulation according to claim 7 comprising an extract from plants of the genus Petasites, wherein the amount of fatty acids has been reduced below 50 % of the natural occurrence in the extract, together with a stabilizer selected from

a) a compound of formula I



wherein n is 0 or 1, R^1 may represent hydrogen when n is 1 only, or R^1 represents alkyl having up to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R^1 represents cycloalkyl, phenyl, or phenoxymethyl, each of which is unsubstituted or substituted in the cyclic moiety by C_1 - C_7 -alkyl or C_1 - C_7 -alkoxy,

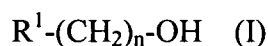
b) a glycol of the formula $HO-[CHR^2-CH_2-O]_m-H$, wherein R^2 is hydrogen or methyl and m is 1 to 500, or of the formula $HO-[CH_2-CH_2-O]_x-[CH_2-CH(CH_3)-O]_y-[CH_2-CH_2-O]_z-H$ wherein x is 50-125, y is 20-70 and z is 50-125,

c) dibenzylether, and

d) a hydrogenated plant oil, like hydrogenated castor oil,
and a pharmaceutical excipient.

9. Pharmaceutical formulation comprising an extract from plants of the genus *Petasites*, a stabilizer selected from

a) a compound of formula I



wherein n is 1, R¹ represents alkyl having 2 to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R¹ represents cycloalkyl, phenyl, or phenoxyethyl, each of which is unsubstituted or substituted in the cyclic moiety by C₁-C₇-alkyl or C₁-C₇-alkoxy,

b) dibenzylether, and

c) a hydrogenated plant oil, like hydrogenated castor oil,
and a pharmaceutical excipient.

10. Pharmaceutical formulation according to any one of claims 7-9 comprising an extract from *Petasites hybridus*.

11. Pharmaceutical formulation according to any one of claims 6 -10 in a non-liquid form.

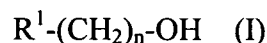
12. Process for removing from an extract from plants of the genus *Petasites* at least one fatty acid promoting the isomerisation of petasin and neopetasin to isopetasin by passing a solution comprising said extract through a suitable weakly alkaline ion exchanger or by neutralizing said fatty acid with a suitable base and extracting it with water.

13. Extract obtainable by the process according to claim 12.

14. Extract from plants of *Petasites hybridus* wherein the amount of fatty acids has been reduced below 50 % of the natural occurrence in the extract.

15. Extract according to claim 13 or 14 stabilized by a stabilizer slowing the speed of the isomerisation of petasin and neopetasin to isopetasin, and the isomerisation of S-petasin and neo-S-petasin to iso-S-petasin selected from

a) a compound of formula I



wherein n is 0 or 1, R¹ may represent hydrogen when n is 1 only, or R¹ represents alkyl having up to 30 carbon atoms wherein one or more non-adjacent carbon atoms may be replaced by oxygen; or R¹ represents cycloalkyl, phenyl, or phenoxymethyl, each of which is unsubstituted or substituted in the cyclic moiety by C₁-C₇-alkyl or C₁-C₇-alkoxy,

b) a glycol of the formula HO-[CHR²-CH₂-O]_m-H, wherein R² is hydrogen or methyl and m is 1 to 500, or of the formula HO-[CH₂-CH₂-O]_x-[CH₂-CH(CH₃)-O]_y-[CH₂-CH₂-O]_z-H wherein x is 50-125, y is 20-70 and z is 50-125,

c) dibenzylether, and

d) hydrogenated castor oil.