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(54) Title: PROCESS FOR THE DIASTEREOISOMERIC RESOLUTION OF 5-METHYLTETRAHYDROFOLIC ACID

(57) Abstract: The invention relates to a process for the diastereoisomeric resolution of 5-methyltetrahydrofolic acid; it discloses also a crystalline amine salt of 5-methyltetrahydrofolic acid and is further directed to a process for the transformation of the single diastereoisomers of the 5-methyltetrahydrofolic amine salts obtained by the process of the invention into the corresponding free acid or into an alkaline earth metal salt. The invention provides a simple, economically advantageous and efficient process and allows to obtain stable and pure (6R) and (6S)-diastereoisomers of 5-methyltetrahydrofolic acid in a good yield and diastereoisomeric purity.

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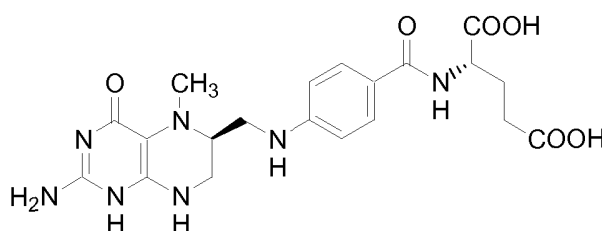
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PROCESS FOR THE DIASTEREOISOMERIC RESOLUTION OF 5-METHYLTETRAHYDROFOLIC ACID

The present invention relates to a process for the diastereoisomeric resolution of 5-methyltetrahydrofolic acid. The invention discloses also a crystalline salt of 5-methyltetrahydrofolic acid and is further directed to a process for the transformation of the single diastereoisomers of the 5-methyltetrahydrofolic salts obtained by the process of the invention into the corresponding free acid or into an alkaline earth metal salt.

5-Methyltetrahydrofolic acid, i.e. (6R,S)-N-[4-[(2-amino-1,4,5,6,7,8-hexahydro-methyl-4-oxo-5-methyl-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid -hereinafter 5-MTHF- and its derivatives have two asymmetric centres, the one belonging to the L-glutamic acid moiety and a second chiral centre on the carbon in position 6 of the pteridinyl moiety.

The configuration of the natural isomer corresponds to (6S)-N-[4-[(2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, having the following formula:



This natural diastereoisomer, found commonly in foods, is the essential endogenous form utilised and stored in the human body whereas the unnatural diastereoisomer is therapeutically ineffective since it is poorly and slowly metabolized and therefore excreted as it is. It is accordingly self-evident the need to administer the natural diastereoisomer. Nevertheless, in view of the difficulties to effectively

separate the diastereoisomers, 5-methyltetrahydrofolic acid is used both as a diastereomer mixture and as a natural isomer, mainly in the form of calcium salts, as pharmaceutical or nutraceutical for the treatment of -for instance-
5 megaloblastic folic acid anaemia, as an antidote for improving the tolerance of folic acid antagonists, specifically aminopterin and methotrexate in cancer therapy ("antifolate rescue"), for enhancing the therapeutic effect of fluorinated pyrimidines and for the treatment of autoimmune diseases such
10 as psoriasis and rheumatic arthritis, for improving the tolerance of certain antiparasitics, such as trimethoprim-sulfamethoxazole, and for reducing the toxicity of dideazatetrahydrofolates in chemotherapy.

In order to either synthesize or isolate the natural
15 diastereoisomer of various derivatives of folic acid, either substituted or reduced, among which 5-MTHF is likely to be the most studied, a number of methods has been disclosed in the last decades.

Given the amphoteric nature of these derivatives of folic acid
20 and also in view of the fact that the isomers to be separated are already diastereoisomers, these resolution methods can be basically classified depending on whether they take advantage of the acid or basic characteristics of such derivatives or of the different solubility of the corresponding
25 tetrahydrofolates as free acids.

Among the processes exploiting the acid characteristics of folic acid derivatives, EP 0537842 discloses -*inter alia*- a process for the separation of 6(R,S)-5-MTHF and 6(R,S)-5-formyltetrahydrofolic acid by precipitating -in water- a
30 mixture of inorganic salts thereof, preferably alkali metal or alkaline earth metal salts, and subsequent treatment with a strong acid, finally adjusting the pH close to neutrality.

Further, US 5,194,611 and US 5,382,581 disclosing salts of N⁵-methyl-5,6,7,8 tetrahydrofolic acid with cyclohexyl amine,
35 diisopropyl amine, benzyl amine, ammonia, ethanol amine,

triethanol amine, 2-dimethyl amino-ethanol, tert-butyl amine, lysine, or arginine, describe a process for the preparation and separation of such salts, in the presence of a mixture of solvents containing water and at least one totally water miscible solvent, -the solvent being preferably in an amount of 50-95%-, into the single (6R)- and (6S)-diastereoisomers, the latter being possibly transformed into the corresponding alkali metal or alkaline earth metal salts.

US 5,457,202 relates to a process for the resolution of (6R,S)-5-methyltetrahydrofolic acid comprising taking up the acid or a water-soluble salt thereof in water and adding a solution of N-ethyl-2-aminomethylpyrrolidine or an optical isomer thereof in water, subsequently precipitating the resulting salt. Heating the reaction mixture to 40-90°C, results in the salt of the 6S-acid dissolution, which is thus separated from the salt of the 6R-acid. The salt of the 6S-acid precipitates again on cooling the solution. Suspension of the latter in water and addition of sodium hydroxide solution results in the sodium salt that can be converted with, for example, an alkaline earth metal hydroxide or chloride into an alkaline earth metal salt such as the calcium salt. The yields of (6S)-5-MTHF are however poor, also in view of the repeated crystallizations required, and the process does not represent a commercially and industrially effective alternative.

IT 1254954 discloses a process for preparing the (6S) diastereoisomer of folinic acid comprising, in the presence of organic solvents, preferably methylethyl ketone and acetone, the salification of the racemic mixture of said acid or of 5-N-formyl-5,6,7,8-tetrahydropteroic acid, with S-(alpha)-methyl-p-nitro-benzylamine or (S)-(-)-alpha-phenylethylamine and the subsequent crystallization of the salt containing the optically active correspondent form.

Among the processes exploiting the basic characteristics of folic acid derivatives US 5,006,655 discloses a process for the preparation of 5,10-methenyl-(6R)-, 5-formyl-(6S)-, or 5-

methyl-(6S)-tetrahydrofolic acid or a salt thereof comprising fractionally crystallizing 5,10-methenyl-(6R,S)-tetrahydrofolic acid or a salt thereof, which is present in at least a 70% by weight concentration, in the presence of organic solvents.

US 5,324,836 discloses a process for the preparation of (6S)- and (6R)-tetrahydrofolic acid and their addition salts with sulphonic acids or with sulphuric acid comprising the steps of reacting (6R,S)-tetrahydrofolic acid with a sulphonic acid or sulphuric acid and fractionally crystallizing the resulting addition salt, preferably in the presence of an oxidation inhibitor.

US 6,858,731 discloses a process for preparing and concentrating (6S, α S) or (6S, α R) tetrahydrofolic acid ester salts and (6S, α S) or (6S, α R) tetrahydrofolic acid comprising preparing or dissolving diastereoisomers of addition salts of tetrahydrofolic acid esters with aromatic sulphonic acids in organic solvents and crystallizing them.

Among the processes exploiting the different solubility of the corresponding tetrahydrofolates as free acids, US 5,489,684 discloses a process for the preparation of (6S)-5,6,7,8-tetrahydrofolic acid having a diastereoisomeric purity of at least 75% comprising the direct crystallization from an aqueous solution of an alkali metal salt of the (6R,S) diastereoisomeric mixture of 5,6,7,8-tetrahydrofolic acid added with a strong non-oxidizing acid or acetic acid at 5-80°C, adjusting the pH at 4.8-5.3.

Further, US 6,271,374 discloses crystalline (6S)-tetrahydrofolic acid having a purity higher than 98% and a process for the preparation thereof comprising the crystallization of (6S)- or (6R,S)-tetrahydrofolic acid in water or in a mixture of water and a water miscible organic solvent.

Some further resolution has been proposed in US 5,698,693 wherein a separation by chiral chromatography or fractional

crystallization carried out on α -esters of (6R,S)-5,6,7,8-tetrahydrofolic acid derivatives, subsequently deprotected, is disclosed.

Chromatography in chiral phase is instead disclosed in EP 0627435 concerning the resolution of diastereoisomeric mixtures of some tetrahydrofolates.

However, either any of these processes show the need of repeated crystallizations, or they require organic solvents or mixtures thereof with water, or they show low yields or diastereoisomeric purity or expensive reagents.

Hence, it is a general object of the present invention to provide a simple, economically advantageous and efficient process, by which a mixture of (6R,S)-diastereoisomers of (6R,S)-N-[4-[(2-amino-1,4,5,6,7,8-hexahydro-methyl-4-oxo-5-methyl-pteridinyl)methyl] amino]benzoyl]-L-glutamic acid may be separated into the corresponding stable and pure (6R) and (6S)-diastereoisomers thereof, in a good yield and diastereoisomeric purity.

According to a first aspect, the present invention relates to a process for the separation of a mixture of diastereoisomers of (6R,S)-5-methyltetrahydrofolic acid or an alkali metal salt thereof comprising the reaction of the mixture in water with at least one organic base selected from the group consisting of 1-phenylethylamine, 1-p-nitrophenylethylamine, 1-p-chlorophenylethylamine, 1-p-methylphenylethylamine, 1-p-methoxyphenylethylamine, 1-(1-naphthyl)ethylamine, 1-(2-naphthyl)ethylamine, either in a (R,S)- or (R)- or (S)-configuration, and diisopropylethylamine, so to obtain the corresponding amine salt, and the recovering of at least one diastereoisomer.

In particular, the process of the invention comprises that the at least one diastereoisomer is recovered by crystallization; preferably, the crystallized diastereoisomer is re-crystallized at least once.

It is also preferred that the at least one diastereoisomer is

transformed into the free acid or into an alkaline earth metal salt.

According to preferred embodiments, the alkaline earth metal salt so obtained can be transformed into the free acid whereas the free acid so obtained can be transformed, in its turn, into an alkali metal or alkaline earth metal salt. The most preferred alkaline earth metal salt is a calcium salt.

The process of the invention allows to cheaply isolate the natural isomer (6S)-5-MTHF substantially pure, this generally meaning a concentration of the diastereoisomer greater than 95 wt%, preferably greater than 99 wt% and showing an extremely high diastereoisomeric purity (higher than 99%).

Further, the process of the invention is carried out in water in the total absence of any other organic solvent both during the crystallization of the amine salt and the recovering of the free acid from such salt.

Another aspect of the present invention relates to a compound which is a crystalline salt of (6R,S)-, (6R)-, (6S)- 5-methyltetrahydrofolic acid with at least one organic base selected from the group consisting of 1-phenylethylamine, 1-p-nitrophenylethylamine, 1-p-chlorophenylethylamine, 1-p-methylphenylethylamine, 1-p-methoxyphenylethylamine, 1-(1-naphthyl)ethylamine, 1-(2-naphthyl)ethylamine, either in a (R,S)- or (R)- or (S)- configuration, and diisopropylethylamine.

In particular, the compound of the invention is selected from the group consisting of (R,S)-1-phenylethylamine 5-methyl-(6R,S)-tetrahydrofolate; (R,S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate; (R,S)-1-phenylethylamine 5-methyl-(6R)-tetrahydrofolate; (R)-1-phenylethylamine 5-methyl-(6R,S)-tetrahydrofolate; (R)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate; (R)-1-phenylethylamine 5-methyl-(6R)-tetrahydrofolate; (S)-1-phenylethylamine 5-methyl-(6R,S)-tetrahydrofolate; (S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate; (S)-1-phenylethylamine 5-methyl-(6R)-

tetrahydrofolate.

The 5-methyltetrahydrofolic moiety of the compound of the invention is preferably in a (6S) configuration.

Still more preferred compounds of the invention are selected
5 from the group consisting of diisopropylethylamine 5-methyl-
(6S)-tetrahydrofolate; (R)-, (S)- and (R,S)-1-phenylethylamine
5-methyl-(6S)-tetrahydrofolate.

In particular, diisopropylethylamine 5-methyl-(6S)-
tetrahydrofolate, (R)-, (S)- and (R,S)-1-phenylethylamine 5-
10 methyl-(6S)-tetrahydrofolate are the most preferred compounds
of the invention.

It can be advantageously noted that the crystalline amine
salts of the invention show a high chemical stability.

The use of the compound of the invention for the separation of
15 a mixture of diastereoisomers of (6R,S)-5-
methyltetrahydrofolic acid or an alkali metal salt thereof is
still another aspect of the present invention.

The compound of the invention shows an extraordinary long
lasting chemical stability, this actually guaranteeing quite
20 unaltered purity, even after months, and that the titre of the
correspondent 5-MTHF moiety results substantially unchanged.

The compound of the invention can be prepared by simply
applying the common general knowledge of the field, as the
skilled man would easily understand; however, as a non-
25 limitative example, the compound of the invention can be
obtained by filtration of the precipitate obtained adding the
(6R,S)-5-methyltetrahydrofolic acid to an aqueous solution
containing diisopropylethylamine, (R)-, (S)- and (R,S)-1-
phenylethylamine, (or vice versa) preferably maintained under
30 nitrogen and stirring the base being in a molar amount of
about 200 to 300% of the acid.

The following examples illustrate the invention without
limiting it.

Example 1

35 Preparation of (R,S)-1-phenylethylamine 5-methyl-(6S)-

tetrahydrofolate dihydrate

Under nitrogen, 13.2 g (109 mmol) of (R,S)-1-phenylethylamine were added to a suspension of 20 g (42 mmol) of 5-methyl-(6R,S)-tetrahydrofolic acid monohydrate in water (100 ml) and
5 heated to 60°C for 2 h. The solution was cooled to room temperature and stirred for 72 h. After cooling to 0°C for 4 h, the suspension was filtered under suction and washed with water (2 x 15 ml). After drying at 40°C for 4 h, 13.4 g of crystalline salt of the title product was obtained which
10 showed a (6S)/(6R) diastereoisomer ratio of 93.3/6.7 (see analytical data).

HPLC data: a) titre as a mixture of 5-methyl-(6S)-tetrahydrofolic acid and 5-methyl-(6R)-tetrahydrofolic acid: calculated 65.5% (on the dry product); found 64.51 (98.5 % of
15 the theoretical value); b) diastereoisomer composition (HAS chiral): 5-methyl-(6S)-tetrahydrofolic acid 93.3%; 5-methyl-(6R)-tetrahydrofolic acid 6.7%;

Specific rotation $[\alpha]^{20}_D = +31.38^\circ$ (C = 1 in water)

NMR (DMSO- d_6)ppm 7.75 (d*, H); 7.57 (d, 2H); 7.4 (d, 4H); 7.30
20 (t, 4H), 7.25 (d, 2H); 6.57 (d, 2H), 6.42 (bs 2H); 6.60-5.80 (m* 11H); 4.08 (m, 3H); 3.18 (bd, 2H); 2.95-2.70 (m 3H); 2.45 (s, 3H); 2.10 (m, 2H); 1.85 (m, 2H); 1.37 (d, 6H). *The signals exchange with D₂O.

Example 2

25 Preparation of (R)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate dehydrate.

Under nitrogen, 13.2 g (109 mmol) of (R)-1-phenylethylamine were added to a suspension of 20 g (42 mmol) of 5-methyl-(6R,S)-tetrahydrofolic acid monohydrate in water (100 ml) and
30 heated to 60°C for 2 h. The solution was cooled to room temperature and stirred for 72 h. After cooling to 0°C for 4 h, the suspension was filtered under suction and washed with water (2 x 15 ml). After drying at 40°C for 4 h, 14.2 g of crystalline salt of the title product were obtained showing a
35 (6S)/(6R) diastereoisomer ratio of 91.1/8.9 (see analytical

data).

HPLC data: a) titre as a mixture of 5-methyl-(6S)-tetrahydrofolic acid and 5-methyl-(6R)-tetrahydrofolic acid: calculated 65.5% (on the dry product); found 64.75 (98.9 % of the theoretical value); b) diastereoisomer composition (HAS chiral): 5-methyl-(6S)-tetrahydrofolic acid 91.1%; 5-methyl-(6R)-tetrahydrofolic acid 8.9%;

DSC two endotherms at 185 (eutectic peak) and 239°C (melting point).

Specific rotation $[\alpha]^{20}_D = +33.4$ (C = 1 in water)

NMR (DMSO- d_6) ppm 7.75 (d*, H); 7.57 (d, 2H); 7.4 (d, 4H); 7.30 (t, 4H), 7.25 (d, 2H); 6.57 (d, 2H), 6.42 (bs 2H); 6.60-5.80 (m* 11H); 4.08 (m, 3H); 3.18 (bd, 2H); 2.95-2.70 (m 3H); 2.45 (s, 3H); 2.10 (m, 2H); 1.85 (m, 2H); 1.37 (d, 6H). *The signals exchange with D₂O.

Example 3

Preparation of (S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate dihydrate

Under nitrogen, 13.2 g (109 mmol) of (S)-1-phenylethylamine were added to a suspension of 20 g (42 mmol) of 5-methyl-(6R,S)-tetrahydrofolic acid monohydrate in water (100 ml) and heated to 60°C for 2h. The solution was cooled to room temperature and stirred for 72 h. After cooling to 0°C for 4 h, the suspension was filtered under suction and washed with water (2 x 15 ml). After drying at 40°C for 4 h, 13.7 g of crystalline salt of the title product were obtained showing a (6S)/(6R) diastereoisomer ratio of 95.8/4.2 (see analytical data).

HPLC data: a) titre as a mixture of 5-methyl-(6S)-tetrahydrofolic acid and 5-methyl-(6R)-tetrahydrofolic acid: calculated 65.5% (on the dry product); found 64.3 (98.2% of the theoretical value); b) diastereoisomer composition (HAS chiral): 5-methyl-(6S)-tetrahydrofolic acid 95.8%; 5-methyl-(6R)-tetrahydrofolic acid 4.2%;

DSC: two endotherms at 183 (eutectic peak) and 245°C (melting

point)

Specific rotation $[\alpha]^{20}_D = +28.8$ ($C = 1$ in water)

NMR (DMSO- d_6) ppm 7.75 (d*, H); 7.57 (d, 2H); 7.4 (d, 4H); 7.30 (t, 4H), 7.25 (d, 2H); 6.57 (d, 2H), 6.42 (bs 2H); 6.60-5.80 (m* 11H); 4.08 (m, 3H); 3.18 (bd, 2H); 2.95-2.70 (m 3H); 2.45 (s, 3H); 2.10 (m, 2H); 1.85 (m, 2H); 1.37 (d, 6H). *The signals exchange with D₂O.

Example 4

Crystallisation of (S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate dihydrate

Under nitrogen, a suspension of 13.2 g (18.8 mmol) of crystalline product, prepared according to Example 3, in water (66 ml) was heated to 60°C for 1 h and then cooled at room temperature for 4h. This procedure was performed four times.

After cooling to 0° for 4 h, the suspension was filtered under suction and washed with water (2 x 10 ml). After drying at 40°C for 4 h, 10.9 g of crystalline salt of the title product was obtained which showed a (6S)/(6R) diastereoisomer ratio of 99.2/0.8 (see analytical data).

HPLC data: a) titre as a mixture of 5-methyl-(6S)-tetrahydrofolic acid and 5-methyl-(6R)-tetrahydrofolic acid: calculated 65.5% (on the dry product); found 64.9 (99.1% of the theoretical value); b) diastereoisomer composition (HAS chiral): 5-methyl-(6S)-tetrahydrofolic acid 99.2%; 5-methyl-(6R)-tetrahydrofolic acid 0.8%;

DSC: one endotherm at 248.1°C (melting point).

Specific rotation $[\alpha]^{20}_D = +28.7$ ($C = 1$ in water).

Example 5

Preparation of 5-methyl-(6S)-tetrahydrofolic acid.

Under nitrogen, a suspension of 10.9 g (15.14 mmol) of crystalline product, prepared according to Example 3, in water (66 ml) was acidified with 10% HCl until pH 3. After cooling to 0°C for 4 h, the suspension was filtered under suction and washed with water (2 x 10 ml). After drying at 40°C for 4 h, 6.9 g of 5-methyl-(6S)-tetrahydrofolic acid was obtained.

HPLC data: a) titre as a mixture of 5-methyl-(6S)-tetrahydrofolic acid and 5-methyl-(6R)-tetrahydrofolic acid: calculated 100%; found 99.3; b) diastereoisomer composition (HAS chiral): 5-methyl-(6S)-tetrahydrofolic acid 99.5%; 5-methyl-(6R)-tetrahydrofolic acid 0.5%;

DSC: endotherm at 256.2 °C.

Specific rotation $[\alpha]^{20}_D = +14.3$ (C = 1 in NaOH 0.01N).

Example 6

Preparation of 5-methyl-(6R,S)-tetrahydrofolic acid.

- 10 Under nitrogen, a suspension of 30 g of amorphous 5-methyl-(6R,S)-tetrahydrofolic acid calcium salt, corresponding to 25.5 g of anhydrous compound (51 mmol), in water (66 ml) was acidified with 10% HCl until pH 3. After cooling to 0°C for 4 h, the suspension was filtered under suction and washed with
- 15 water (2 x 10 ml). After drying at 40°C for 4 h, 24.0 g of 5-methyl-(6R,S)-tetrahydrofolic acid was obtained.

- HPLC data: a) titre as a mixture of 5-methyl-(6S)- and 5-methyl-(6R)-tetrahydrofolic acid: calculated 100%; found 99.3; b) diastereoisomer composition (HAS chiral): 5-methyl-(6S)-
- 20 tetrahydrofolic acid 50.5%; 5-methyl-(6R)-tetrahydrofolic acid 49.5%;

DSC: endotherm at 248.3 °C.

Specific rotation $[\alpha]^{20}_D = +19.2$ (C = 1 in NaOH 0.01N).

STABILITY

- 25 The stability of the compounds listed in the following table, under powder form and in sealed aluminium foil bag, was tested, keeping the samples in airtight container protected from light and measuring the purity and the titre thereof after 6 and 12 months.

Compound	Starting values		After 6 months		After 12 months	
	Purity	Titre	Purity	Titre	Purity	Titre
(R)-PEA (6S)-5-MTHF	99.8	64.5	99.3	64.35	99.0	64.1
(S)-PEA (6S)-5-MTHF	99.7	64.3	99.6	64.2	99.5	64.0

5 An expiration date of 24 months could be safely established for the compound of the invention. Any of the compounds listed in the above table showed a high stability since both their purity and titre, even after 12 months, resulted to fall well within the required specifications. In particular, the stability of the acid moiety was observed by HPLC, detecting the purity and titre thereof.

CLAIMS

1. A process for the separation of a mixture of diastereoisomers of (6R,S)-5-methyltetrahydrofolic acid or an alkali metal salt thereof comprising the reaction of the
5 mixture in water with at least one organic base selected from the group consisting of 1-phenylethylamine, 1-p-nitrophenylethylamine, 1-p-chlorophenylethylamine, 1-p-methylphenylethylamine, 1-p-methoxyphenylethylamine, 1-(1-naphthyl)ethylamine, 1-(2-naphthyl)ethylamine, either in a
10 (R,S)- or (R)- or (S)- configuration, and diisopropylethylamine, so to obtain the corresponding amine salt, and the recovering of at least one diastereoisomer.
2. A process according to the previous claim, comprising recovering the at least one diastereoisomer by
15 crystallization.
3. A process according to the previous claim, wherein the crystallized diastereoisomer is re-crystallized at least once.
4. A process according to any of the previous claims, wherein the at least one diastereoisomer is transformed into the free
20 acid or into an alkaline earth metal salt.
5. A process according to the previous claim, wherein the free acid is transformed into an alkali metal or alkaline earth metal salt.
6. A process according to claim 4 or 5, wherein the alkaline
25 earth metal salt is a calcium salt.
7. A process according to any of claims 4-6, wherein the alkaline earth metal salt is transformed into the free acid.
8. A crystalline compound which is a salt of (6R,S)-, (6R)-, (6S)- 5-methyltetrahydrofolic acid with at least one organic
30 base selected from the group consisting of 1-phenylethylamine, 1-p-nitrophenylethylamine, 1-p-chlorophenylethylamine, 1-p-methylphenylethylamine, 1-p-methoxyphenylethylamine, 1-(1-naphthyl)ethylamine, 1-(2-naphthyl)ethylamine, either in a
(R,S)- or (R)- or (S)- configuration, and
35 diisopropylethylamine.

9. The compound according to the previous claim, which is selected from the group consisting of (R,S)-1-phenylethylamine 5-methyl-(6R,S)-tetrahydrofolate; (R,S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate; (R,S)-1-phenylethylamine 5-methyl-(6R)-tetrahydrofolate; (R)-1-phenylethylamine 5-methyl-(6R,S)-tetrahydrofolate; (R)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate; (R)-1-phenylethylamine 5-methyl-(6R)-tetrahydrofolate; (S)-1-phenylethylamine 5-methyl-(6R,S)-tetrahydrofolate; (S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate; (S)-1-phenylethylamine 5-methyl-(6R)-tetrahydrofolate.

10. The compound according to claim 8 or 9, wherein the 5-methyltetrahydrofolic moiety is in a (6S) configuration.

11. The compound according to any of claims 8-10, which is selected from the group consisting of diisopropylethylamine 5-methyl-(6S)-tetrahydrofolate; (R)-, (S)- and (R,S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate.

12. The compound according to any of claims 8-11, which is diisopropylethylamine 5-methyl-(6S)-tetrahydrofolate.

13. The compound according to any of claims 8-12, which is (R)-, (S)- and (R,S)-1-phenylethylamine 5-methyl-(6S)-tetrahydrofolate.

14. Use of the compound according to any of claims 8-13, for the separation of a mixture of diastereoisomers of (6R,S)-5-methyltetrahydrofolic acid or an alkali metal salt thereof.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/052034

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D475/04 A61K31/519

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 194 611 A (MARAZZA FABRIZIO [CH] ET AL) 16 March 1993 (1993-03-16) cited in the application column 3, line 51 - column 5, line 12 column 5, line 40 - column 7, line 2 examples 5,6	1-14
X	US 5 457 202 A (SCHEIB KLAUS [DE] ET AL) 10 October 1995 (1995-10-10) cited in the application column 1, line 44 - line 57 examples 1-4 claim 1	1-14
E	WO 2008/031284 A (NAN JING RHINE PHARM TECH INC [CN]; CHEN XIN [CN]; ZHU GUANGXU [CN]; C) 20 March 2008 (2008-03-20) the whole document	1-14

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

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

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

14 November 2008

Date of mailing of the international search report

21/11/2008

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2008/052034

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