Title: COMPOUND WITH VITAMIN K ACTIVITY, PARTICULARLY AS ADDITIVE FOR FEEDS, AND PREPARATION OF THE COMPOUND

Abstract: A new indication of use for bis(menadione bisulphite)piperazine (MBP) and a method for synthesizing the compound is described. MBP has a substantial vitamin K-like activity, which however, differently from other compounds currently in use for the same purpose, is associated with a considerable stability in conditions of intense physical stress, with high activity per unit weight, and a low danger index (assessed by means of an EC protocol that is adopted universally in the field for this purpose). The described synthesis method is based on the low water-solubility constant of MBP, so that as a consequence of the mixing of a menadione bisulphite salt and a second piperazine salt one obtains a complete precipitation of the salt of interest, which can thus be separated and purified easily.
COMPOUND WITH VITAMIN K ACTIVITY, PARTICULARLY AS ADDITIVE FOR FEEDS, AND PREPARATION OF THE COMPOUND

Technical Field

The present invention relates to the use of a compound derived from menadione as an agent with vitamin K activity, particularly in the form of an additive for zootechnical feeds, and to a method for synthesizing the compound.

Background Art

The requirements of stability, high activity and safety have always been highly desirable goals of producers and users of substances with vitamin K activity since the discovery of said vitamin and the diffusion of the first substances marketed for this purpose.

The first synthetic analogue of vitamin K was menadione (commonly termed 2-methyl-1,4-naphthoquinone), discovered in 1940, which had a high vitamin K activity but also, and most of all, had poor stability and was highly dangerous, so that the direct use of this molecule never had a significant industrial application.

A derivative of menadione, menadione sodium bisulfite (MSB), claimed in US-2,367,037, and a diluted form thereof, MSBC, widely used in the US market, despite having a considerable antihemorrhagic activity and being less dangerous, still had limited stability with respect to light, heat and humidity, especially in solution and in the presence of a higher-than-neutral ambient pH; unfortunately, these conditions are common in premixes or feeds for animals stored for a long time and in less than perfect conditions.

A considerable improvement was achieved with the discovery of menadione dimethylpyrimidinol bisulfite (MPB), disclosed in US-3,328,169, and subsequently with menadione nicotinamide bisulfite, which is more stable than MSB, MSBC and MNB, and also has a vitamin PP activity (reference should be made, in this regard, to IT 1097391).
However, both MPB and MNB are characterized by a significant danger level, particularly for operators, as a consequence of direct contact, causing irritations and burns of the skin, eyes and respiratory tract.

Disclosure of the Invention

The aim of the present invention is therefore to provide a compound for use as a substitute of vitamin K that overcomes the drawbacks of the prior art.

Within this aim, an object is to provide a compound for use as a substitute of vitamin K that has high stability, high antihemorrhagic activity per unit weight, and a low danger level.

Another object is to provide a composition that comprises the compound described above, particularly for use in the zootechnical and human field, that overcomes the drawbacks of the prior art and in particular is stable over time and in the most disparate preservation and utilization conditions.

Another object is to provide a method for producing a compound as described above that is characterized by high yields and is economically advantageous.

This aim and these and other objects are achieved by the use of bis(menadione bisulfite)piperazine (MBP) for the preparation of vitamin K integrators.

The aim and objects of the invention are also achieved by a method for producing bis(menadione bisulfite)piperazine (MBP), which comprises the step of placing in contact, in a suitable solvent, a first compound having the formula (I):

\[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2
\end{array}
\]

\[2^+\]

\[2/\text{m} \cdot (Y)^{\text{m}^-}\]
Formula (I)

where \( m \) is an integer and is the valency of \( Y \), and \( Y \) is the conjugate base of any strong mineral acid, said base being preferably \( (\text{Cl})^\cdot \), \( (\text{H}_2\text{PO}_4)^\cdot \), \( (\text{HPO}_4)^{2\cdot} \), \( (\text{PO}_4)^{3\cdot} \), \( (\text{HSO}_4)^\cdot \), \( (\text{SO}_4)^{2\cdot} \), \( (\text{HSO}_3)^\cdot \), \( (\text{SO}_3)^{2\cdot} \) and mixtures thereof, with a second compound selected among:

i) one or more compounds having the formula (II):

\[
\begin{array}{c}
\text{CH}_3 \\
\text{SO}_3 \\
\text{X}^{n+} \\
\text{Y}^n
\end{array}
\]

Formula (II)

where \( n \) is an integer and is the valency of \( X \), and \( X \) is the conjugate acid of any Lewis base, \( X \) is preferably sodium, potassium, ammonium, or

ii) a compound having the formula (III)

\[
\begin{array}{c}
\text{CH}_3 \\
\text{O} \\
\text{O}
\end{array}
\]

Formula (III)

with the condition that when \( Y \) is \( (\text{HSO}_3)^\cdot \), said second compound is the compound having the formula (III).

In a first aspect, the present invention relates to a new indication for the use of a compound that can be traced back chemically to menadione, is highly stable, and has a high antihemorrhagic activity per unit weight and a low danger index according to the 92/32/EC directive.

The term "integrator" is used to designate a composition that comprises MBP and is intended to be administered to an animal, preferably
orally, with the goal of integrating the quantity of vitamin K taken by the animal for example through its diet, or of contrasting vitamin K deficiencies, restoring a physiological level of said vitamin.

The compound according to the present invention is the piperazine salt (or diethylene diamine) of menadione bisulfite (also known as bis (menadione bisulfite)piperazine, MBP and, according to the IUPAC standards, as bis(1,2,3,4-tetrahydro-2-methyl-1,4-dioxo-2-naphthalene sulfonate) of hexahydropyrazine), a salt that is constituted by one mole of piperazine for every two moles of menadione bisulfite and whose structural formula is shown hereafter:

```
\[
\begin{array}{c}
\text{CH}_3 \\
\text{SO}_3 \\
\end{array}
\quad \text{CH}_3 \\
\text{SO}_3 \\
\end{array}
\quad \text{H}_2\text{N} \\
\text{NH}_2
\]
```

MBP is disclosed in Patents EP-1220608 and US-6,596,064 with the name of menadione(bis) piperazine bisulfite(II), but exclusively in relation to use thereof as a biocidal and antifouling agent with a low ecotoxicity index, for example for use in marine paints.

It has now instead been found, surprisingly, that MBP also has a marked vitamin K activity, combined with high stability, a high biological activity per unit weight, and a low danger index, and therefore its use as a vitamin substitute, for example in zootechnical feeds or medicinal preparations, has been found to be extremely advantageous.

If compared with other substances that have long been used as substitutes or integrators of vitamin K (commonly termed VKAS), MBP has shown unexpectedly a distinctly higher stability, both during accelerated-stability tests performed on the compound as such and in longer-term tests performed on common edible preparations, such as standard feeds for the zootechnical field comprising the compounds to be evaluated in each
instance.

In particular, the tests have highlighted the considerable stability of MBP and of feeds that contain it even after storage for a long time in conditions that are considered unfavorable, such as high temperature and humidity, in which the other compounds with vitamin K-like activity degrade rapidly.

Higher stability also leads to a higher biological activity (and therefore higher therapeutic effectiveness) per unit weight.

The use of MBP is particularly advantageous also in view of its danger index, assessed according to the 92/32/EC directive; said index is surprisingly low if compared with the values obtained with molecules that are chemically similar to MBP and are currently used as VKAS. On the basis of the parameters of the cited directive, compounds such as MBP and MNB are in fact classified as dangerous/irritant for the eyes, skin and respiratory tract, while MBP has been found to be neither dangerous nor irritant (in this regard, reference should be made to the examples cited hereinafter).

In a preferred embodiment of the invention, the integrator comprising MBP is an edible preparation, advantageously a feed or a concentrated composition for feeds, particularly for animals of zootechnical interest. In this embodiment, MBP can be used as such is or, more advantageously, combined with additives and ingredients that are common in the field, in order to prepare a first composition, which can be then diluted by the end user by adding other specific ingredients, depending on the animal for which the preparation is intended. A preferred edible preparation comprises MBP, preferably in an amount comprised between 1 mg/kg and 1000 mg/kg of preparation as is or combined with other ingredients.

In a different embodiment of the invention, the integrator comprising MBP is a preparation for prevention and treatment, in an animal, of vitamin K deficiencies and of pathological conditions associable with said
deficiencies. Preferred pathological conditions associable with vitamin K deficiencies are neonatal hemorrhage, hemorrhage induced by ingestion of vitamin-K antagonists, hemorrhage induced by feeds contaminated by mycotoxins, hemorrhage induced by feeds contaminated by dicumarol (sweet clover disease). In this embodiment, MBP is present preferably in a quantity comprised between 0.5 mg/kg and 5000 mg/kg of preparation, and is combined advantageously with one or more pharmaceutically acceptable excipients known in the field, for example to improve its transport and absorption.

According to the invention, the term "animals" is used to designate members of all existing higher animal classes and preferably oviparous animals and mammals, preferably human beings and, among zootechnical species, fish, ruminants and monogastric animals, preferably bovines, ovines, goats, swine, poultry and rabbits.

In a second aspect, the present invention relates to a composition that comprises particularly bis(menadione bisulfite)piperazine.

In a preferred embodiment, said composition is an edible preparation for animals and comprises bis(menadione bisulfite)piperazine (MBP), preferably in an amount comprised between 100 mg/kg and 10000 mg/kg of composition, optionally but advantageously combined with at least one typical excipient of feeds chosen among:

a) vitamins,
   - preferably vitamin A, vitamin D₃, vitamin E, vitamin B₂, vitamin B₁₂, vitamin B₆, and mixtures thereof,

b) animal and vegetable flours
   - preferably corn flour, soybean meal, ground rice, alfalfa meal, peanut meal, meatmeal, beet meal, barley meal,

c) inorganic compounds,
   - preferably dicalcium phosphate, ground CaCO₃, NaCl and mixtures thereof,
d) amino acids and mineral salts.

In a different preferred embodiment, said composition is intended for the production of a medicament for preventing and treating, in an animal, vitamin K deficiencies and pathological conditions associable with said deficiencies. In this embodiment, the composition comprises menadione piperazine bisulfite (MBP), preferably in a quantity comprised between 0.5 mg/kg and 5000 mg/kg of composition, optionally but advantageously combined with at least one ingredient selected from the group that consists of pharmaceutically acceptable vehicles and carriers.

If needed, it is also possible to obtain a composition suitable for use as a vitamin K integrator by mixing excipients listed specifically for the production of edible preparations such as zootechnical feeds, preferably the excipients a) to d) cited above, with pharmaceutically acceptable vehicles and carriers.

In a third aspect, the present invention relates to a method for preparing bis(menadione bisulfite)piperazine (MBP).

Said method comprises the step of placing in contact, in a suitable solvent, preferably water, a compound of formula (I) as defined above with one or more compounds of formula (II) or with a compound of formula (III) as defined above, wherein the choice among compounds of formula (II) and of formula (III) essentially depends on the piperazine salt (compound of formula (I)) that one intends to use. Since the solubility constant of MBP is particularly low, it precipitates as soon as it forms, and therefore can be isolated and purified easily with high yields and cheaply. Precipitation can be optionally facilitated by means of methods that are known in the field (such as for example the addition of suitable co-solvents).

Preferred compounds of formula (I) are adducts constituted by piperazine and a conjugate base of a strong acid, said acid being selected from the group consisting of halogen acids, preferably hydrochloric acids, phosphoric acid, sulfuric acid, sulfurous acid, and mixtures thereof. If the
acid is polyprotic, the conjugate base salified with piperazine can be any protic form of said acid. Y is preferably selected from the group that consists of halide, preferably (Cl⁻, (H₂PO₄)⁻, (HPO₄)²⁻, (PO₄)³⁻, (HSO₄)⁻, (SO₄)²⁻, (HSO₃)⁻, (SO₃)²⁻ and mixtures thereof.

A particularly advantageous compound of formula (II) is menadione sodium bisulfite (MSB), or an adduct of menadione bisulfite with potassium, ammonium or another cation, which produces an adduct that has a sufficient solubility in the solvent in which the reaction occurs, particularly water.

Preferably, the step of placing in contact the compounds of formulas (I), (II) or (III) comprises mixing a concentrated solution of the piperazine salt in a 1:2 molar ratio with a concentrated aqueous solution of the compound of formula (II) or (III), thus achieving the precipitation of bis (menadione bisulfite)piperazine, which is then filtered easily and collected with high purity and with high yields.

Other characteristics and advantages of the present invention will become better apparent from the description of the following preferred embodiments, intended exclusively by way of non-limiting example.

Example 1

Preparation of bis(menadione bisulfite)piperazine:

300 g (3.484 moles) of anhydrous piperazine are dissolved in 3.5 liters of water; 900 g of 30% HCl (7.2 moles) are added thereto, completely salifying the piperazine.

2315 g of MSB with a 52.5% menadione titer (7.06 moles) are dissolved in 4.3 liters of water. The piperazine solution is added slowly under agitation to the solution of MSB at ambient temperature. Agitation is performed for 1 h 30 min. The result is a white crystalline precipitate, which when filtered and dried amounts to 1610 grams.

Bis(menadione bisulfite)piperazine is scarcely water-soluble, has a melting point of 216-220 °C, contains no more than 1% H₂O (K.F.), and has
the following composition under elementary analysis:

<table>
<thead>
<tr>
<th></th>
<th>Found:</th>
<th>Theoretical:</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>52.1%</td>
<td>52.47%</td>
</tr>
<tr>
<td>H</td>
<td>4.9%</td>
<td>5.04%</td>
</tr>
<tr>
<td>N</td>
<td>4.2%</td>
<td>4.70%</td>
</tr>
<tr>
<td>S</td>
<td>10.2%</td>
<td>10.79%</td>
</tr>
</tbody>
</table>

The menadione content is 56.8%.

Example 2

Demonstration of the production of integrators for zootechnical feeds by combining MBP with the common excipients used for this purpose was intended.

In the preparation of feeds, normally one works in dry and cold conditions, preparing first of all an active concentrate a), which is then incorporated in the composition designated hereinafter by b), which in turn represents a medium for a feed integrator, thus obtaining a normal integrator, which is diluted at the time of use with the respective feeds suitable for each animal in the intended proportions.

The following concentrated preparations for feeds were prepared:

<table>
<thead>
<tr>
<th></th>
<th>a1)</th>
<th>a2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>3,500,000 IU</td>
<td>1,000,000 IU</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>400,000 IU</td>
<td>300,000 IU</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>3,500 mg</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>400 mg</td>
<td>1200 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>2 mg</td>
<td>1 mg</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>600 mg</td>
<td>600 mg</td>
</tr>
<tr>
<td>MBP</td>
<td>4000 mg</td>
<td>1000 mg</td>
</tr>
</tbody>
</table>

The two concentrates were then mixed with a medium that comprised the following ingredients:
- Corn flour 400 g/kg
- Soybean meal 200 g/kg
- Ground rice 100 g/kg
- Alfalfa meal 100 g/kg
- Peanut meal 50 g/kg
- Meatmeal 50 g/kg
- Beet meal 25 g/kg
- Barley meal 25 g/kg
- Dicalcium phosphate 25 g/kg
- Ground CaCO₃ 15 g/kg
- NaCl 10 g/kg.

Amino acids and mineral salts were then added to the mixtures thus prepared.

The resulting integrators can then be diluted, in turn, at the time of use with specific feeds suitable for the animals to which they are to be administered.

**Example 3**

Demonstration of the stability of menadione bisulfite piperazine is now intended.

A first test for determining rapidly the stability of the product is the accelerated stability test: the product being tested is mixed intimately (2-4% weight/weight) with a standard medium (in this case, aluminum silicate) which has a high humidity (10-15%), a basic pH (suspension obtained with 1 g in 10 cc of H₂O) of approximately 10, and stored at 55 °C for several days. This is a standard procedure for comparing stability with respect to humidity, heat, pH, obtaining results in a short time. In these conditions, MSB degrades rapidly over a few days.

In the specific case, the following procedure was used.

1.5 g of bis(menadione bisulfite)piperazine, prepared according to
example 1, are mixed with 50 g of aluminum silicate containing 11.25% of humidity (K.F.). The preparation is placed in a sealed container and is kept at 55 °C in a thermostat for 10 days. The same procedure is performed for a sample of menadione nicotinamide bisulfite and menadione sodium bisulfite.

After this period, the level of the three compounds that is still present is determined, and it has been found that at the end of the accelerated stability test, 90% of the MBP is still present, against 75% of MNB and 6% of MSB.

<table>
<thead>
<tr>
<th>Days</th>
<th>MSB(%)</th>
<th>MNB(%)</th>
<th>MBP(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>84</td>
<td>93</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>83.5</td>
<td>93</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>75</td>
<td>90</td>
</tr>
</tbody>
</table>

Accordingly, MBP has an unexpectedly high stability in conditions generally considered as highly unfavorable even for compounds with vitamin K-like activity that are considered stable, such as MNB.

Example 4

Demonstration of the vitamin K activity of the compound according to the invention is now intended.

Vitamin K activity was measured in chicken by administering to groups of chicks increasing levels of menadione, MNB, MPB and MBP for a period of 14 days. After this period, and for each dosage, a blood sample was taken, and the prothrombin time (coagulation rate) was determined according to Quick's method. This time decreases as the dose of VKAS increases, because it is an indicator of the vitamin K activity of said substance. According to this method, it was possible to identify the pharmacologically equivalent quantities of the tested substances, where "equivalent" is used to indicate the quantities that determined similar
prothrombin times.

The estimate of the National Research Council (NRC-Nutrient Requirements for Poultry, 8th ed., 1984, NAP, Washington, DC) of the requirement of vitamin K in chicken is approximately 500 μg/mg, a value which refers to the requirement of menadione and is generally used as a comparison parameter for evaluating the activity of commercially available VKAS.

The following table summarizes the results of the test, expressed as equivalent quantities of the tested substances (TP = prothrombin time):

<table>
<thead>
<tr>
<th>Menadione mg/kg</th>
<th>MPB μg/kg</th>
<th>MNB μg/kg</th>
<th>MBP μg/kg</th>
<th>TP (MBP)</th>
<th>TP (MNB)</th>
<th>TP (MBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>11</td>
<td>8.6</td>
<td>28</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>25</td>
<td>55</td>
<td>55</td>
<td>43</td>
<td>25</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>50</td>
<td>110</td>
<td>110</td>
<td>86</td>
<td>25</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>400</td>
<td>880</td>
<td>880</td>
<td>690</td>
<td>19</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>500</td>
<td>1100</td>
<td>1100</td>
<td>860</td>
<td>15</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

As can be seen from this table, the vitamin K requirement for chicken is met by 860 μg/kg of MBP, versus 1100 μg/kg of MPB and MNB, indicating a higher activity per administered unit weight of compound.

Example 5

Demonstration of the low danger level of MBP is now intended.

All the substances introduced on the European market and therefore also known and used substances with vitamin K-like activity (MSB, MPB, MNB) must be classified according to the 92/32/EC Directive according to
their danger level and labeled accordingly.

The main test for determining the danger level is the determination of acute oral toxicity in mice (i.e., the minimum dose that causes mortality of 50% of the test animals, LD$_{50}$); the LD$_{50}$ for menadione has been set at 500 mg/kg of body weight (Molitor and Robinson, 1940), for MSB at 2500 mg/kg of body weight (ARZNAD. 17, 1339, (1967)), for MSB and MPB at 1600 mg/kg of body weight (measured on chicken, not on mice) (Oduho et al.: J. of Nutrition, 123, 737(1993)). For bis(menadione bisulfite)piperazine (MBP), even at 2000 mg/kg of body weight, no mortality in the tested subjects is recorded, and LD$_{50}$ is therefore far higher than 2000 mg/kg. Moreover and contrary to the other products, bis(menadione bisulfite) piperazine, subjected to specific tests for eye, skin and respiratory irritation, exhibits no positive reaction.

The results are summarized in the following table.

<table>
<thead>
<tr>
<th></th>
<th>LD$_{50}$ (mg/kg)bw</th>
<th>Eye irritation</th>
<th>Skin irritation</th>
<th>Respiratory tract irritation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menadione</td>
<td>500</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>MSB</td>
<td>2500</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>MPB</td>
<td>1600 (chicken)</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>MNB</td>
<td>1600 (chicken)</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>MBP</td>
<td>&gt;&gt;2000</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

The low danger index of MBP with respect to other VKAS is therefore evident.

Although only some preferred embodiments of the invention have been described in the text, the person skilled in the art will understand immediately that it is in any case possible to obtain other equally
advantageous and preferred embodiments.

The disclosures in Italian Patent Application No. MI2004A000679 from which this application claims priority are incorporated herein by reference.
CLAIMS

1. The use of menadione piperazine bisulfite (MBP) to prepare vitamin K integrators.

2. The use according to claim 1, wherein said integrator is an edible preparation.

3. The use according to claim 2, wherein said edible preparation is a preparation for animals of zootechnical interest.

4. The use according to claim 3, wherein MBP is present in an amount comprised between 1 and 1000 mg per kilogram of integrator.

5. The use according to claim 2, wherein MBP is used in combination with at least one food excipient.

6. The use according to claim 5, wherein the at least one food excipient is selected from the group that consists of:
   a) vitamins,
      – preferably vitamin A, vitamin D₃, vitamin E, vitamin B₂, vitamin B₁₂, vitamin B₆, and mixtures thereof,
   b) animal and vegetable flours
      – preferably corn flour, soybean meal, ground rice, alfalfa meal, peanut meal, meatmeal, beet meal, barley meal,
   c) inorganic compounds,
      – preferably dicalcium phosphate, ground CaCO₃, NaCl and mixtures thereof,
   d) amino acids and mineral salts.

7. The use according to claim 1, wherein said integrator is a preparation for preventing and treating, in an animal, vitamin K deficiencies and pathological conditions associable with said deficiencies.

8. The use according to claim 7, wherein the preferred pathological conditions associable with vitamin K deficiencies are neonatal hemorrhage, hemorrhage induced by ingestion of vitamin-K antagonists, hemorrhage
induced by feeds contaminated by mycotoxins, hemorrhage induced by feeds contaminated by dicumarol (sweet clover disease).

9. The use according to claim 7, where MBP is present in an amount comprised between 0.5 mg and 5000 mg per kilogram of preparation.

10. The use according to any one of claims 3 and 7, wherein the animals are selected from the group that consists of human beings, fish, bovines, ovines, goats, swine, poultry and rabbits.

11. A composition, particularly for use as a vitamin K integrator, comprising MBP and at least one excipient selected from the group that consists of:

a) vitamins,
   - preferably vitamin A, vitamin D₃, vitamin E, vitamin B₂, vitamin B₁₂, vitamin B₆, and mixtures thereof,

b) animal and vegetable flours
   - preferably corn flour, soybean meal, ground rice, alfalfa meal, peanut meal, meatmeal, beet meal, barley meal,

c) inorganic compounds,
   - preferably dicalcium phosphate, ground CaCO₃, NaCl and mixtures thereof,

d) amino acids and mineral salts,

e) one or more pharmaceutically acceptable vehicles and carriers.

12. The composition according to claim 11, wherein the at least one excipient is selected among excipients a) to d) and MBP is present in a quantity comprised between 1 mg and 1000 mg per kilogram of composition.

13. The composition according to claim 11, wherein the at least one excipient is selected among excipients e) and MBP is present in a quantity comprised between 0.5 mg and 5000 mg per kilogram of composition.

14. A method for producing bis(menadione bisulfite)piperazine
(MBP), comprising the step of placing in contact, in a suitable solvent, a first compound of formula (I):

\[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2 \\
2^+ \\
2/m \cdot (X)^{m-}
\end{array}
\]

Formula (I)

where \( m \) is an integer and is the valency of \( Y \), and \( Y \) is the conjugate base of any strong mineral acid, with a second compound selected among:

i) one or more compounds having the formula (II):

\[
\begin{array}{c}
\text{CH}_3 \\
\text{SO}_3^- \\
(X)^{n+} \\
\end{array}
\]

Formula (II)

where \( n \) is an integer and is the valency of \( X \), and \( X \) is the conjugate acid of any Lewis base, or

ii) a compound having the formula (III)

\[
\begin{array}{c}
\text{CH}_3
\end{array}
\]

Formula (III)

with the condition that when \( Y \) is \((\text{HSO}_3)^-\) and said second compound is the compound having the formula (III).

15. The method according to claim 14, wherein \( Y \) is selected from the
group that consists of halide, preferably (Cl\(^{-}\)), (H\(_2\)PO\(_4\))\(^{-}\), (HPO\(_4\))\(^{2-}\), (PO\(_4\))\(^{3-}\), (HSO\(_4\))\(^{-}\), (SO\(_4\))\(^{2-}\), (HSO\(_3\))\(^{-}\), (SO\(_3\))\(^{2-}\) and mixtures thereof.

16. The method according to claim 14, wherein X is selected among sodium, potassium and ammonium.

17. The method according to claim 14, wherein the compound of formula (II) is menadione sodium bisulfite (MSB).

18. The method according to claim 14, comprising the additional steps of:
   – precipitating MBP; and
   – separating and purifying the compound of interest.

19. The method according to claim 14, wherein the solvent in which the process occurs is water.

20. The method according to claim 14, wherein the compound of formula (I) is in a 1:2 molar ratio with the compounds of formula (II) or (III).
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/122 A23L/302 A23K1/16 C07C50/14 C07D295/02

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)

IPC 7 A61K A23K A23L C07C 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, PAJ, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>3 April 1969 (1969-04-03) the whole document page 2, lines 14,15</td>
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<td>US 2 367 302 A (MOORE MARJORIE B ET AL) 16 January 1945 (1945-01-16) column 1, line 5 - column 2, line 38 column 3, lines 57-71 claims 1,7,8 claim 1</td>
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*E* document member of the same patent family

Date of the actual completion of the international search

31 May 2005

Date of mailing of the international search report

06/06/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx 31 651 epos nl, Fax: (+31-70) 340-3016

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Couzy, F
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