Title: PROCESS FOR THE PURIFICATION OF SOPHORA ALKALOIDS

Abstract: A method of purifying alkaloids extracted from plants of the Sophora genus (Sophora alkaloids), characterised in that the method comprises a purification step in which the Sophora alkaloids are placed on a macroporous absorption resin with an eluent comprising at least 40wt% water.
PROCESS FOR THE PURIFICATION OF SOPHORA ALKALOIDS

FIELD OF THE INVENTION

5 The invention relates to a process for purification of alkaloids derivable from plants of the *Sophora* genus (*Sophora* alkaloids).

BACKGROUND AND PRIOR ART

10 *Sophora*, a genus of the Leguminosae family, contains several species which are important sources of traditional Chinese medicines. For example, the roots of *Sophora flavescens* Ait. (Chinese name "Kushen"), the roots of *Sophora tonkinensis* (Chinese name "Shandougen"), the seeds of *Sophora moorecroftiana* (Chinese name "Shashenghuai"), and the seeds of *Sophora alopecuroides* (Chinese name "Kudouzi") are used in traditional Chinese medicines for the treatment of eczema, colpitis, acute pharyngolaryngeal infection, sore throat, acute dysentery and gastrointestinal haemorrhage.

CN 1978446 and CN 1172933 disclose an isolation method for oxymatrine and/or matrine involving the use of organic solvents.

25 CN 1687063 discloses an isolation method for oxymatrines in which the plant extract is purified using a Supercritical Fluid Extraction (SFE) tank, a costly and complicated process.

A method of purification of matrine is described in CN 1285592. This method involves reducing the extract with hydrazine and active carbon, followed by an acid wash through a cation ion exchange resin and then neutralisation with ammonia.

The present invention addresses the problem of improved alkaloid extraction/purification of alkaloid from any part of the Sophora genus plant, thus ensuring a sustainable supply. In addition to this the present invention also uses an environmentally purification/extraction without the need for toxic solvents.

SUMMARY OF THE INVENTION

Accordingly the present invention relates to a method of purifying alkaloids extracted from plants of the Sophora genus (Sophora alkaloids), characterised in that the method comprises a purification step in which the Sophora alkaloids are placed on a macroporous absorption resin with an eluent comprising at least 40wt% water.
DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

Extraction and purification Method

The roots/seeds of the plant of sophora genus are preferably extracted with water. It is advantageous if the weight ratio of herb to water is 1:10. The extraction temperature is preferably to 20-80°C. The water extract is preferably concentrated to a defined volume, more preferably a 1:1 weight ratio of water to herb).

The present invention relates to the purification of the Sophora alkaloids by placing on a macroporous absorption resin with an eluent comprising at least 40wt% water, more preferably an eluent comprising at least 50wt% water.

In a particularly preferred embodiment of the invention the extract is eluted on at least one macroporous resin with at least 80 wt% water, more preferably 90 wt% water, more preferably 100 wt% water.

It is further preferred if the purification step has at least three successive purification steps on three differing absorption resins.

Preferred macroporous absorption resins are selected from the group consisting of the following resins:

1. Resins having a polystyrene backbone functionalised with acyl amide. A preferred resin of this type is ADS-
21, (Trade name. Produced by "Tianjin Nankai Hecheng S&T Co. Ltd) having the following structure:

When using resins of this type it is preferred if the ratio of herb to resin is 1:1 to 1:10, more preferably 1:2.

The preferred solvent is water.

2. Resins having a polystyrene backbone functionalised with amidocyanogen. A preferred resin of this type is S-8 (Trade name. Produced by "Tianjin Nankai Hecheng S&T Co. Ltd," having the structure given below: 

\[
\begin{align*}
\text{C}_n \text{H}_2 \text{N} & \text{C} \text{H}_2 \text{C} \text{H}_2 \text{C}_n \\
\text{R}_1 & \text{C} \text{H}_2 \text{C} \text{H}_2 \text{C} \text{H}_2 \\
\text{R}_2 & \text{C} \text{H}_2 \text{C} \text{H}_2 \text{C}_n 
\end{align*}
\]
The preferred weight ratio of herb to this type of resin is 1:1 to 1:10, more preferably 1:3.

A preferred eluent for resins of this type is water.

3. Resins having a polystyrene backbone functionalised with ethene. A preferred resin of this type is HZ-816 (Trade name. Produced by Shanghai Huazhen Sci. & Tech. Co. Ltd,) having the structure given below:
The weight ratio of herb to resin is preferably 1:1 to 1:10, more preferably 1:2.

Preferred eluents for use with this resin are water or a 50% ethanol/50% water.

It is preferred if the purification uses the above 3 resin types consecutively, more preferably in the order that they are listed above.

It is preferred if the purification comprises a reducing step. The reduction step may be followed by an oxidizing step.

The reducing step preferably comprises reducing the alkaloid with hydrogen preferably with a Raney nickel catalyst.

The oxidation step preferably uses hydrogen peroxide.
As a final step the alkaloid is preferably re-crystallized, preferably from an organic solvent, more preferably from petroleum ether or acetone.

5 **Sophora Alkaloids**

The process of the present invention relates to the extraction/purification of one or more Sophora alkaloids.

10 It is particularly preferred if the alkaloids are tetracyclic quinolizidine alkaloids of the general structural formula (I) or formula II:

![Chemical Structure](attachment:image.png)
in which $X$ is O or a lone pair.

A tetracyclic quinolizidine alkaloid of formula (I) is which $X$ is 0 is generally termed (++)-oxymatrine. The alkaloid may also be termed oxymatrine, or (++)-matrine N-oxide.

A tetracyclic quinolizidine alkaloid of formula (I) is which $X$ is a lone pair is generally termed (++)-matrine. The alkaloid may also be termed matrine or matridin-15-one.

(++)-Oxymatrine and/or (++)-matrine and/or oxysophocarpine are particularly preferred.

The invention will be further illustrated by the following, non-limiting Examples, in which all percentages quoted are by weight based on total weight unless otherwise stated.
Example

Using the root of *S. flavesens* as raw material

1kg root of *S. flavesens* is extracted with 10L water for three times. Extract temp is Room temp and time: 10h per time. After filtration, all of the water extraction is then concentrated to 2L.

2L water extract is loaded to 2L ADS-21 macroporous absorption resin and then eluted with 5L water.

5L water fraction (from ADS-21) is then loaded to 3L S-8 directly. After 5L water fraction pass through S-8, using another 6L water to elute S-8 to wash alkaloids out as much as possible. Totally 11L water fraction is obtained after S-8.

H L water fraction (from S-8) is then loaded to 2L HZ-816 directly. After all of the H L water fraction pass through HZ-816, using another 4L water to elute HZ-816 to remove impurities. And then HZ-816 is finally eluted with 5-6L 50% EtOH.

50% EtOH is concentrated to dry to get 40g total alkaloid.

According to HPLC analysis, oxymatrine in total alkaloid is 65% and oxysohcarpaine is 16%.

40g total alkaloid is dissolved in 100ml water, and then reduced with hydrogen with raney Ni as catalyst. Reaction temp is room temp and reaction time is 8 hours.
The reaction solution is filtrated and concentrated to dry (36g). 36g reaction product is then re-crystallized with petrol ether to get 26g matrine with more than 95% purity.

26g matrine is dissolved in water, add 30-40ml 30% H2O2 (in water) to oxidize matrine to oxymatrine. Reaction temp is 60°C and time is 20-24h till no matrine exists in solution detected by Thin-Layer-Chromatography (TLC).

The solution is adjust to pH=8-9 using NaOH and heated to 80°C to remove H2O2. After no H2O2 exists (detected using KI-Amylum test paper), the solution is then concentrated to get final product (22g) with oxymatrine more than 95%. The yield rate of oxymatrine to raw material is 2.2%, much higher than the yield rate (0.5 - 0.8%) in current patent and process used in factory.

Using the seed of S. moorcroftiana as raw material

1kg seeds of S. moorcroftiana is extracted with 10L water for three times. Extract temp: Room temp; Time: 1Oh/time. After filtration, all of the water extraction is then concentrated to IL.

(Because the content of oxymatrine in the seeds of S. moorcroftiana is higher than that in the root of S. flavesens, the amount of resin used in this example is accordingly higher than example 1).
Il water extract is loaded to 3L ADS-21 macroporous absorption resin. The ADS-21 resin is then eluted with 9L water.

9L water fraction (from ADS-21) is then loaded to 5L S-8 resin directly. After 9L water fraction passes through S-8, using another 10L water to elute S-8. Totally 19L water fraction is obtained after S-8.

19L water fraction (from S-8) is then loaded to 3L HZ-816 directly. After all of the 19L water fraction passed through HZ-816, use another 6L water to elute HZ-816 to remove impurities. And then HZ-816 is finally eluted with 8-9L 50% EtOH.

50% EtOH is concentrated to dry to get 50g total alkaloid.

According to HPLC analysis, oxymatrine in total alkaloid is 83% and oxysophocarpine is 5%.

50g total alkaloid is then re-crystallized with acetone to get final product 38g.

According to HPLC analysis, the content of oxymatrine in final product is 96%. The yield rate is 3.8%. Currently no relate data was reported to get oxymatrine from the seed of 5. moorcroftiana.
1. A method of purifying alkaloids extracted from plants of the Sophora genus (Sophora alkaloids), characterised in that the method comprises a purification step in which the Sophora alkaloids are placed on a macroporous absorption resin with an eluent comprising at least 40wt% water.

2. A method according to claim 1 in which the eluent comprises at least 50 wt% water.

3. A method according to claim 1 or claim 2 which comprises at least successive purification steps on three differing absorption resins.

4. A method according to any preceding claim in which the macroporous resins are selected from the group consisting of resins having a polystyrene backbone functionalised with acyl amide; resins having a polystyrene backbone functionalised with amidocyanogen and polystyrene backbone functionalised with ethene.

5. A method according to any preceding claim which further comprises a reducing step.

6. A method according to claim 5 which further comprises an oxidizing step.
7. A method according to claim 5 or claim 6 which further comprises a re-crystallization step.

8. A method according to any preceding claim in which the alkaloids are tetracyclic quinolizidine alkaloids of the general structural formula (I) or II:

\[
\begin{align*}
\text{X} & = 0 \text{ or a lone pair} \\
\text{X} & = \text{a lone pair} \\
\text{X} & = \text{a lone pair}
\end{align*}
\]

9. A method according to claim 8 in which the alkaloid is (+)-oxymatrine and/or (+)-matrine and/or oxysphocarpine.
A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D471/22 C07B63/00

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 C07B

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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Further documents are listed in the continuation of Box C. See patent family annex.

"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)
"D" 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.

Date of the actual completion of the international search

26 March 2009

Date of mailing of the international search report

03/04/2009

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