Title: PROCESS FOR THE RECOVERY OF SHIKIMIC ACID

Abstract: Process for the recovery of shikimic acid from a fermentation broth wherein at least part of the fermentation broth is dried, the dried material is subjected to an extraction with an organic solvent whereby a shikimic acid solution is obtained. Optionally the cells are removed from the fermentation broth before drying. Preferably at least part of the fermentation broth is acidified before drying to a pH between 0 and 5, more preferably between 2 and 3. Optionally before drying at least part of the fermentation broth is subjected to a heat treatment at a temperature of at least 70 °C. Preferably in the extraction an organic solvent with a dielectric constant larger than 5 is used, more preferably a solvent is used which forms an azeotrope with water.
PROCESS FOR THE RECOVERY OF SHIKIMIC ACID

The invention relates to a process for the recovery of shikimic acid from a fermentation broth.

Shikimic acid is a well-known compound that for instance is a suitable intermediate in the preparation of pharmaceuticals, for instance neuraminidase inhibitors for the treatment of influenza.

The recovery of shikimic acid from a fermentation broth is described in K.M. Draht et al. J. Am Chem. Soc. 1999, 121, 1603-1604. In the known process the cells are discarded from the fermentation broth, the resulting supernatant is treated with activated carbon and subsequently eluted through a cation exchange column, after which the shikimic acid is isolated.

It has appeared, however, that the product quality obtained with the known process is not satisfactory. Moreover the process itself is not sufficiently robust.

The present invention aims at a robust process wherein shikimic acid with high purity (more than 90%) can be obtained. This is according to the invention achieved in a process wherein the fermentation broth is dried and the dried material is subjected to an extraction (with an organic solvent). Preferably the cells are first removed from the fermentation broth after which the resulting supernatant is dried and subjected to an extraction.

The fermentation broth containing shikimic acid can be obtained in a manner well known in the art by fermentor cultivation, in particular fed-batch fermentor cultivation, wherein a carbon source is biocatalytically converted to shikimic acid.

The fermentation broth can be dried as such, but preferably the cells are separated prior to the drying process, so that only part of the fermentation broth (the supernatant), remains. The cells can be removed from the fermentation broth in a method well known in the art, for example by filtration, microfiltration, centrifugation, preferably by microfiltration.

Optionally the supernatant can be treated with activated carbon, for instance Norit SX ultra, or with a hydrophobic resin for instance XAD 1600 (Rohm&Haas) to remove impurities.

Optionally at least part of the fermentation broth, for instance the fermentation broth or the supernatant, can be subjected to a heat treatment in
order to convert byproducts of shikimic acid into products which do not interfere
with the recovery of shikimic acid. The temperature at which the heat treatment is
performed is preferably between 60 and 150 °C, in particular between 70°C and
105°C.

The pH at which the extraction is carried out is preferably
between 0 and 5, more preferably between 1 and 4, in particular between 2 and 3.
At any moment before drying the fermentation broth or supernatant, pH may be
adjusted, if desired, to facilitate extraction of the free shikimic acid from the dried
material. In a preferred embodiment of the invention the fermentation broth or the
supernatant can be brought to the desired pH by addition of an acid, for instance
sulphuric acid, phosphoric acid, hydrochloric acid, or an organic acid, preferably
with a pKₐ lower than 4.5, for instance oxalic acid.

The fermentation broth or supernatant can be dried by any
method known in the art. Simple evaporation may be used, but this may present
practical problems on industrial scale. Preferably another drying method is
applied, for instance freeze drying, spraydrying, or drying by way of granulation.
By drying by way of granulation is understood the addition, for instance by
spraying, of the fermentation broth or supernatant to a dryer, in particular a fluid
bed dryer, containing a solid support, for instance a filter aid particularly dicalite,
an activated carbon particularly Norit SX ultra or a cellulose particularly Avicel. In
order to improve the drying efficiency, the broth or the supernatant may be
concentrated first, for example by evaporation or by reversed osmosis.
The water content after drying preferably is less than 10%, more preferably less
than 5%, in particular less than 3%.

The fermentation broth or supernatant as such may tend to form
an oily substance after drying. Optionally, therefore, compounds may be added to
the fermentation broth or supernatant to afford formation of the dried material as a
solid. Compounds which may be added are activated carbon such as Norit SX
ultra, cellulose such as Avicel, filter aids such as dicalite, inorganic salts such as
sodium sulphate or sodiumhydrogensulphate.

The extraction of shikimic acid from the dried (solid) material is
performed using an organic solvent, preferably an organic solvent with a dielectric
constant larger than 5, more particularly larger than 10. Examples of suitable
organic solvents to be used in the extraction are ketones, for example acetone, 2-
butanone, diethylketone and methyl-isobutylketone, and esters, for example
methylacetate and ethylacetate, and alcohols, in particular C1-C5 alcohols, for example methanol, ethanol, n-butanol.

Optionally water may be present during the extraction. Presence of water enhances the solubility of shikimic acid in the organic solvent. The water content is for instance between 0 and 10% (which means between 0 and 100 g/l), preferably between 0 and 5% (0 and 50 g/l), most preferable between 0 and 3% (0 and 30 g/l). Preferably solvents are used for extraction of shikimic acid which enable azeotropic removal of water during evaporation. Suitable solvents are for instance 2-butane, diethylketone, methyl-isobutylketone, ethylacetate or n-butanol.

The temperature at which the extraction is performed is preferably between 0 and 50°C, in particular between 20 and 35°C, because higher temperatures lead to extraction of impurities and lower temperatures lead to low extraction yields or high consumption of solvent.

In a preferred embodiment the extraction is performed by eluting solvent over a bed of solid dried supernatant. The eluate may be collected in several fractions. Optionally fractions with high concentration of shikimic acid may be separated from fractions with low concentration of shikimic acid. In such embodiment usually the fractions with high concentration of shikimic acid are used for evaporation/crystallization/isolation of shikimic acid, while the fractions with low concentration of shikimic acid are used for extraction of the next batch of dried supernatant.

Preferably the extraction is performed countercurrently, for instance by washing countercurrently on, for instance, a band filter.

The shikimic acid solution obtained after extraction may be treated with activated carbon, in order to further enhance the purity of the shikimic acid. As activated carbon may be used for instance Norit SX Ultra.

The shikimic acid may subsequently be isolated, for instance by crystallization, whereby the extract is evaporated and the product shikimic acid crystallizes. Optionally seeds of shikimic acid may be added during the evaporation procedure in order to facilitate controlled crystallization, leading to product with higher purity. The product can be isolated by means of filtration or centrifugation, and optionally dried.

In order to further increase the purity of the shikimic acid the
shikimic acid may be recrystallized, for instance by solving it in an organic solvent. Suitable organic solvents are in principle the same solvents as listed above with regard to the extraction. The temperature at which the recrystallization is performed is not critical and is preferably chosen between room temperature and the boiling temperature of the solvent. The solution of shikimic acid can optionally be filtered to remove insoluble matter. After dissolving shikimic acid and optional filtration, solvent may be evaporated, whereby, in the resulting residu, shikimic acid crystallizes. The product can be isolated, for instance by filtration or centrifugation, and subsequently dried.

Optionally water is added to the organic solvent to enhance the solubility of shikimic acid, and thereby limit the quantity of solvent used. Preferably solvents are used which allow removal of water by azeotropic distillation, as listed above. In order to limit the solubility of shikimic acid in the residu after evaporation of solvent, the water content in the mixture should be low. In this way high yields can be obtained.

Preferably the recrystallization is performed using seed crystals of shikimic acid.

Preferably the recrystallization is performed with the same solvent as the extraction. More preferably, the mother liquor after isolation of the shikimic acid after recrystallization is used for extraction of dried supernant in order to increase the yield of the recovery process.

The invention is further elucidated by the following examples without, however, being restricted thereto.
EXAMPLES

Example 1
Microfiltration of broth containing shikimic acid, followed by evaporation and heat treatment

A broth obtainable as described in K.M. Draths, D.R Knop, J.W. Frost, J. Am. Chem. Soc., 1999, 121, 1603-1604 was filtered using a Membbralox 20 nm membrane of 3.6 m². 1323 l broth, containing 28.85 kg shikimic acid and 5.00 kg quinic acid, was filtered. Retentate is circulated at a flow of 4 m³/h. In order to keep the flux at an acceptable level, water was fed to the retentate after collection of 890 l permeate. A total of 185 l water was added. After collection of a total of 1020 l permeate + diafiltrate, the operation was stopped. The permeate+diafiltrate contained 21.61 kg shikimic acid and 3.76 kg quinic acid. The mixture was concentrated at reduced pressure to a volume of 200 l, and subsequently heated to reflux (at atmospheric pressure, temperature 108 °C) during 4 h.

Example 2.
Freeze drying of acidified broth filtrate, followed by extraction with acetone and crystallization

An aqueous mixture containing shikimic acid, obtained as described in example 1 (250 ml, pH=6.2) was acidified to pH=2.6 with 3 M sulphuric acid (160 ml consumed). A precipitate was removed by filtration. 50 ml of filtrate (containing 2.98 g shikimic acid, 5.15 g acetic acid and 0.52 g quinic acid) was taken from the filtrate, and 5 g filter aid (dicalite 4108) was added. The mixture was freeze dried. The solid residu was extracted with acetone (100 ml) at room temperature. The filtrate (90 ml) contained 1.07 g shikimic acid and 0.03 g quinic acid. The filtrate was stored at 3°C. After several weeks, crystals were formed (assay shikimic acid 95%, quinic acid 1.3%).

Example 3.
Freeze drying of acidified broth filtrate, followed by extraction with acetone, evaporation and crystallization

An aqueous mixture containing shikimic acid, obtained as described in example 1 (94 ml, pH=6.2, containing 8.70 g shikimic acid, 15.4 g
acetic acid and 1.48 g quinic acid) was acidified to pH=2.6 with 96% sulphuric acid (8.5 ml consumed). A precipitate was removed by filtration. 15 g filter aid (dicalite 4108) was added, and the mixture was freeze dried. The solid residu was suspended in 1000 ml acetone, and the mixture was filtered. The wet cake was washed with 100 ml acetone, and both filtrates were combined. Active carbon (9.5 g, Norit SX Ultra) was added, and the mixture was stirred during 15 minutes. The mixture was filtered, and the wet cake was washed with 50 ml acetone. The filtrates were combined (total volume 1020 ml). The solution was evaporated in vacuum at 30-35°C to a volume of 270 ml. The solution was cooled to room temperature, a small amount of shikimic acid seed (obtained as described in example 2) was added, and the mixture was stored overnight at room temperature. The crystals were isolated and washed. 0.8 g (assay shikimic acid 95%) product was obtained. By evaporation of the filtrate to a volume of 100 ml, seeding of the residu and storage overnight at room temperature, another 2.4 g of product (assay shikimic acid 96%) was obtained.

Example 4
Coal treatment of acidified broth filtrate, followed by spray drying

An aqueous mixture containing shikimic acid, obtained as described in example 1 (2.5 l, pH=6.2, containing 231 g shikimic acid, 410 g acetic acid and 39 g quinic acid) was diluted with 7.5 l water. Active carbon (Norit SX Ultra, 300 g) was added, followed by addition of 96% sulphuric acid to pH=2.4 (280 ml consumed). The mixture was heated to 90°C during 2 h, and filtered. The filtrate was cooled to room temperature. The filtrate was subjected to spray drying in a Niro Spray Dryer FU 11. Flow in 10 l/h, inlet temperature 120°C, outlet temperature 70°C.
A powder (992 g) was obtained, assay shikimic acid 16.4%, quinic acid 2.9%.

Example 5
Extraction of spray dried material with methylethylketone, followed by evaporation and crystallization

Spray dried material obtained as described in example 4 (60 g) was suspended in 600 ml methylethylketone + 7.2 ml water. The mixture was stirred at room temperature during 90 minutes, and subsequently filtered. The wet cake was suspended in 600 ml methylethylketone + 7.2 ml water. The mixture
was stirred at room temperature during 90 minutes, and subsequently filtered. The wet cake was suspended in 600 ml methylethylketone + 7.2 ml water. The mixture was stirred at room temperature during 90 minutes, and subsequently filtered. The filtrates were analyzed by HPLC. Results:

<table>
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<tr>
<th>filtrate nr.</th>
<th>shikimic acid (g/l)</th>
<th>quinic acid (g/l)</th>
</tr>
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<tr>
<td>1</td>
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<td>4.98</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>2.91</td>
<td>0.20</td>
</tr>
</tbody>
</table>

The combined filtrates (1740 ml, containing 8.40 g shikimic acid and 0.40 g quinic acid) were evaporated at atmospheric pressure in a round bottom flask equipped with stirrer and distillation head. After evaporation of 1250 ml, the residu was seeded with shikimic acid crystals. Evaporation was stopped at a volume of 250 ml of the residu. Time of evaporation 1½ h. The residu was cooled to room temperature in the course of one hour while stirring, and subsequently stirred for 2 hours at room temperature. The mixture was filtered, and the wet cake was washed with two bed volumes of dry methylethylketone. The wet cake was dried. 7.92 g of product was obtained (assay shikimic acid 76%, assay quinic acid 1%).

**Example 6**

Recrystallization of shikimic acid

Shikimic acid, obtained as described in example 5, (7.00 g, assay shikimic acid 76%, assay quinic acid 1%) was suspended in methylethylketone (750 ml) + water (10 ml). The mixture was heated to reflux during 10 minutes, cooled to 60 °C, and the turbid solution was filtered. The filtrate was evaporated at atmospheric pressure to a volume of 400 ml, seeded with shikimic acid, and evaporation was continued to a volume of the residu of 200 ml. Time of evaporation 1.5 h. The mixture was cooled to room temperature in the course of 1 h, and stirred for another 2 h. The mixture was filtered, and the wet cake was washed with two bed volumes of dry methylethylketone. The wet product was dried, yield 5.36 g, assay shikimic acid 85%, assay quinic acid 0.5%.

**Example 7**
Column extraction of spray dried material with methylethylketone

Spray dried material obtained as described in example 4 (60 g) was mixed with 80 ml methylethylketone + 1 ml water. The mixture was transferred to a column with a diameter of 40 mm. The height of the column of solid material was 80 mm. A mixture of 1600 ml methylethylketone and 21.6 ml water was fed to the top of the column, and the eluate (at a rate of 8-10 ml/min) was collected in several fractions. The fractions were analyzed by HPLC. Results:

<table>
<thead>
<tr>
<th>Fr. nr.</th>
<th>volume (ml)</th>
<th>shikimic acid (g/l)</th>
<th>quinic acid (g/l)</th>
<th>Fr. nr.</th>
<th>volume (ml)</th>
<th>shikimic acid (g/l)</th>
<th>quinic acid (g/l)</th>
</tr>
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<td>100</td>
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<td>0.34</td>
<td>11</td>
<td>100</td>
<td>7.50</td>
<td>0.18</td>
</tr>
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<td>3</td>
<td>50</td>
<td>8.48</td>
<td>0.38</td>
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<td>0.02</td>
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<td>0.23</td>
</tr>
<tr>
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<td>17</td>
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<td>0.01</td>
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</tr>
<tr>
<td>9</td>
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<td>10.43</td>
<td>0.19</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Example 8

Column extraction of spray dried material with methylethylketone, followed by evaporation and crystallization

Spray dried material obtained as described in example 4 (60 g) was mixed with 80 ml methylethylketone + 1.2 ml water. The mixture was transferred to a column with a diameter of 40 mm. A mixture of 1100 ml methylethylketone and 16.5 ml water was fed to the top of the column, and the eluate (at a rate of 8 ml/min) was collected (1100 ml). The eluate was evaporated at atmospheric pressure to a volume of 225 ml water in the course of 1.5 h. The mixture was cooled to room temperature in the course of 1 h, and stirred at room temperature for 2h. The mixture was filtered, and the wet cake was washed with 2 times 20 ml dry methylethylketone. The wet cake was dried. 8.27 g of product was
obtained, assay shikimic acid 98.0%, assay quinic acid 0.7%.

Example 9

Spray drying of heat treated broth

A broth obtainable as described in K.M. Draths, D.R. Knop, J.W. Frost, J. Am. Chem. Soc., 1999, 121, 1603-1604 was used as starting material. To 6.8 kg broth (containing 195 g shikimic acid, 35 g quinic acid and 185 g acetic acid) sulphuric acid (96%) was added to a pH of 2.6 (300 g consumed). The mixture was heated to 95 °C during 2½ h. The mixture was cooled, and spray dried in a spray dryer (Niro Spray Dryer FU 11). Flow in 10 l/h, inlet temperature 130 °C, outlet temperature 75 °C. The spray dried material contained 14.7% shikimic acid and 3.0% quinic acid.

Example 10

Column extraction of spray dried broth, followed by coal treatment, evaporation and crystallization

Spray dried material obtained as described in example 9 (120 g) was mixed with 250 ml methylethylketone + 3 ml water. The mixture was transferred to a column with a diameter of 60 mm. A mixture of 2000 ml methylethylketone and 24 ml water was fed to the top of the column, and the eluate (at a rate of 16-20 ml/min) was collected (2000 ml). Activated carbon (32 g) was added to the eluate, and the mixture was stirred for 2 h at room temperature. The mixture was filtered. The filtrate was evaporated at atmospheric pressure to a volume of 400 ml in the course of 1 h. After evaporation of 800 ml methylethylketone, shikimic acid seed crystals were added to the mixture. The mixture was cooled to room temperature in the course of 1 h, and stirred at room temperature for 2 h. The mixture was filtered, and the wet cake was washed with 2 times 40 ml dry methylethylketone. The wet cake was dried. 13.72 g of product was obtained, assay shikimic acid 92%, assay quinic acid 1%.

Example 11

Coal treatment of acidified broth filtrate, followed by spray drying

An aqueous mixture containing shikimic acid, obtained as described in example 1 (2.5 l, pH=6.2, containing 231 g shikimic acid, 410 g acetic acid and 39 g quinic acid) was diluted with 7.5 l water. Active carbon (Norit
SX Ultra, 600 g) was added, followed by addition of 96% sulphuric acid to pH=2.4 (250 ml consumed). The mixture was heated to 94°C during 2 h, and filtered. The filtrate was cooled to room temperature. The filtrate was subjected to spray drying in a Niro Spray Dryer FU 11. Flow in 10 l/h, inlet temperature 130°C, outlet temperature 70 °C.

A powder was obtained, assay shikimic acid 17.8%, quinic acid 3.2%.

**Example 12.**

**Extraction of spray dried material with different solvent and with different amounts of water**

Spray dried material obtained as described in example 11 (12.5 g) was suspended in 250 ml solvent. The mixture was stirred at room temperature during 60 minutes and a sample was taken for HPLC. Subsequently water (amount: see table) was added, the mixture was stirred for an hour, and a sample was taken for HPLC analysis. This procedure was repeated.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>water added (g/l)</th>
<th>[shikimic acid] (g/l)</th>
<th>[quinic acid] (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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</tr>
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CLAIMS

1. Process for the recovery of shikimic acid from a fermentation broth wherein the fermentation broth is dried and the dried material is subjected to an extraction with an organic solvent whereby a shikimic acid solution is obtained.

2. Process according to claim 1, wherein the cells are removed from the fermentation broth before drying the resulting supernatant.

3. Process according to claim 2, wherein the cells are removed by filtration, microfiltration or centrifugation.

4. Process according to any of claims 1-3 in which the fermentation broth or the supernatant is acidified before drying to a pH between 0 and 5.

5. Process according to claim 4, wherein the pH is between 2 and 3.

6. Process according to any of claims 1-5 wherein before drying the fermentation broth or the supernatant is subjected to a heat treatment at a temperature of at least 70 °C.

7. Process according to any of claims 2-6, wherein the supernatant is treated with activated carbon or a hydrophobic resin before drying.

8. Process according to any of claims 1-7, wherein the fermentation broth or the supernatant is dried by freeze drying, spray drying or granulation.
9. Process according to any of claims 1-8, wherein the fermentation broth or the supernatant is dried in the presence of a compound selected from the group of activated carbon, cellulose, filter aids and inorganic salts.

10. Process according to any of claims 1-9, wherein in the extraction an organic solvent with a dielectric constant larger than 5 is used.

11. Process according to claim 10, wherein water is added to the extraction solvent to a concentration of between 0 and 100 g/l, preferably between 0 and 50 g/l, most preferably between 0 and 30 g/l.

12. Process according to claim 10 or 11, wherein in the extraction a solvent is used which forms an azeotrope with water.

13. Process according to claim 12, wherein the solvent is 2-butanone and the amount of water is between 0 and 30 g/l.

14. Process according to any of claims 1-13, wherein the extraction is performed by eluting solvent over solid dried supernatant.

15. Process according to claim 14, wherein the eluate is collected in separated fractions with varying concentration of shikimic acid and wherein shikimic acid is isolated from one or more of the fractions with higher concentrations and one or more of the fractions with lower concentrations of shikimic acid is used for extraction of the next batch of dried supernatant.
16. Process according to any of claims 1-15, wherein the extraction is performed countercurrently.

17. Process according to any of claims 1-16 wherein the shikimic acid solution in the organic solvent is treated with activated carbon.

18. Process according to any of claims 1-17 wherein the shikimic acid is isolated from the solution.

19. Process according to claim 18, wherein shikimic acid is crystallized from the solution by evaporation of the solvent.

20. Process according to claim 19 wherein the crystallisation is performed in the presence of seed crystals.

21. Shikimic acid crystals with a shikimic acid content of more 90% by weight, obtainable using the process of any of claims 1-20.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C51/48 C07C51/47 C07C51/43 C07C51/42 C07C62/32
C12P7/42

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 C07C C12P

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)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search 29 November 2001

Date of mailing of the international search report 06/12/2001

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