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(72) Inventeur/Inventor:
SAUTER, MARKUS, DE
(73) Propriétaire/Owner:
BOEHRINGER INGELHEIM PHARMA GMBH & CO. KG,
DE
(74) Agent: FETHERSTONHAUGH & CO.

(54) Titre : PROCEDE DE PRODUCTION AMELIORE D'ACIDE BETULINIQUE
(54) Title: IMPROVED PROCESS FOR OBTAINING BETULINIC ACID

(57) **Abrégé/Abstract:**

The invention relates to an improved method for obtaining highly pure, crystalline betulinic acid from a methanolic extract of ground plane bark and/or plane rind. The invention also relates to a highly pure, crystalline betulinic acid, which is solvated with an equivalent of ethanol.



ABSTRACT

The invention relates to an improved method for obtaining highly pure, crystalline betulinic acid from a methanolic extract of ground plane bark and/or plane rind. The invention also relates to a highly pure, crystalline betulinic acid, which is solvated with an equivalent of ethanol.

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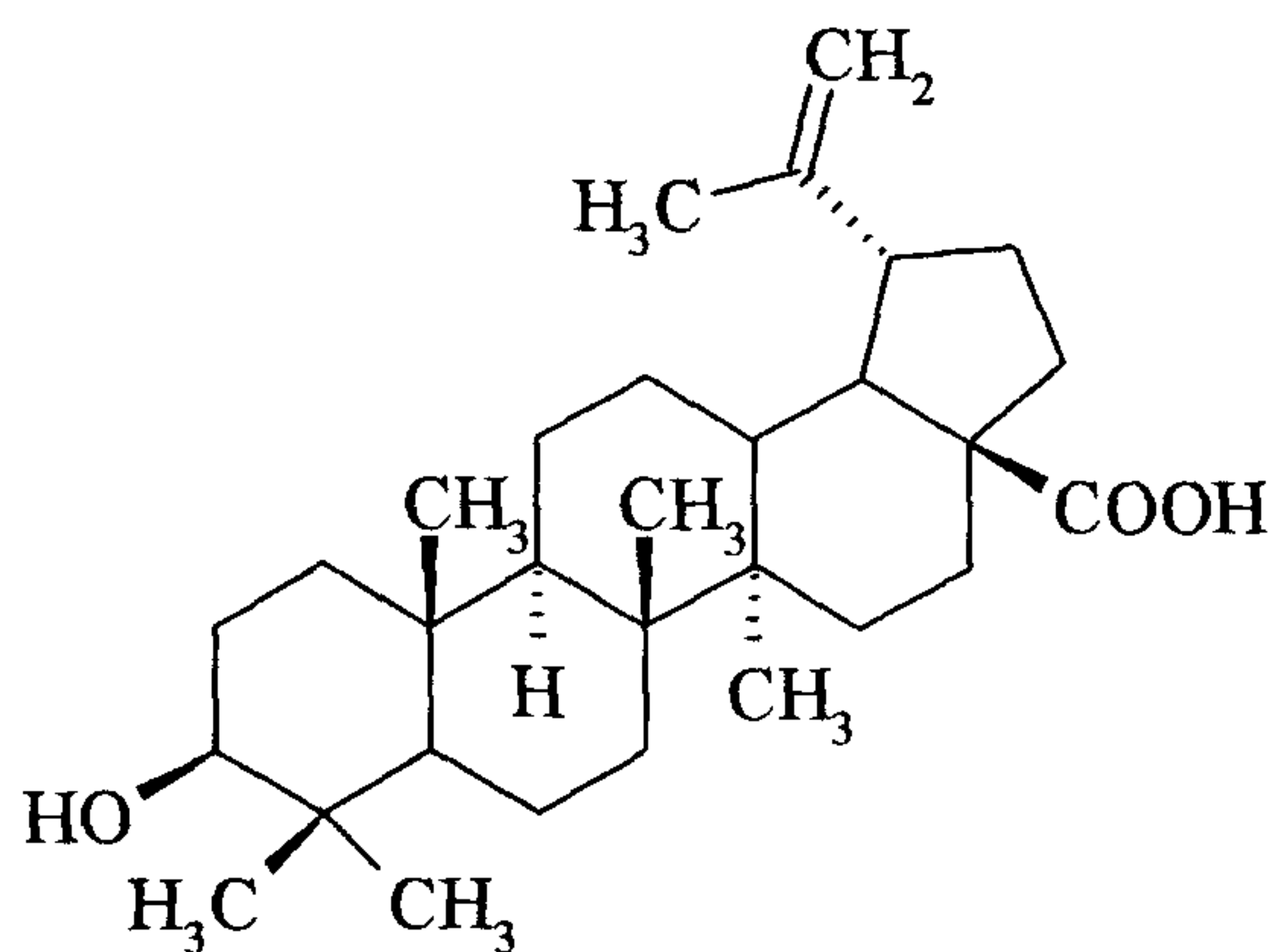
Improved process for obtaining betulinic acid

Description

The invention relates to an improved process for obtaining highly pure, crystalline betulinic acid
5 acid from a methanolic extract of ground plane bark.

Background to the invention

Betulinic acid is 3 β -hydroxy-lup-20(29)-ene-28-acid of formula



It is known that betulinic acid is effective against the growth of melanoma cells (e.g. Pisha et
10 al., Nature Medicine 1, 1995, 1046 ff) as well as against other cancer cells (e.g. Sunder et al.,
US Patent US 6,048,847). In addition its amides are supposed to be suitable for use against
HIV (e.g. Evers et al., J. Med. Chem. 39, 1996, 1056 ff; or Soler et al., J. Med. Chem. 39,
1996, 1069 ff). Consequently betulinic acid is in great demand.

15 Apart from being produced synthetically (e.g. L. Ruzicka et al., Helv. Chim. Acta 21, 1938,
1076 ff) betulinic acid may also be obtained from various plants, particularly trees, such as for
example from the outer bark or cortex of *Picramnia pentandra* (e.g. Herz et al.,
Phytochemistry 11, 1972, 3061 ff), from the cortex of *Arbutus menziesii* (Robinson et al.,
Phytochemistry 9, 1970, 907 ff) and from the cortex of *Ziziphus mauritiana* (e.g. Pisha et al.,
20 Nature Medicine 1, 1995, 1046 ff).

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It is difficult to isolate betulinic acid from these starting materials. By contrast it would appear more promising to obtain it from the cortex and/or bark of the plane tree (*Platanus acerifolia*). DE 197 13 768 proposes a process for obtaining betulinic acid, in which a powder obtained from plane bark is extracted with a medium polarity solvent such as for example

5 dichloromethane, chloroform or diethyl ether.

However, this process is unsuitable for the industrial recovery of large amounts of betulinic acid, as very large volumes of the medium polarity solvent have to be used to extract the betulinic acid (7 litres of dichloromethane are used to 150 g of powdered plane bark).

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Bruckner et al., J. Chem. Soc. 1948, 948-951 describe a process for obtaining betulinic acid from plane bark in which the ground bark is extracted with methanol, the extract obtained is evaporated down, and the concentrate is repeatedly recrystallised from methanol in the presence of charcoal. However, the betulinic acid thus obtained still contains a large number

15 of impurities.

The aim of the present invention was therefore to provide an improved process for the large-scale recovery of highly pure, crystalline betulinic acid from plane bark and/or plane cortex which avoids the disadvantages of the known methods.

20 Detailed description of the invention

Surprisingly it has now been found that highly pure crystalline betulinic acid can be obtained from a methanolic extract of ground plane bark and/or plane cortex by evaporating the extract down and crystallising it from an alcohol if the residue from the methanolic extraction is washed with a nonpolar diluent before the crystallisation.

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The present invention thus relates to a process for obtaining highly pure, crystalline betulinic acid from a methanolic extract of ground plane bark and/or plane cortex by evaporation of the extract and crystallisation from an alcohol, characterised in that before the crystallisation the residue is washed with a nonpolar diluent.

The invention further relates to highly pure crystalline betulinic acid which is solvated with one equivalent of ethanol.

5 The Western plane (*Platanus occidentalis*) has proved particularly suitable as the plane tree in question.

Suitable alcohols for recrystallising the crude betulinic acid are generally aliphatic alcohols with 1 to 4 C-atoms, particularly methanol, ethanol or isopropanol or mixtures thereof, most preferably ethanol.

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Suitable nonpolar diluents are generally aliphatic, cycloaliphatic or aromatic hydrocarbons or mixtures thereof, preferably aliphatic hydrocarbons with 5 to 8 carbon atoms, particularly pentane, hexane or heptane, most preferably n-hexane, cycloaliphatic hydrocarbons with 5 to 8 carbon atoms, particularly cyclopentane, cyclohexane, methylcyclohexane, or aromatic
15 hydrocarbons with 6 to 9 carbon atoms, particularly toluene or xylene.

Where, in the interests of simplicity, the ratio of two substances is given hereinbefore or hereinafter in the form "x to y-fold amount", where x and y denote the lower and upper limits, respectively, of the amount specified, this refers to "x to y" parts by weight of one substance
20 in relation to one (1) part by weight of the other substance.

Preferred embodiments of the invention are:

(A) processes in which the following steps for washing and isolating the residue of the methanolic extract are carried out successively:

- 25 (i) heating the residue in a nonpolar diluent;
(ii) separating the residue from the diluent;
(iii) heating the residue in ethanol, clarifying the ethanolic phase by the addition of activated charcoal and separating the activated charcoal from the ethanolic phase at a temperature between 60 and 95 °C; and
30 (iv) crystallising and separating the betulinic acid from the ethanolic phase.

(B) processes in which the ground plane cortex is heated to boiling with 2 to 5 times the amount of methanol for 0.5 to 5.0 hours, extracted, the extract obtained is filtered at a temperature between 40 and 60 °C, evaporated down to 30 to 70 % of its volume and crystallised at a temperature between – 10 and + 10 °C.

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In an embodiment, the present invention provides a process for obtaining highly pure, crystalline betulinic acid from a methanolic extract of ground plane cortex and/or plane bark by concentrating the extract and crystallising it from an alcohol, wherein a) the ground plane cortex is heated to boiling once with
5 2 to 5 times the amount of methanol for 0.5 to 5.0 hours, extracted, the extract obtained is filtered at a temperature between 40 and 60°C, evaporated down to 30 to 70% of its volume and crystallised at a temperature between -10 and +10°C and b) the following steps are carried out successively for washing and isolating the residue of the methanolic extract; (i) heating the residue in a nonpolar diluent;
10 (ii) separating the residue from the diluent; (iii) heating the residue in ethanol, clarifying the ethanolic phase by the addition of activated charcoal and separating the activated charcoal from the ethanolic phase at a temperature between 60 and 95°C; and (iv) crystallising and separating the betulinic acid from the ethanolic phase.

15 The term "betulinic acid" as used above and hereinafter includes both betulinic acid as such and the hydrates and solvates thereof, preferably betulinic acid which is solvated with 1 to 2 equivalents of an alcohol, particularly one equivalent of ethanol.

(C) processes in which the residue of the methanolic extract is taken up in 2 to 8 times the amount of an aliphatic hydrocarbon, preferably cyclohexane, methylcyclohexane or *n*-hexane, particularly *n*-hexane, and the mixture is heated to boiling.

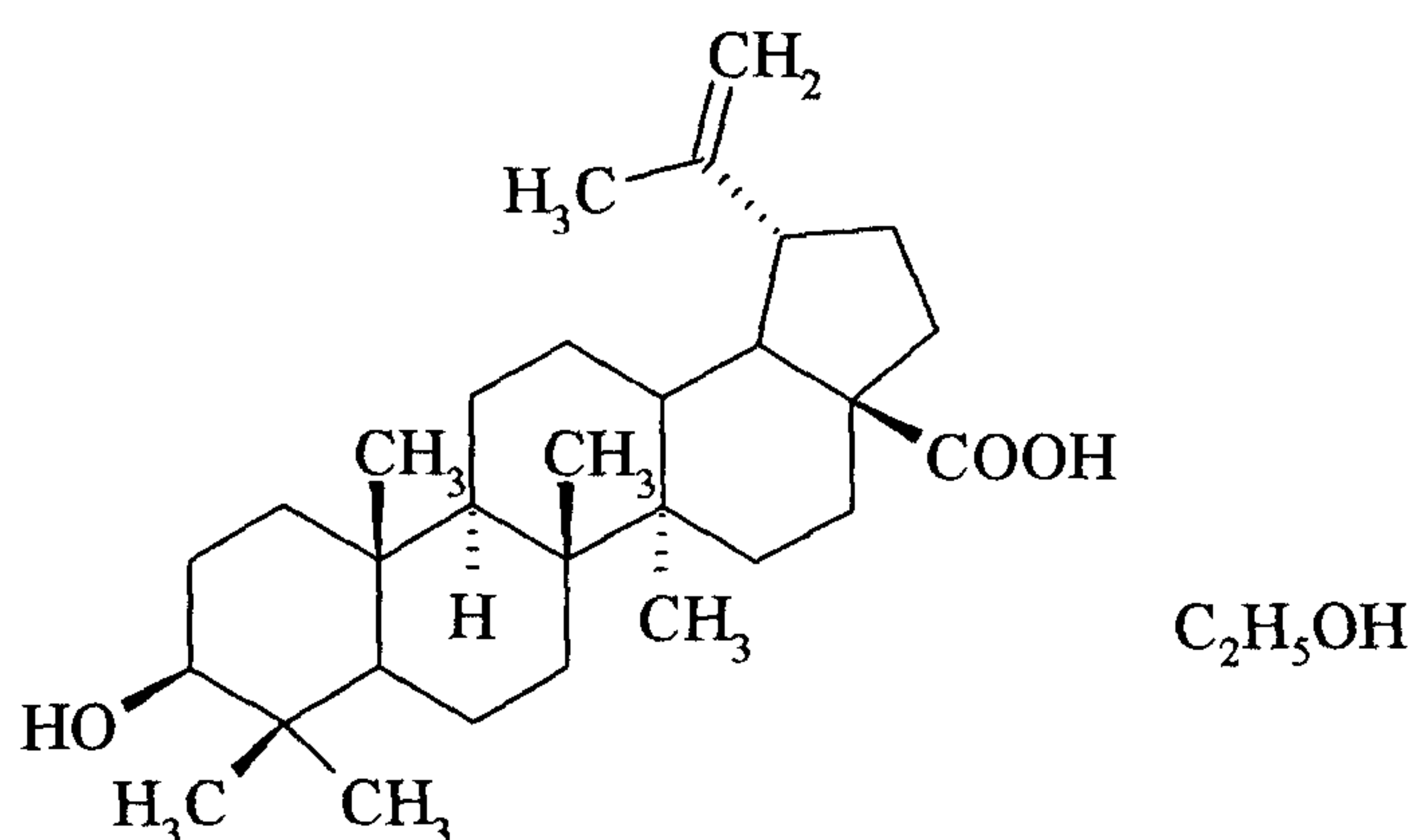
(D) processes in which in step (ii) the residue is separated from the diluent by centrifuging or suction filtering.

(E) processes in which in step (iii) the residue is taken up in 20 to 100 times, preferably 30 to 80 times, particularly 40 to 60 times the amount of ethanol and heated to boiling.

(F) processes in which in step (iv) the ethanolic phase is cooled to temperatures of -10 to $+10$ °C, preferably 0 to $+5$ °C, the betulinic acid precipitated is separated off by centrifuging or suction filtering and dried.

(G) processes in which the betulinic acid obtained is in the form of the monoethoxide.

Highly pure crystalline betulinic acid which is solvated with one equivalent of ethanol is particularly preferred, particularly the betulinic acid ethoxide of formula



In a most particularly preferred embodiment of the process according to the invention the powdered plane cortex is suspended in 2 to 5 times, preferably 3 to 4 times as much methanol and heated for 1 to 5 hours, particularly about 2 hours, preferably by refluxing. The suspension is filtered at 40 to 60 °C, particularly at about 50 °C, the methanol extract is evaporated down to 60 to 40 %, particularly about 50 % of its volume. The concentrated extract is cooled to 0 to 10 °C, particularly about 5 °C, filtered off and washed with cold methanol. The residue is preferably dried *in vacuo* at 40 to 70 °C, particularly at about 50 °C.

In this way, the crude betulinic acid is obtained as a colourless powder (about 2.0 to 2.5 %, based on plane cortex). This is suspended in 4 to 8 times, particularly 5 to 6 times as much *n*-hexane, cyclohexane or methylcyclohexane and heated for 0.5 to 2 hours, particularly 1 hour, particularly by refluxing. The suspension obtained is centrifuged or suction filtered,

washed with 1.5 to 4.5 times, particularly about 3 times as much hot *n*-hexane, cyclohexane or methylcyclohexane, and the residue is dried overnight at about 40°C, preferably *in vacuo*.

The betulinic acid thus obtained is dissolved in 30 to 50 times as much ethanol, with heating.

- 5 Activated charcoal is added and the mixture is filtered while hot. As it cools, crystallisation begins. The mixture is cooled to 0 to 10 °C, particularly 5°C, suction filtered or centrifuged and washed with cold ethanol.

Highly pure crystalline betulinic acid is obtained, which is solvated with one mol of ethanol.

- 10 The following Example serves to illustrate a process for obtaining betulinic acid which is carried out by way of example. It is intended solely as a possible procedure provided as an illustration, without restricting the invention to its contents.

Example**1A Extraction of the plane cortex**

4500 g of plane cortex powder (ground in a cutter mill) are suspended in 15 l of methanol and
5 refluxed for 2 h. The suspension is filtered at about 50 °C, the methanol extract is evaporated
down to 50% of its volume (7.5 l). The concentrated extract is cooled to 5°C, filtered off and
washed with 2 l of cold methanol. The residue is dried *in vacuo* at 50°C.

104 g of crude betulinic acid are obtained as a colourless powder (2.3 %, based on plane
cortex). HPLC: 90.6 %

1B Stirring out from nonpolar solvent

93 g of betulinic acid obtained according to Example 1A are suspended in 600 ml of *n*-hexane
and refluxed for 1 hour. The suspension is suction filtered through a nutsche, washed with
300 ml of hot *n*-hexane, and the residue is dried overnight at 40°C *in vacuo*.

15 89.5 g (96.2 %) of betulinic acid are obtained as a colourless powder. HPLC: 94.75 %

Cyclohexane, methylcyclohexane or mixtures of these solvents may also be used analogously.

1C Recrystallisation from ethanol

89 g of betulinic acid obtained according to Example 1B are refluxed and dissolved in 4 l of
20 ethanol. 40 g of activated charcoal are added and the mixture is filtered hot through a
nutsche. After cooling to ambient temperature, crystallisation sets in. The mixture is cooled to
5°C, suction filtered and washed with 500 ml of cold ethanol. 63.5 g (71.3 %) of crystalline
betulinic acid is obtained, which is solvated with 1 mol of ethanol. HPLC: 93.8 % (percentage
area 100%); melting point = 296-298°C, $[\alpha]_D = +8$, $c = 0.9$ in pyridine.

1D Working up the mother liquor

4 l of the ethanolic mother and washing liquor from Example 1C are evaporated down to 1
litre *in vacuo*, suction filtered at 5°C and washed with cold ethanol.

20 g (22.5 %) of betulinic acid are obtained. HPLC: 96.5 %

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CLAIMS:

1. Process for obtaining highly pure, crystalline betulinic acid from a methanolic extract of ground plane cortex and/or plane bark by concentrating the extract and crystallising it from an alcohol, wherein
 - 5 a) the ground plane cortex is heated to boiling once with 2 to 5 times the amount of methanol for 0.5 to 5.0 hours, extracted, the extract obtained is filtered at a temperature between 40 and 60°C, evaporated down to 30 to 70% of its volume and crystallised at a temperature between -10 and +10°C; and
 - b) the following steps are carried out successively for washing and
10 isolating the residue of the methanolic extract:
 - (i) heating the residue in a nonpolar diluent;
 - (ii) separating the residue from the diluent;
 - (iii) heating the residue in ethanol, clarifying the ethanolic phase by the addition of activated charcoal and separating the activated charcoal from the
15 ethanolic phase at a temperature between 60 and 95°C; and
 - (iv) crystallising and separating the betulinic acid from the ethanolic phase.
2. Process according to claim 1, wherein the residue of the methanolic extract is taken up in 2 to 8 times the amount of aliphatic hydrocarbon and the
20 mixture is heated to boiling.
3. Process according to claim 2, wherein the aliphatic hydrocarbon is η -hexane.
4. Process according to any one of claims 1 to 3, wherein in step (ii) the residue is separated from the diluent by centrifuging.

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5. Process according to any one of claims 1 to 4, wherein in step (iii) the residue is taken up in 20 to 100 times the amount of ethanol and heated to boiling.
6. Process according to any one of claims 1 to 5, wherein in step (iv) the ethanolic phase is cooled to temperatures of -10 to +10°C and the betulinic acid
5 precipitated is separated off by centrifuging and dried.
7. Process according to claim 6, wherein in step (iv) the ethanolic phase is cooled to temperatures of 0 to +5°C.
8. Process according to any one of claims 1 to 7, wherein the betulinic acid thus obtained is present as the monoethoxide.