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(54) Title: METHOD FOR PURIFICATION OF ACARBOSE

(57) Abstract: The present invention relates to a novel process for the preparation of acarbose. Said process comprises the steps of: 1) acidifying a fermentation broth containing an acarbose; 2) removing particulates from the fermentation broth; 3) adsorbing the acarbose on a cation-exchanger in the presence of an anion of a weak acid; 4) eluting the acarbose from the cation-exchanger with at least one of a sodium chloride solution and a salt solution; 5) precipating the acarbose with a solvent; and 6) recovering the precipitated acarbose.

METHOD FOR PURIFICATION OF ACARBOSE

CROSS REFERENCE TO RELATED APPLICATIONS

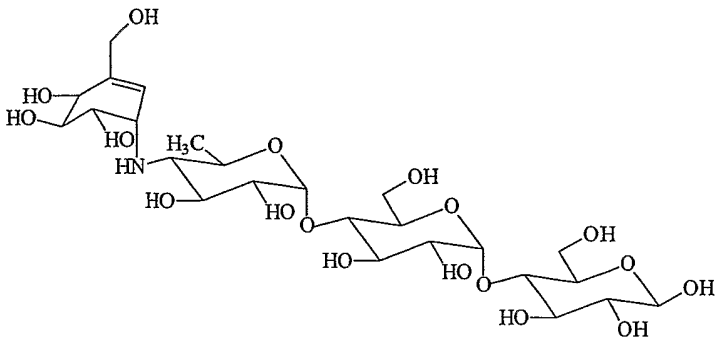
This is a continuation-in-part application of the U.S. Conventional Application (Serial No. 09/924,271) filed on August 7, 2001 which claims the benefit of the Provisional Application Serial No. 60/223,492 filed August 7, 2000, the disclosures of which are incorporated by reference in their entireties herein.

FIELD OF THE INVENTION

The present invention relates to a novel process for the purification of acarbose.

BACKGROUND OF THE INVENTION

Acarbose, also known as O-4,6-Dideoxy-4[[[1S-(1α, 4α,5β,6α)]-4,5,6-trihydroxy-3-(hydroxmethyl)-2-cyclohexen-1-yl]amino]-α-D-glycopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucose, or 4",6"-dideoxyl-4"-[(1S)-(1,4,6/5)-4,5,6-trihydrox-3-hydroxymethyl-2-cyclohexenylamino]maltotriose, has the following formula (I).



Acarbose is a potent α-glucosidase inhibitor that reduces sugar absorption in the gastrointestinal tract. It is used as an orally administered anti-diabetic drug sold under the trademark GLUCOBAY® and is available for the treatment of diabetes mellitus in humans.

U.S. Pat. No. 4,062,950 and Ger. Pat. No. 2,347,782 describe the isolation of acarbose from strains of *Actinoplanes*. These processes employ the use of ion-exchangers to adsorb acarbose from fermentation broths; but the ion-exchange steps contain nitrate anion. The presence of nitrate anion causes impurities to adsorb onto the ion-exchange resins and thus contaminates the acarbose. The presence of impurities also complicates the purification process because additional purification steps are needed to remove these impurities.

There is a need for an improved process for purification for acarbose. It is desirable to develop a purification process for acarbose whereby an increased purity of acarbose can be obtained with simplified purification steps.

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides a process for the purification of acarbose using ion-exchange chromatography; specifically, a cation-exchanger; and more specifically, a cation-exchanger in the presence of a weak acid.

According to another aspect, the present invention involves the use of a strong cation-exchanger in the

presence of an anion of a weak acid to adsorb acarbose.

The present invention provides a method of purifying acarbose, which comprises the steps of:

1) acidifying a fermentation broth containing an acarbose;
2) removing particulates from the fermentation broth;
3) adsorbing the acarbose on a cation-exchanger in the presence of an anion of a weak acid;
4) eluting the acarbose from the cation-exchanger with at least one of a sodium chloride solution and a salt solution;
5) precipitating the acarbose with a solvent; and
6) recovering the precipitated acarbose.

The present invention provides a method of purifying acarbose, which comprises the steps of:

1) acidifying a fermentation broth containing an acarbose;
2) removing particulates from the fermentation broth;
3) adsorbing the acarbose on an anion-exchanger in the presence of an anion of a weak acid;
4) eluting the acarbose from the anion-exchanger;
5) adsorbing the eluted acarbose on a cation-exchanger in the presence of the anion of a weak acid;
6) eluting the acarbose from the cation-exchanger with at least one of a sodium chloride solution and a salt solution;
7) precipitating the acarbose with a solvent; and
8) recovering the precipitated acarbose.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the term "anion" refers to a negatively-charged ion and the term "cation" refers to a positively-charged ion.

As used herein, "ion exchange chromatography" refers to a separation method that employs charged ion-exchanger for binding and eluting a target molecule (e.g., acarbose).

As used herein, a "cation-exchanger" is a type of charged ion-exchanger that possesses a net negative charge which binds acarbose. One skilled in the art will appreciate that a strong ion-exchanger is one which remains almost fully ionized over a wide pH range whereas a weak exchanger is ionized over a small pH range. The terms "strong cation-exchanger" and "strong acid cation-exchanger" are used interchangeably and they refer to the same types of cation-exchangers.

As used herein, the term "a salt solution" refers to a solution at least one of chloride salt, sulfate salt, nitrate salt, acetate salt and the like.

As used herein, "a solution of chloride salt" refers to sodium chloride, potassium chloride, calcium chloride and the like.

As used herein, "a solution of sulfate salt" refers to sodium sulfate, potassium sulfate, calcium sulfate and the like.

As used herein, "a solution of nitrate salt"

refers to sodium nitrate, potassium nitrate, calcium nitrate and the like.

As used herein, "a solution of acetate salt" refers to sodium acetate, potassium acetate, calcium acetate and the like.

Among the strong acid cationic exchange resins which may be used are those having sulfonic acid ($SO3^-$ H^+) groups. These include commercial products, e.g., Amberlite® IR-118, IR-120, 252H; Amberlyst® 15, 36; Amberject® 1200 (H) (Rohm and Haas); Dowex® 50 wX series, Dowex® HCR-W2, Dowex® 650C, Dowex® Marathon C, Dowex® DR-2030, and Dowex® HCR-S, ion exchange resin (Dow Chemical Co.); Diaion® SK 102 to 116 resin series (Mitsubishi Chemical Corp.) and Lewatit SP 120 (Bayer). The preferred strong acid cationic exchange resins are Amberlite® 120, Dowex® 50 WX and Diaion® SK series.

Preferred cation-exchangers also include Amberlite®. Amerblite ion-exchanger employs a polystyrene resin matrix. Amberlite® 252 resin in H^+ form is an example for cation-exchanger in acid form. Preferred cation-exchanger is Amberlite® 252 in H^+ form.

Cation ion-exchangers further include sulpho, sulphomethyl (i.e., methyl sulfonate), and sulphopropyl forms. Preferable cation-ion exchangers include the functional group of methyl sulfonate. Exemplary strong cation-exchangers include Mini S® (methyl sulfonate), Mono S® (methyl sulfonate), SP Sepharose® (methyl sulfonate), SOURCE 15S®, 30S® (methyl sulfonate) and the like.

Weak cation ion-exchange resins include those which have carboxylic acid groups as well as carboxy and carboxymethyl forms. Preferable weak cation-exchangers include the functional group of -COOH. An exemplary weak cation-exchanger is CM Sepharose Fast Flow®.

As used herein, an "anion-exchanger" refers to anion-exchange resins that possess a net positive charge. Preferred anion-exchange resins include resins that contain a quarternary amine functional group. Diethylaminoethyl (DEAE) exchangers and carboxymethyl (CM) exchangers are usually used as anion exchangers.

As used herein, the term "an anion of a weak acid" refers to an anion of organic acids or phosphate. The anion of weak acid is selected from the group consisting of tartarate, succinate, citrate, acetate, formate, malonate, oxalate, phthalate, benzoate and phosphate.

As used herein, the term "weak acid" specifically refers to an acid selected from the group consisting of tartaric acid, succinic acid, citric acid, acetic acid, formic acid, malonic acid, oxalic acid, phthalic acid, benzoic acid and phosphoric acid.

As used herein, the term "particulates" refers to cellular debris and other particles that are present in a fermentation broth. Particulates also include mycelium.

As used herein, the term "M" refers to a molar concentration in moles/liter.

As used herein, the yield % is based on w/w. Each peak has an area on a HPLC chromatogram. "Area %" refers to the peak area of purified product divided by the total area of all peaks multiplied by 100.

As used herein, the term "yield of anion exchange" (See Table 1)refers to the yield % of acarbose prior to the cation-exchange step.

As used here, the term "summarized yield" refers to yield of anion-exchange multiplied by yield of cation-exchange.

According to one embodiment, the present invention provides a purification process for acarbose employing an appropriate anion which is selected from the group consisting of tartarate, succinate, citrate, acetate, formate, malonate, oxalate, phthalate, benzoate, and phosphate.

According to another embodiment, the present invention provides a process of purifying acarbose employing the presence of an anion of a weak acid during the cation-exchanger. When the anion of a weak acid is present, it is found that the impurities present in the fermentation broth cannot adsorb onto the strong acid cation-exchanger. Consequently, only acarbose adsorbs onto the strong acid cation-exchanger, and results in a better purification. This results in selective adsorption of acarbose. Accordingly, we found a novel phenomenon that adsorption of acarbose without the impurities.

According to another embodiment, the present

invention provides the acarbose adsorbing onto a strong acid cation-exchanger without previous desalting. In contrast, when counter-ions such as chloride, nitrate and the like are used, it is found that desalting is required.

According to another embodiment, the present invention provides an unexpected phenomenon where it is found that the specific type of anion can influence the selectivity and adsorption capacity of the cation-exchanger.

According to another embodiment, the present invention provides a process for purifying acarbose employing the use of multiple ion-exchangers. Fermentation broth is allowed to adsorb onto multiple ion-exchangers successively. In particular, acarbose is eluted from an anion-exchanger prior to the adsorption onto a cation-exchanger. The use of successive exchangers has proved to be effective in purifying acarbose.

A preferred embodiment for an anion-exchanger is an anion-exchanger where its resin is in OH^- form.

A preferred embodiment for an anion that is used in the anion-exchange is an anion that includes tartarate, succinate, citrate, acetate, formate, malonate, oxalate, phthalate, benzoate, and phosphate.

A preferred embodiment for an cation-exchanger is a strong cation-exchanger. The presently most preferred embodiment includes a cation-exchanger that is a strong cation exchange resin in acid form.

According to another embodiment, the present invention employs a cation-exchanger whereby a strong cation-exchanger resin is in calcium form.

According to another embodiment, the particulates present in the fermentation broth are removed. The techniques to remove the particulates includes the sedimentation as well as filtering as one of skill in the art would appreciate. Fermentation broth containing acarbose can be filtered prior to the application onto the cation-exchangers. The filtration of fermentation broth removes any particulates and cell debris. Preferably, the filter is a pre-coat vacuum drum filter. One skilled in the art would appreciate the use of other filters of a similar kind and can serve a similar function as to pre-clear the fermentation broth prior to the chromatography purification. Most preferably, the filtration of fermentation broth is repeated at least twice.

According to another embodiment, the fermentation broth containing acarbose is adjusted to an acidic pH prior to filtration. Preferably, prior to the first filtration, the pH of the fermentation broth is adjusted to a pH of about 4.0 to a pH of about 6.0 with a mineral acid or a weak acid.

A "mineral acid" is defined herein as a strong acidic solution such as hydrochloric acid, sulphuric acid, nitric acid, phosphoric acid and the like.

A "weak acid" is selected from the group consisting of tartaric acid, succinic acid, citric acid, acetic acid, formic acid, malonic acid, oxalic

acid, phthalic acid, benzoic acid, and phosphoric acid. A preferred embodiment for a weak acid is acetic acid.

According to another embodiment, the present invention relates to a process of purifying acarbose using two ion-exchangers. Preferably, the first ion-exchanger is an anion-exchanger. Most preferably, the first anion-exchanger is in the acetate, tartarate or succinate forms.

Preferably, the second ion-exchanger is a strong cation-exchanger. Most preferably, the second cation-exchanger is a strong cation-exchanger in acid form.

According to another embodiment, the present invention relates to a process of purifying acarbose, wherein acarbose adsorbed onto a cation-exchanger is eluted with either hydrochloric acid or weak acids.

According to another embodiment, the present invention relates to a process of purifying acarbose, wherein acarbose that is adsorbed onto a cation-exchanger is eluted with either a sodium chloride solution or a salt solution of sulfate, nitrate or acetate.

According to another embodiment, the present invention relates to a process of purifying acarbose with an increased yield. Particularly, the invention provides eluting adsorbed acarbose from a cation-exchanger with a salt solution wherein the yield of ion-exchange purification is higher. Typically, the yield is higher than 85%.

According to another embodiment, the present invention relates to a process of purifying acarbose, wherein a solvent is used for the precipitation of acarbose from the eluant. Preferably the solvent includes alcohol, a mixture of alcohols and acetone, acetonitrile, ester of acetic acid, ester of formic acid, ester of propionic acid or the like.

The present invention is described in further detail with reference to the following examples. However, the present invention is by no means restricted to these specific examples.

EXAMPLES

EXAMPLE 1

A fermentation broth of 122 kg was acidified with sulfuric acid to about pH 4.0-4.5. The acidified fermentation broth was filtered on pre-coat vacuum drum filter. The filtered mycelium was washed with water. The fermentation broth contained 537 gram active substance. The filtration yield was 91% (w/w). The volume of the filtrate was 227 liters.

The pH of the acidified filtrate was adjusted to about 2.0-2.2 with sulfuric acid and it was filtered again pre-coat drum filter. The volume of the filtrate was 223 liters. The filtration yield was 94% (w/w).

The pH of the filtrate of about 2.0-2.2 was adjusted to about 4.0-7.0 with anion-exchange resin in basic form. The yield of the pH adjust was 94.5% (w/w).

The adjusted filtrate was poured through on ion-exchange column. The ion-exchange column contained 20 liters anion-exchange resin in acetate form. The flow rate was 12.5 liters/hour. The effluent flow was conducted without desalinating continuously to another ion-exchange column containing 22 liters strong acid cation-exchanger in acid form. The ion-exchange was finished with 50 liters rinsing water.

The active substance that were bound or adsorbed onto the ion-exchange resin was eluted with 0.02 M hydrochloric acid. The eluants were collected into different fractions using a fraction collector. A main fraction of the eluants contained 374 gram active substance. The volume of the main fraction was 37.5 liters.

The summarized yield of the adsorption and elution was 87% (w/w).

The main fraction was analyzed by HPLC. HPLC method was as follows: Supercoil LC-NH_2 column; 5 μM; mobile phase: 1.2 gram KH_2PO_4 and 0.7 gram Na_2HPO_4 in 1,000 mL water; detection: UV2=210 nm. There was less than 10% related substances on HPLC. The pH of the main fraction was adjusted to about 4.0-5.0 with anion-exchange resin in basic form.

EXAMPLE 2

Another purification of acarbose was performed with the following procedures.

A part (480 mL) of the pH adjusted main fraction

was taken for purification. This fraction contained 4.9 gram acarbose.

Two ion-exchange columns connected in series were used.

The first ion-exchange column contained 60 ml anion-exchange resin in tartarte form. The second column contained 60 ml strong acid cation-exchanger in acid form. The applied flow rate was 40 ml/hour. The ion-exchange was finished with 120 mL rinsing water.

The adsorbed active substance was eluted from the second column with 0.02 M hydrochloric acid. The main fraction contained 4.4 gram acarbose. The main fraction was analyzed by HPLC. There were less than 2% related substances on the HPLC chromatogram. The main fraction was concentrated after removing chloride ions with anion exchange resin in basic form. The concentration of acarbose was about 50% (w/w).

The acarbose was precipitated in the presence of ethanol. The crystals were filtered and dried. The 4 gram product contained less than 1% related substances.

EXAMPLE 3

Another purification of acarbose was performed with the following procedures.

A part (480 mL) of the pH adjusted main fraction (final solution of Example 1) was taken for purification. This part contained 4.8 gram acarbose.

Two ion-exchange columns connected in series were used.

The first ion-exchange column contained 60 mL anion-exchange resin in succinate form. The second column contained 60 mL strong acid cation-exchanger in acid form. The applied flow rate was 40 mL/hour. The ion-exchange was finished with 120 mL rinsing water.

The adsorbed active substance was eluted from the second column with 0.02 M hydrochloric acid. The main fraction contained 4.3 grams acarbose. The main fraction was analyzed with HPLC analysis method. There were less than 2% related substances on the HPLC chromatogram. The main fraction was concentrated after removing chloride ions with anion exchange resin in basic form. The concentration of acarbose was about 50% by w/w.

The acarbose was precipitated in the presence of ethanol. The crystals were filtered and dried. The 3.9 gram product contained less than 1% related substance.

EXAMPLE 4

The purification of acarbose illustrated in the above-mentioned Example 1 were using strong ion-exchanger in the presence of an anion of weak acids such as acetate, tartarte or succinate.

We found that other anion of weak acids can also influence the purification of acarbose during the ion-exchange chromatography. Table 1 summarizes the

comparison of the efficiency of other anion of weak acids. Before the step of adsorbing acarbose onto the cation-exchanger, an anion exchanger was used to change the anion content of the filtrate from an existing anion (a stronger anion such as sulphate, chloride, nitrate and the like) to an anion of a weak acid.

Optimal effects of other anion of weak acids on the cation-exchange chromatography in acarbose purification is seen in Table 1.

EXAMPLE 5

A fermentation broth of 60 kg was acidified with acetic acid to pH about 4.0-6.0. Acid was added to fermentation broth and mixed. The acidified fermentation broth was filtered on pre-coat vacuum drum filter. The filtered mycelium was washed with water. The fermentation broth contained 160 gram active substance. The filtration yield was 91% (w/w) using a HPLC method. The volume of the filtrate was 88 litres.

The filtrate was poured through on ion-exchange column. The ion-exchange column contained 8 litres strong acid cation-exchanger in acid form (Amberlite® 252 in H^+ form). The ion-exchange was finished with 8 litres rinsing water.

The active substance that were bound or adsorbed onto the ion-exchange resin was eluted with 0.02 M hydrochloric acid. The flow-rate was 1 litre/hour. Preferred solution is hydrochloric acid. Preferred concentration is 0.0002 M - 0.03 M. Most preferred concentration is 0.005 M - 0.02 M. The eluants were

collected into different fractions using a fraction collector. A main fraction of the eluants contained 124 gram active substance.

The yield of ion-exchange purification process was 85% w/w as determined by HPLC.

The main fraction was analyzed by HPLC. Acarbose had a purity of 94.5 area %. There were less than 10% impurity content. The details of HPLC were as follows: HPLC column used: Supercosil LC-NH_2; particle size: 5μM; length: 250mm; diameter: 4.6 mm; mobile phase: 1.2 gram KH_2PO_4 and 0.7 gram Na_2HPO_4 in 1,000 mL water (pH: 6.5); injection volume: 20μL; and detection: UV2=210nm.

EXAMPLE 6

A fermentation broth of 150 kg was acidified with acetic acid to pH about 4.0 to about 6.0. Acid was added to fermentation broth and mixed. The acidified fermentation broth was filtered on pre-coat vacuum drum filter. The filtered mycelium was washed with water. The fermentation broth contained 927 gram active substance. The filtration yield was 89% (w/w) using a HPLC method. The volume of the filtrate was 246 liters.

The filtrate was poured through on ion-exchange column. The ion-exchange column contained 25 liters strong acid cation-exchanger in acid form. The flow rate was 9 liters/hour. The ion-exchange was finished with 40 liters rising water.

The active substance that was bound or adsorbed

onto the cation-exchange resin was eluted with 0.002 M sodium chloride (NaCl) solution for 2 days, then with 0.1 M NaCl solution for 20 hours. The eluants were collected into different fractions using a fraction collector. A main fraction of the eluants contained 685 gram active substance.

The yield of ion-exchange purification process was 83.0% (w/w) as determined by HPLC.

The main fraction was analyzed by HPLC. Acarbose had a purity of 96 area %.

It will be appreciated that the instant specification and claims are set forth by way of illustration and not limitation, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

Table 1

ACARBOSE

Effect of anions of a weak acid on cation-exchange

(Amberlite® 252 resin in H^+ form, 15 cm resin height, eluant : 0.02 N HCl in each case, all fractions were combined)

Anion of a weak acid	Borate	Tartarate	Succinate	Citrate	Acetate	Formate	Maleinate	Malonate	Oxalate
Sample name of solution containing anion	292/376	292/377	292/378	292/379	292/380	292/381	292/382	292/383	292/384
Acarbose (area %) in the solution containing anions of weak acids	9.675	11.93	8.887	10.3	10.254	10.597	1.633	6.89	7.351
Yield of anion exchanger (%)	97.0	94.9	-	95.4	96.2	100.0	99.5	100.0	100.0
Sample name of combined fractions after cation-exchange	289/983	298/984	289/985	289/986	289/987	289/988	289/989	289/990	289/991
Acarbose (area %) in the combined fractions after cation-exchange	31.15	76.95	74.486	68.395	71.280	70.102	7.639	55.437	68.[illegible]
Yield of cation-exchanger (%)	7.3	82.9	-	63.4	88.2	69.1	5.9	31.0	33.[illegible]
Summarized yield of the two steps (%)	7.1	78.6	74.2	60.5	84.8	69.1	5.8	31.0	33

Table 1

ACARBOSE

Effect of anions of a weak acid on cation-exchange

(Amberlite® 252 resin in H^+ form, 15 cm resin height, eluant : 0.02 N HCl in each case, all fractions were combined)

Anion of a weak acid	Phthalate	Benzoate	Chloride	Phosphate	Sulfate	Nitrate			
Sample name of solution containing anions	289/1001	289/1002	289/1003	289/1004	289/1005	289/1006			
Acarbose (area %) in the solution containing investigated anion	1.609	2.157	12.246	12.992	11.942	1.856			
Yield of anion exchanger (%)	95.8	93.9	97.3	99.1	-	97.9			
Sample name of combined fractions after cation-exchange	292/412	292/413	292/414	292/415	292/416	292/417			
Acarbose (area %) in the combined fractions after cation-exchange	34.045	80.412	51.773	69.896	57.819	57.443			
Yield of cation-exchanger (%)	49.4	appr. 85-90	9.3	27.8	-	6.9			
Summarized yield of the two steps (%)	47.3	80-85	9.1	27.5	9.2	6.8			

WHAT IS CLAIMED IS:

1. A process for purifying acarbose, comprising the steps of:
1) acidifying a fermentation broth containing an acarbose;
2) removing particulates from the fermentation broth;
3) adsorbing the acarbose on a cation-exchanger in the presence of an anion of a weak acid;
4) eluting the acarbose from the cation-exchanger with at least one of a sodium chloride solution and a salt solution;
5) precipitating the acarbose with a solvent; and
6) recovering the precipitated acarbose.

2. The process of claim 1, wherein the fermentation broth is acidified to a pH about 4 to about 6.

3. The process of claim 1, wherein the fermentation broth is acidified to a pH about 5.

4. The process of claim 1, wherein the fermentation broth is acidified with a weak acid.

5. The process of claim 4, wherein the weak acid is acetic acid.

6. The process of claim 4, wherein the weak acid is selected from the group consisting of tartaric acid, succinic acid, citric acid, formic acid, malonic acid, oxalic acid, phthalic acid, benzoic acid, phosphoric acid and the derivatives thereof.

7. The process of claim 1, wherein the particulates

are removed with a filter.

8. The process of claim 1, wherein the filter is pre-coat vacuum drum.

9. The process of claim 1, wherein the cation-exchanger is a strong acid cation-exchanger.

10. The process of claim 9, wherein the strong acid cation-exchanger is a resin in acid form.

11. The process of claim 1, wherein the anion of a weak acid is selected from the group consisting of tartarate, succinate, citrate, acetate, formate, malonate, oxalate, phthalate, benzoate, phosphate and the derivatives thereof.

12. The process of claim 1, wherein the acarbose is eluted from the cation-exchanger with a sodium chloride solution.

13. The process of claim 12, wherein the sodium chloride solution has a concentration of about 0.002 M to about 0.03 M.

14. The process of claim 12, wherein the sodium chloride solution has a concentration of about 0.005 M to about 0.02 M.

15. The process of claim 1, wherein the acarbose is eluted from the cation-exchanger with a salt solution selected from the group consisting of sodium chloride, potassium chloride and calcium chloride.

16. The process of claim 1, wherein the acarbose is eluted from the cation-exchanger with a salt solution selected from the group consisting of sodium sulfate, potassium sulfate and calcium sulfate.

17. The process of claim 1, wherein the acarbose is eluted from the cation-exchanger with a salt solution selected from the group consisting of sodium nitrate, potassium nitrate and calcium nitrate.

18. The process of claim 1, wherein the acarbose is eluted from the cation-exchanger with a salt solution selected from the group consisting of sodium acetate, potassium acetate and calcium acetate.

19. The process of claim 1, wherein the solvent used for precipitation is selected from the group consisting of alcohols, mixture of alcohols, acetone, acetonitrile, ester of acetic acid, ester of formic acid and ester of propionic acid.

20. A process for purifying acarbose, comprising the steps of:
1) acidifying a fermentation broth containing an acarbose;
2) removing particulates from the fermentation broth;
3) adsorbing the acarbose on an anion-exchanger in the presence of an anion of a weak acid;
4) eluting the acarbose from the anion-exchanger;
5) adsorbing the eluted acarbose on a cation-exchanger in the presence of the anion of a weak

acid;

6) eluting the acarbose from the cation-exchanger with at least one of a sodium chloride solution and a salt solution;

7) precipitating the acarbose with a solvent; and

8) recovering the precipitated acarbose.

21. Pure acarbose as prepared in accordance with the process of claim 1.

22. A pharmaceutical formulation comprising pure acarbose as prepared in accordance with the process of claim 1, wherein the acarbose has a purity of at least about 94%.

23. A pharmaceutical formulation comprising pure acarbose as prepared in accordance with the process of claim 1, wherein the acarbose has a purity of at least about 96%.

24. Pure acarbose as prepared in accordance with the process of claim 20.

25. A pharmaceutical formulation comprising pure acarbose as prepared in accordance with the process of claim 20, wherein the acarbose has a purity of at least about 94%.

26. A pharmaceutical formulation comprising pure acarbose as prepared in accordance with the process of claim 20, wherein the acarbose has a purity of at least about 96%.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/02705

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07H 15/00, 17/00; C12P 19/28
US CL : 536/17.2; 435/85
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)
U.S. : 536/17.2; 435/85

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 practicable, search terms used)
USPAT, US-PGPUB, EPO, JPO, DERWENT; search terms: acarbose, acetic, strong acid, acid, acidify

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 01/30796 A1 (CHONG KUN DANG PHARMACEUTICAL ORP.) 03 May 2001(03.05.01), see entire document.	20-26 ---------- 1-19
X --- Y	WO 99/077720 A2 (UNIVERSITY OF MASSACHUSETTS LOWELL) 18 February 1999(18.02.99), see entire document, especially page 4, lines 7-15.	20-26 ---------- 1-19

☐ 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 application or patent 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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2002 (14.06.2002)	08 JUL 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Francisco C Prats Telephone No. 703-308-0196

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