CRUDE EXTRACTS FROM ANDROGRAPHIS PANICULATA

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Notice: This patent is subject to a terminal disclaimer.

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Related U.S. Patent Documents

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Division of application No. 12/717,260, filed on Mar. 4, 2010, now Pat. No. Re. 42,718.

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U.S. Cl.: 424/725

Field of Classification Search: None

References Cited

U.S. PATENT DOCUMENTS

6,258,526 B1 3/2002 Mergens et al.
7,625,945 B2 12/2009 Yan et al.

FOREIGN PATENT DOCUMENTS

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Communication pursuant to Article 94(3) EPC in European Patent Application No. 05742174.5, dated Sep. 27, 2011.


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ABSTRACT

This invention relates to a method of inhibiting TNFα or IL-1β expression with an extract of Andrographis paniculata. The extract contains andrographolide, 14-deoxy-andrographolide, 14-deoxy-11,12-dehydrogen-andrographolide, and neoandrographolide.

6 Claims, No Drawings
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Ex parte Subramanyam (BPAI, Mar. 29, 2010).


Communication pursuant to Article 94(3) EPC in EP 1747008, dated Feb. 24, 2011.


English translation of JP 2000034233 A—2000.*


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CRUDE EXTRACTS FROM ANDROGRAPHIS PANICULATA

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

CROSS REFERENCE TO RELATED APPLICATIONS

[Pursuant to 35 USC § 119(e), this application claims priority to U.S. Provisional Application Ser. No. 60/566,477, filed Apr. 28, 2004, the contents of which are incorporated herein by reference.] Notice: More than one reissue application has been filed for the reissue of U.S. Pat. No. 7,341,748. The reissue applications are reissue application Ser. No. 12/717,260, and the present application filed herewith. This application is a divisional reissue application of reissue application Ser. No. 12/717,260, filed Mar. 4, 2010, now U.S. Pat. No. Re. 42,718, which is a broadening reissue application of U.S. Pat. No. 7,341,748, which issued on Mar. 11, 2008, from U.S. application Ser. No. 11/116,678, filed Apr. 27, 2005, and claims the benefit of U.S. Provisional Application No. 60/566,477, filed Apr. 28, 2004, all of which are incorporated herein by reference.

BACKGROUND


SUMMARY

This invention is based on a surprising discovery that an extract of Andrographis paniculata inhibits expression of both TNFα and IL-1β. The extract, obtained from the aerial part of Andrographis paniculata, contains andrographolide, 14-deoxy-andrographolide, 14-deoxy-11,12-dehydroandrographolide, and neandrographolide. Preferably, the extract contains 2-20% by weight andrographolide, 1-6% by weight 14-deoxy-andrographolide, 1-12% by weight 14-deoxy-11,12-dehydroandrographolide, and 1-5% by weight neandrographolide. More preferably, the extract contains 3-8% by weight andrographolide, 3-5% by weight 14-deoxy-andrographolide, 7-9% by weight 14-deoxy-11,12-dehydroandrographolide, and 2-4% by weight neandrographolide. It is particularly preferred that the extract contain 4.2% by weight andrographolide, 4.4% by weight 14-deoxy-andrographolide, 8% by weight 14-deoxy-11,12-dehydroandrographolide, and 2.1% by weight neandrographolide.
One aspect of this invention relates to a method of inhibiting expression of TNFα or IL-1β in a subject. The method includes administering to the subject an effective amount of the above-described extract.

Another aspect of this invention relates to a method of treating a disorder related to TNFα or IL-1β, i.e., inflammatory bowel disease (including Crohn’s disease and ulcerative colitis), chronic heart failure, diabetes mellitus, systemic lupus erythematosus, polymyositis/denmyelositis, psoriasis, acute myelogenous leukemia, AIDS dementia complex, hematosepsis, septic shock, graft-versus-host disease, uveitis, asthma, acute pancreatitis, or periodontal disease. The method includes administering to a subject in need of the treatment an effective amount of the above-described extract.

Also within the scope of this invention is a composition containing the extract of this invention described above for use in treating TNFα related disorders and IL-1β related disorders as well as the use of such a composition for the manufacture of a medicament for treating these disorders. Details of several embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description, and also from the claims.

DETAILED DESCRIPTION

This invention includes methods of inhibiting expression of TNFα or IL-1β, treating a TNFα-related disorder, and treating an IL-1β-related disorder by administering to a subject in need thereof an effective amount of the above-described extract. The term “an effective amount” refers to the amount of the extract which is required to confer one of the above-described effects in the subject. Effective amounts may vary, as recognized by those skilled in the art, depending on parameters such as administration route, dosage, and the possibility of co-administration with other agents. The term “treatment” refers to administering the extract to a subject that has a TNFα-related disorder or an IL-1β related disorder, or has a predisposition toward the disorder, with the purpose of curing, healing, alleviating, relieving, altering, remediating, ameliorating, improving, or affect the disorder, the symptoms of the disorder, or the predisposition toward the disorder.

To prepare an extract for use in this invention, one can immerse the aerial parts of Andrographis paniculata in one or more suitable solvents, e.g., ethanol, methanol, and acetone; separate the liquid from the solid residue; and concentrate the liquid. The extract thus obtained may be further processed. For example, one can remove impurities or modify the ratio of the components by chromatography.

To practice one of the above-described methods, one administers to a subject in need thereof orally, rectally, parenterally, by inhalation spray, or via an implanted reservoir a composition that is either the above-mentioned extract alone or a mixture of the extract and a pharmaceutically acceptable carrier. The term “parenteral” as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intracuticular, intrarterial, intrasynovial, intradermal, intrathecal, intraleisonal and intracranial injection or infusion techniques.

An oral composition can be any orally acceptable dosage form including, but not limited to, tablets, capsules, emulsions and aqueous suspensions, dispersions and solutions. Commonly used carriers for tablets include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added to tablets. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions or emulsions are administered orally, the active ingredient can be suspended or dissolved in an oily phase combined with emulsifying or suspending agents. If desired, certain sweetening, flavoring, or coloring agents can be added.

A sterile injectable composition (e.g., aqueous or oleaginous suspension) can be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that can be employed are mannitol, water, Ringer’s solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium (e.g., synthetic mono- or di-glycerides). Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents.

An inhalation composition can be prepared according to techniques well known in the art of pharmaceutical formulation and can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

A topical composition can be formulated in form of oil, cream, lotion, ointment and the like. Suitable carriers for the composition include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohols (greater than C12). The preferred carriers are those in which the active ingredient is soluble. Emulsifiers, stabilizers, humectants and antioxidants may also be included as well as agents imparting color or fragrance, if desired. Additionally, transdermal penetration enhancers may be employed in these topical formulations. Examples of such enhancers can be found in U.S. Pat. Nos. 3,989,816 and 4,444,762. Creams are preferably formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which mixture the active ingredient, dissolved in a small amount of an oil, such as almond oil, is dispersed. An example of such a cream is one which includes about 40 parts water, about 20 parts beeswax, about 40 parts mineral oil and about 1 part almond oil. Ointments may be formulated by mixing a solution of the active ingredient in a vegetable oil, such as almond oil, with warm soft paraffin and allowing the mixture to cool. An example of such an ointment is one which includes about 30% almond and about 70% white soft paraffin by weight.

A carrier in a pharmaceutical composition must be “acceptable” in the sense of being compatible with the active ingredient of the formulation (and preferably, capable of stabilizing it) and not deleterious to the subject to be treated. For example, solubilizing agents, such as cyclodextrins (which form specific, more soluble complexes with one or more of active compounds of the extract), can be utilized as pharmaceutical excipients for delivery of the active compounds. Examples of other carriers include colloidal silicon dioxide, magnesium stearate, cellulose, sodium lauryl sulfate, and D&C Yellow #10.

A suitable in vitro assay can be used to preliminarily evaluate the efficacy of the above-described extract in inhibiting expression of TNFα or IL-10 expression. The extract can further be examined for its efficacy in treating a TNFα related disorder or an IL-1β related disorder by in vivo assays. For
example, the extract can be administered to an animal (e.g., a mouse model) having a TNFα or IL-1β related disorder and its therapeutic effects are then assessed. Based on the results, an appropriate dosage range and administration route can also be determined.

Without further elaboration, it is believed that the above description has adequately enabled the present invention. The following specific examples are, therefore, to be construed as merely illustrative, and not exhaustive of the remainder of the disclosure in any way whatsoever. All of the publications, including patents, cited herein are hereby incorporated by reference in their entirety.

Preparation of an Extract of Andrographis paniculata

Dried powder of the aerial part of Andrographis paniculata (1 kg) was suspended in 85% ethanol. The suspension was refluxed for two hours and filtered. The residue was extracted with 85% ethanol again. The combined ethanol solutions were cooled and concentrated to afford 105 g of the desired extract. HPLC analysis shows that the extract contained 4.0% andrographolide.

In vitro Assay

An in vitro assay was conducted to evaluate the efficacy of the Andrographis paniculata extract in inhibiting expression of TNFα and IL-1β expression. Peripheral blood monocytes (PBMC) cells were isolated from fresh blood using the Ficoll-Paque Plus (Amersham Bioscience) according to the protocol recommended by the manufacturer. The cells were suspended in RPMI 1640 media containing 10% FBS at a concentration of 1x10^6 cells/ml and seeded in a 96-well plate (1x10^5 cells total in each well). Each reaction was carried out in three wells.

10 µl of the Andrographis paniculata extract in DMEM was added into each well (final concentrations: 0.1, 0.3, 1, 3, 10, and 30 µg/ml). Wells containing dexamethasone (Cal-Biochem.) at the final concentration of 10 µM were used as positive control. Wells containing 10 µl of the media were used as negative control. The plate was incubated at 37° C. under 5% CO₂ for 15 minutes. After 10 µl aliquots of 100 µg/ml lipopolysaccharide were added to all wells except for the negative control, the plate was incubated at 37° C. under 5% CO₂ overnight.

The plate was spun at 1000 rpm for 15 minutes and the supernatants were collected. Concentrations of TNFα and IL-1β were measured using the TNFα ELISA (Enzyme Linked Immunosorbent Assay) Kit and IL-1β ELISA Kit (Jingmei Biotechnology).

The inhibition ratio was calculated as follows:

\[
\text{Inhibition Ratio (\%)} = \left(1 - \frac{C_{\text{LPS}} - C_{\text{Control}}}{C_{\text{LPS}} - C_{\text{Control}}} \right) \times 100
\]

where \(C_{\text{extract}}\) is the concentration of TNFα or IL-1β in PBMC cells treated with the extract and LPS, \(C_{\text{LPS}}\) is the concentration of TNFα or IL-1β in PBMC cells treated with LPS and dexamethasone, and \(C_{\text{Control}}\) is the concentration of TNFα or IL-1β in PBMC cells without being treated with LPS or the extract.

The results show that the extract significantly inhibited expression of both TNFα and IL-1β.

In vivo Assays

In vivo assays were conducted to evaluate the efficacy of the Andrographis paniculata extract in treating inflammatory bowel disease (IBD). Balb/c male mice (18-24 g) were anaesthetized with 1% pentobarbital sodium at 0.05 mg/10 g. To induce IBD, 1.5 mg of 2,4,6-trinitrobenzenesulfonic acid (TNBS; Sigma) in 50% ethanol was administered slowly to each mouse (except blank control mice) via a catheter. Blank control mice only received 0.1 ml of 50% ethanol. The mice were treated with the extract of Andrographis paniculata 24 hours and 2 hours prior to the TNBS administration and daily for 5 days after the administration. The body weight of each mouse was monitored every day before and after the TNBS administration. The mice were sacrificed 24 hours after the last administration of the extract. Colonos were removed and weighed. Furthermore, the colon weight to body weight ratio was calculated and adhesion between colon and other organs was also monitored.

Samples of colon tissues located precisely 2 cm above the anal canal were obtained, fixed in 10% buffered phosphate, embedded in paraffin, sectioned, and stained with hematoxylin/eosin. The degree of inflammation on microscopic cross sections was graded from 0 to 4 (0: no signs of inflammation; 1: a very low level of inflammation; 2: a low level of leukocyte infiltration; 3: a high level of leukocyte infiltration, a high vascular density, and a thickened colon wall; and 4: transmural infiltrations, loss of goblet cells, a high vascular density, and a thickened colon wall).

The results show that when mice were treated with 150 mg/kg TNBS alone, they had severe illness characterized by diarrhea, profound and sustained weight losses, a significant increase of the colon weight to body weight ratio, and a mortality rate of 50%. Macroscopic examination indicates that the colon of each of mice had transmural inflammation in all layers of the bowel wall. In contrast, when mice were treated with the extract of Andrographis paniculata (500 mg/kg/day) prior to the induction of IBD, they had a reduced overall mortality rate, less severe wasting syndrome, a lower colon weight to body weight ratio, and a lower IBD score. The bowel wall was sleek and was not adhesive with surrounding tissues.

In a separate assay, male Wistar rats were used to evaluate the efficacy of the Andrographis paniculata extract in treating IBD following a procedure similar to that described above. To induce IBD, the rats were administered with 2,4-dinitrobenzenesulfonic acid, instead of TNBS.

Similar results were obtained. Specifically, rats treated with the Andrographis paniculata extract had a reduced overall mortality rate, less severe wasting syndrome, a lower colon weight to body weight ratio, and a lower IBD score, compared with those not treated with the extract.

OTHER EMBODIMENTS

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are also within the scope of the following claims.

What is claimed is:

1. A method of treating an inflammatory bowel disease in a subject in need thereof, comprising administering to the subject an effective amount of an extract of Andrographis paniculata, wherein the extract contains 2-20% by weight andrographolide, 1-6% by weight 14-deoxy-andrographolide, 1-12% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neoandrographolide.

2. The method of claim 1, wherein the extract contains 3-8% by weight andrographolide, 3-5% by weight 14-deoxy-andrographolide, 7-9% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 2-4% by weight neoandrographolide.
3. The method of claim 2, wherein the extract contains 4.2% by weight andrographolide, 4.4% by weight 14-deoxy-andrographolide, 8% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 2.1% by weight neandrographolide.

4. The method of claim 1, wherein the inflammatory bowel disease is Crohn's disease.

5. The method of claim 1, wherein the inflammatory bowel disease is ulcerative colitis.

6. The method of claim 5, wherein the extract contains 3-8% by weight andrographolide, 3-5% by weight 14-deoxy-andrographolide, 7-9% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 2-4% by weight neandrographolide.

7. The method of claim 6, wherein the extract contains 4.2% by weight andrographolide, 4.4% by weight 14-deoxy-andrographolide, 8% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 2.1% by weight neandrographolide.

8. An extract of Andrographis paniculata for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising: 2-20% by weight andrographolide, 14-deoxy-andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and further comprising in said extract for treating effectively an inflammatory bowel disease, 1-5% by weight neandrographolide.

9. The extract of claim 8, wherein said extract comprises 3-8% by weight andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 2-4% by weight neandrographolide.

10. An extract of Andrographis paniculata, for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising: 2-20% by weight andrographolide, 14-deoxy-andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and further comprising in said extract for treating effectively an inflammatory bowel disease, 1-5% by weight neandrographolide.

11. The extract of claim 10, wherein said extract comprises 3-8% by weight andrographolide, 14-deoxy-andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 2-4% by weight neandrographolide.

12. A pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising an extract of Andrographis paniculata, comprising: 2-20% by weight andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neandrographolide and further comprising a pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, a pharmaceutically acceptable carrier.

13. A pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising an extract of Andrographis paniculata, comprising: 2-20% by weight andrographolide, 14-deoxy-andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neandrographolide and further comprising a pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, a pharmaceutically acceptable carrier.
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In claim 12, column 8, lines 16-19 “comprising a pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,” should read --comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,— As corrected, claim 12 would read:

12. A pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising an extract of *Andrographis paniculata*, comprising: 2-20% by weight andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neoandrographolide and further comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, a pharmaceutically acceptable carrier.

In claim 13, column 8, lines 26-28 “comprising a pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,” should read --comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,— As corrected, claim 13 would read:

13. A pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising an extract of *Andrographis paniculata*, comprising: 2-20% by weight andrographolide, 14-deoxy-andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neoandrographolide and further comprising in said pharmaceutical

Signed and Sealed this
Fifth Day of March, 2013

Teresa Stanek Rea
*Acting Director of the United States Patent and Trademark Office*
In the Claims:

In claim 12, column 8, lines 16-19 “comprising a pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,” should read --comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,-- As corrected, claim 12 would read:

12. A pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, comprising an extract of *Andrographis paniculata*, comprising: 2-20% by weight andrographolide, 1.0-7% by weight 14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neandrographolide and further comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, a pharmaceutically acceptable carrier.

In claim 13, column 8, lines 26-28 “comprising a pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,” should read --comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof,-- As corrected, claim 13 would read:

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14-deoxy-11,12-dehydrogen-andrographolide, and 1-5% by weight neoandrographolide and further comprising in said pharmaceutical composition for treating effectively an inflammatory bowel disease in a subject in recognized need thereof, a pharmaceutically acceptable carrier.