APPLICATION ACCEPTED AND AMENDMENTS ALLOWED 18.1.91

FORM 1
REGULATION 9

COMMONWEALTH OF AUSTRALIA

609116

PATENTS ACT 1952-1973

APPLICATION FOR A PATENT

I/We SANSHO SEIYAKU CO., LTD.

of 26-7, Oike 2-chome, Onojo-shi, Fukuoka-ken, JAPAN

hereby apply for the grant of a Patent for an invention entitled:

EXTERNAL PREPARATIONS OF MELANOGENESIS-INHIBITORY AGENT

which is described in the accompanying complete specification.

My/Our address for service is:

GRIFFITH HASSEL & FRAZER
71 YORK STREET
SYDNEY N.S.W. 2000
AUSTRALIA

DATED this 20th day of April, 1988.

SANSHO SEIYAKU CO., LTD.

By his/their Patent Attorneys

GRIFFITH HASSEL & FRAZER

TO: THE COMMISSIONER OF PATENTS
COMMONWEALTH OF AUSTRALIA

LODGED AT SUB-OFFICE
20 APR 1988
Sydney

2521A:rk

ASSIGNEE - APPLICANT

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952 (AS AMENDED)

DECLARATION IN SUPPORT OF AN APPLICATION FOR A PATENT

(Name of applicant) In support of an Application made by SANSHO SEIYAKU CO., LTD.

(Title) for a patent for an invention entitled EXTERNAL PREPARATIONS OF MELANOGENESIS-INHIBITORY AGENT

(Full name of signatory) I, Tsuneo Jinnai

(Address of signatory) of 26-7, Oike 2-chome, Onojo-shi, Fukuoka-Ken, JAPAN

do solemnly and sincerely declare as follows:-

I. I am authorised by the abovementioned applicant for the the patent to make this Declaration on its behalf.

2. The name and address of each actual inventor of the invention is as follows:

Yoshitaka Higa

of 754-2, Kokuba, Dazaifu-shi, Fukuoka-ken, JAPAN

and the facts upon which the applicant is entitled to make this application are as follows:

(Insert details of assignment etc.) The applicant is the assignee of the said invention from the said inventor

(Delete paragraphs 3 and 4 for Non-Convention application) 3. The basic application(s) as defined by Section 141 of the Act was (were) made as follows:

Country ..... on .....

in the name(s) .....

and in ..... on .....

in the name(s) .....

and in ..... on .....

and in the name(s) .....

4. The basic applications(s) referred to in the preceding paragraph of this Declaration was(were) the first application(s) made in a Convention country in respect of the invention the subject of this application.

(Place and date of signing) Declared at Fukuoka-ken, JAPAN this 5th day of April, 1988.

Signed: Tsuneo Jinnai

Position: .....

(12) PATENT ABRIDGMENT (11) Document No. AU-B-14776/88
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 609116

(54) Title
EXTERNAL PREPARATIONS OF MELANOGENESIS-INHIBITORY AGENT

International Patent Classification(s)
(51)[4] A61K 035/50 A61K 031/35

(21) Application No. : 14776/88 (22) Application Date : 20.04.88

(43) Publication Date : 26.10.89

(44) Publication Date of Accepted Application : 26.04.91

(71) Applicant(s)
SANSHO SEIYAKU CO., LTD.

(72) Inventor(s)
YOSHITAKA HIGA

(74) Attorney or Agent
GRIFFITH HACK & CO., GPO Box 4164, SYDNEY NSW 2001

(56) Prior Art Documents
AU 36585/88 A61K 31/35
AU 21523/88 A61K 31/35
AU 15410/88 A61K 31/35

(57) Claim

1. An external preparation of melanogenesis-inhibitory agent containing, as active ingredients, a placental extract of pregnant cow and kojic acid or a derivative thereof, wherein the placental extract results from washing the placenta of a pregnant cow with water and removing blood therefrom, followed by extracting with water and wherein the amount of kojic acid or a derivative thereof is 1 to 30% based on the weight of the placental extract of a pregnant cow and the sum of these two active ingredients is 0.1 to 3% based on the total weight of said external preparation.

609116

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952 Form 10

COMPLETE SPECIFICATION

FOR OFFICE USE

Short Title:

Int. Cl:

Application Number:
Lodged:

This document contains the amendments made under Section 49 and is correct for printing.

Complete Specification–Lodged:
Accepted:
Lapsed:
Published:

Priority:

Related Art:

TO BE COMPLETED BY APPLICANT

Name of Applicant: SANSHO SEIYAKU CO., LTD.

Address of Applicant: 26–7, Oike 2–chome, Onojo–shi, Fukuoka–ken, JAPAN

Actual Inventor: Yoshitaka HIGA

Address for Service: GRIFFITH HASSEL & FRAZER
71 YORK STREET
SYDNEY NSW 2000
AUSTRALIA

Complete Specification for the invention entitled:

EXTERNAL PREPARATIONS OF MELANOGENESIS–INHIBITORY AGENT

The following statement is a full description of this invention, including the best method of performing it known to me/us:–

2521A:rk

EXTERNAL PREPARATIONS OF MELANOGENESIS-INHIBITORY AGENT

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to external preparations containing, as active ingredients, a placental extract of pregnant cow and kojic acid or a derivative thereof.

Description of the Prior Art

Peroxides, such as hydrogen peroxide, magnesium peroxide, sodium peroxide and zinc perborate, had long been used as the active ingredient in the so-called bleach-mask cosmetics --- cosmetics to make the human skin fair. But these peroxides, which are very unstable compounds, were poor in storage stability, had difficulty in formulation together with base materials, and were not satisfactory in the intended effect. Cosmetics containing vitamin C, cystein or colloidal sulfur were later introduced for the same purpose, but the effect of these cosmetics was also unsatisfactory. Besides these, are also known bleach-mask cosmetics using kojic acid (Japanese Patent Publication No. 56-18569), and bleach-mask cosmetics using kojic acid derivatives (Japanese Patent Kokai No. 56-79616, No. 56-7710, No. 56-7776 and No. 59-33207). Cosmetics using a placental extract are also known (Japanese Patent Publication No. 35-15399). In addition, bleach-mask cosmetics using a placental extract (from a fully-

matured human placenta, for example) and kojic acid or a derivative thereof have also been disclosed (Japanese Patent Kokai No. 61-202806).

SUMMARY OF THE INVENTION

The object of this invention is to provide external preparations employing a placental extract of pregnant cow which differs from a fully-matured human placenta extract in working mechanism and exceeds in effect as a melanogenesis-inhibitory agent and kojic acid or a derivative thereof, that is better than conventional preparations.

Intensive studies on this subject have led us to find that this object can be achieved by utilizing the synergistic effect between a placental extract of pregnant cow and kojic acid or a derivative thereof. An external preparation containing a placental extract of pregnant cow and kojic acid or a derivative thereof showed an outstanding effect against melanin formation in a test on B16 cells derived from mouse melanoma and in a clinical test. This invention was accomplished on the basis of these findings.

Thus, this invention relates to external preparations containing, as active ingredients, a placental extract of pregnant cow and kojic acid or a derivative thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the infrared absorption spectrum of the placental extract of pregnant cow, which is an active ingredient of the external preparations of this invention.

Figure 2 is the ultraviolet absorption spectrum of the same.

Figure 3 is a graph illustrating the tyrosinase isozyme activity of the same.

Figure 4 is a graph illustrating the tyrosinase isozyme activity of an extract of fully-matured human placenta.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The placental extract of pregnant cow used in this invention is an aqueous solution, or a freeze-dried product thereof, which is obtained by taking out the placenta from a pregnant cow, washing it with water, removing the blood, chopping the washed placenta into small pieces (the resulting debris may be stored in frozen state, when desired), extracting the soluble debris thus obtained with water, and removing impurities from the aqueous extract.

Extraction can be effected, for example, according to the following procedure: cutting the placenta into small pieces, followed by removal of the blood; mechanically disrupting the cut pieces by means of a mixer or the like; and extracting the debris thus

obtained with water at about 40 to 45°C for 3 to 5 hours, followed by centrifugal separation. The crude placental extract is adjusted to pH of 6.4 to 5 and heated at 50 to 60°C for about 10 minutes, the precipitate which separates out is filtered off through a sheet of filter paper, and the filtrate is adjusted to pH 6.0 to 7.5.

Manufacturing Example

Placentas of pregnant cows were washed with water, cut into pieces about 1 cm square, squeezed in water to remove the blood, and dewatered thoroughly. The pieces thus treated (40.00 Kg) were further cut into smaller fragments, 80.0 liters of pure water and 240 g of methyl p-oxybenzoate were added, the mixture was treated in a mixer, and the pulpy suspension thus formed was heated at 40 to 45°C for three hours with gentle stirring to effect extraction. The extract thus obtained was centrifuged at 8000 rpm, the supernantant collected was adjusted to pH 4.5 and heated at 50°C for ten minutes with stirring to precipitate proteins having isoelectric points at this pH, which were filtered off through a sheet of filter paper. The filtrate was adjusted to pH 7.0 by addition of 10% caustic soda solution, and the mixture was again filtered, giving 80 Kg of placental extract.

The placental extract of pregnant cow thus obtained shows unique melanogenesis-inhibitory action

different in working mechanism from that of the known extract of fully-matured human placenta. It is a melanogenesis-inhibitory substance with a nitrogen content of 105 mg/100ml and having IR and UV absorption spectra as shown in Figures 1 and 2, respectively.

Tests on its melanogenesis-inhibitory action and the results obtained are described below.

(Test on Melanogenesis-inhibitory Action)

The placental extract of pregnant cows prepared in the above Manufacturing Example was dissolved in Eagle's MEM medium containing 10% fetal bovine serum, and B16 mouse melanoma cells (hereinafter abbreviated as B16 cells) were grown in this culture medium. After five days of culture, the cells lost color almost completely. Observation of these color-faded B16 cells under an optical microscope revealed a decreased number of cells positive to the dopa reaction and to the premelanosome reaction, while electron-microscopic observation showed a markedly decreased number of melanin-developed melanosomes and a large number of morphologically changed premelanosomes. In a recovery test, in which the color-faded cells were cultured in a medium not containing the placental extract of pregnant cow for five days, premelanosome recovered favorably as observed under an electron microscope, and was restored completely after five days.

The tyrosinase isozyme activity of the above B16 cells cultured on a medium containing the placental

extract of pregnant cow gradually diminished during the culture period. The tyrosinase isozyme activity in electrophoresis diminished for all the types of isozyme, T_1, T_2 and T_3.

Tyrosinase is an important enzyme participating in the formation of melanin and is present in living bodies as three types of isozyme, T_1, T_2 and T_3. It is first created as type-T_2 isozyme, then modified to type T_1 and to type T_3 in that order, and finally present in granules in which melanin is formed (premelanosomes) as type T_3.

As described above, the melanogenesis-inhibitory action of the placental extract of pregnant cow is characterized by the activity of tyrosinase activity generally diminishing with the passage of time for all of the types, T_1, T_2 and T_3.

On the other hand, a similar test was conducted on the melanogenesis-inhibitory action of the known extract of fully-matured human placenta against B16 cells by the same method as above. Electron-microscopic observation showed a decreased number of premelanosomes. The test of tyrosinase activity of B16 cells cultured in a medium containing the above extract revealed a sharp decrease in the activity of type-T_2 isozyme in the earlier stage of cultivation, followed by a decrease in the activity of isozymes T_1 and T_2 in the later stage.

These test results are shown in Figure 3 (placental extract of pregnant cow) and in Figure 4 (extract of fully-matured human placenta).

It is clear from the above results that a placental extract of pregnant cow and a per se known extract of fully-matured human placenta are distinct from each other in the melanogenesis-inhibitory action.

As examples of the kojic acid derivatives used in this invention, may be its fatty acid monoesters such as kojic acid monocaprylate and kojic acid monostearate mentioned (Japanese Patent Kokai No. 56-77272); its fatty acid diesters such as kojic acid dipalmitate, kojic acid dioleate and kojic acid distearate (Japanese Patent Kokai No. 56-7776); and other types of esters such as kojic acid monocinnamate and kojic acid monobenzoate (Japanese Patent Kokai No. 59-33207).

The mixing ratio of the placental extract of pregnant cow to kojic acid or a derivative thereof is most preferably such that the amount of the latter is 1 to 30% based on the weight of the former.

The sufficient amounts of these two active ingredients to be contained in the external preparations of this invention are preferably in the range from 0.1 to 10 weight % and in the range from 0.1 to 3 weight %, respectively, based on the total weight of the preparation.

The external preparations of this invention are principally applied in the form of emulsions, lotions and ointment, but may also be used as cosmetics, such as lotions, emulsions, cream and packs. These may be

AUSTRALIAN PATENT OFFICE

prepared by commonly employed methods using ordinary bases and additives.

Described below are test examples to show the melanogenesis-inhibitory action of the external preparations of this invention.

(1) Test using Bl6 cells

Bl6 cells were added to each of the media listed below and cultured for six days.

(Zone A-I) A medium containing 1/8 the volume of the placental extract of pregnant cow prepared in Manufacturing Example

(Zone A-II) A medium containing 0.5mM kojic acid

(Zone A-III) A medium containing 0.5mM kojic acid and 1/8 the volume of the placental extract of pregnant cow prepared in Manufacturing Example

(Zone A-IV) A medium containing 1/4 the volume of the placental extract of pregnant cow prepared in Manufacturing Example

(Zone A-V) A medium containing 1.0mM kojic acid

(Zone A-VI) A medium containing 1.0mM kojic acid and 1/4 the volume of the placental extract of pregnant cow prepared in Manufacturing Example

The grown cells in each zone were dispersed by the addition of trypsin and collected by centrifugation (1000 rpm × 5 minutes), and the degree of melanism was visually judged. The result is shown in Table 1.

Table 1

Test Zone	A-I	A-II	A-III	A-IV	A-V	A-VI
Degree of melanism	±	±	+++	±~+	±~+	+++

-: Black (control), ±: Slightly faded

+: Moderately faded, ++: Considerably faded

+++: Decolorized almost completely

The above test results clearly show that the external preparations of this invention are highly effective in fading the color of B16 melanoma cells.

Melanin formation is considered to follow the steps of Tyrosine → DOPA → Dopaquinone → Dopachrome → Melanin, in which tyrosinase participates in the reactions of Tyrosine → DOPA → Dopaquinone. Kojic acid and derivatives thereof retard the action of tyrosinase, and the placental extract of pregnant cow retard the formation of tyrosinase in cells, thereby inhibiting melanogenesis. In this way, the two kinds of active ingredients in the external preparations of this invention synergistically inhibit melanin formation through different working mechanisms.

(2) Coating test on human skin

A coating test was conducted at three hospitals (A, B and C) on patients suffering from liver spots (25 persons in each) using the ointment of this invention.

Final evaluation was made on the basis of visual observation three months later. The result is as shown below.

(Evaluation standard)

Ineffective: No color fading observed

Slightly effective: Slight color fading

Effective: Apparent color fading

Markedly effective: Complete or almost complete cure

(Test result)

In total 75 patients; "Ineffective": 6 patients, "Slightly effective": 18 patients, "Effective": 36 patients, "Markedly effective": 15 patients.

Sum of "Slightly effective", "Effective" and "Markedly effective": 69 patients (Efficacy rate: 92%).

As is apparent from the foregoing, the placental extract of pregnant cow contained in the external preparations of this invention as an active ingredient is a very effective melanogenesis-inhibitory substance, which acts through a mechanism different from that of the known extract of fully-matured human placenta and markedly diminishes the overall activity of tyrosinase isozymes (T_1, T_2 and T_3) that are enzymes essential to the formation of melanin in living bodies. The external preparations of this invention, which utilize the synergistic effect between this unique substance and

kojic acid or a derivative thereof (a known melanogenesis-inhibitory substance), are effective for prohibiting remarkably generation of melanin and preventing from pigmentation diseases, such as liver spots by application of external preparation in this invention to the affected part.

Example 1 (Ointment)

A mixture of 2.00 g polyethylene glycol (40 E.O.) monostearate, 5.00 g self-emulsifiable glycerol monostearate, 5.00 g stearic acid, 1.00 g behenyl alcohol, 10.00 g liquid paraffin, 10.00 g glycerol trioctanoate and 0.20 g methyl p-oxybenzoate was heated until clear. To this solution, was added a heated solution of 5.00 g 1,3-butylene glycol and 48.80 g pure water with stirring to effect emulsification. After cooling, 1.0 g kojic acid and 6.0 g placental extract of pregnant cow were added to the emulsion, and the mixture was stirred well, cooled and charged in suitable containers.

Example 2 (Emulsion)

A mixture of 1.00 g polyoxyethylene-sorbitan(20 E.O.) monostearate, 0.50 g polyoxyethylene-sorbitan (60 E.O.) tetraoleate, 1.00 g lipophilic glycerol monostearate, 0.50 g stearic acid, 0.50 g behenyl alcohol, 4.00 g avocado oil, 4.00 g glycerol trioctanoate and 0.20 g methyl p-oxybenzoate was heated until clear. To this solution, was added a heated

solution of 5.00 g 1,3-butylene glycol, 0.14 g xanthan gum, 0.5 g kojic acid, 4.0 g placental extract of pregnant cow and 76.16 g pure water with stirring to effect emulsification. After cooling, a small amount of perfume was added to the emulsion, and the mixture was stirred well, cooled and charged in suitable containers.

Example 3 (Lotion)

Pure water was added to a mixture of 0.2 g kojic acid, 5.0 g placental extract of pregnant cow, 0.10 g methyl p-oxybenzoate, 0.01 g hyaluronic acid and a small amount of perfume to make up a total weight of 100 g, and the resulting mixture was stirred well and charged in a suitable container.

The external preparations of this invention may be applied at suitable doses depending on the conditions of the affected part, and are preferably coated on the affected part three times a day after washing.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An external preparation of melanogenesis-inhibitory agent containing, as active ingredients, a placental extract of pregnant cow and kojic acid or a derivative thereof, wherein the placental extract results from washing the placenta of a pregnant cow with water and removing blood therefrom, followed by extracting with water and wherein the amount of kojic acid or a derivative thereof is 1 to 30% based on the weight of the placental extract of a pregnant cow and the sum of these two active ingredients is 0.1 to 3% based on the total weight of said external preparation.

2. An external preparation as claimed in claim 1, wherein the amounts of placental extract of pregnant cow and kojic acid are in the range from 0.1 to 10 wt% and 0.1 to 3 wt% respectively based on the total weight of the preparation.

3. An external preparation of melanogenesis-inhibitory agent substantially as herein described with reference to any one of the Examples.

DATED this 4th day of January 1991.

SANSHO SEIYAKU CO., LTD
By their Patent Attorneys
GRIFFITH HACK & CO.

AUSTRALIAN PATENT OFFICE

0828s/MS

FIG. 1

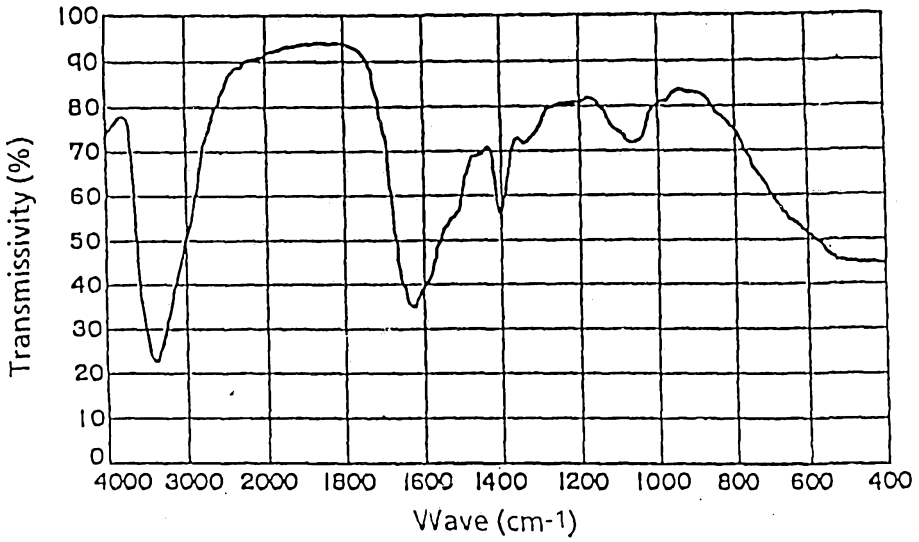
Transmissivity (%)
100
90
80
70
60
50
40
30
20
10
0
4000 3000 2000 1800 1600 1400 1200 1000 800 600 400
Wave (cm-1)

FIG. 2

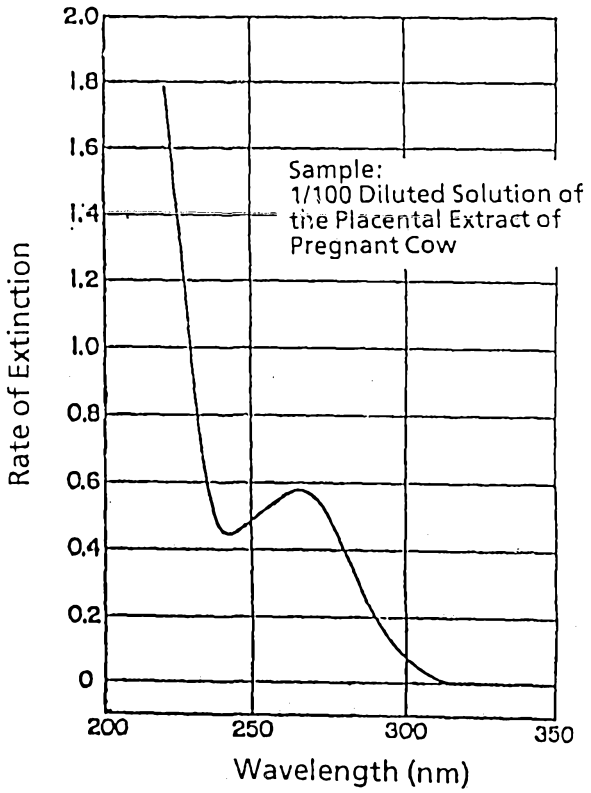
Rate of Extinction
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
200
250
300
350
Wavelength (nm)
Sample:
1/100 Diluted Solution of
the Placental Extract of
Pregnant Cow

FIG. 3

Tyrosinase isozyme activity

Placental Extract of Pregnant Cow

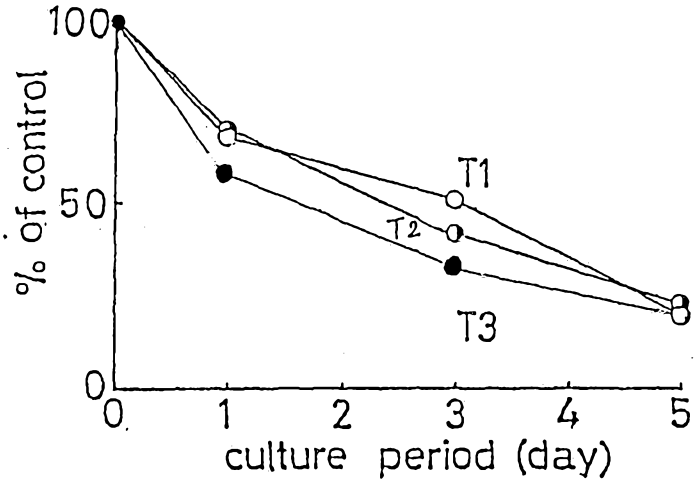

FIG. 4

Tyrosinase isozyme activity

Fully-matured Human Placenta Extract