(12) UK Patent Application (19) GB (11) 2 075 834 A

(21) Application No **8109495**
(22) Date of filing **26 Mar 1981**
(30) Priority data
(31) **490293**
(32) **2 Apr 1980**
(33) **Spain (ES)**
(43) Application published
25 Nov 1981
(51) **INT CL^3**
A61K 35/78
(52) Domestic classification
A5B E
(56) Documents cited
GB 2024622A
GB 256768
The extra pharmacopocia, Martindale, 26th edition 1972 pp 150-151
(58) Field of search
A5B
(71) Applicants
Conrad Limited,
The Banck House,
Frederick Street,
Nassau,
Bahamas.
(72) Inventors
Francisco Ramón Alvarado Salgado
(74) Agents
Marks & Clerk,
57/60 Lincoln's Inn Fields,
London WC2A 3LS.

(54) **Arthritis treatment with fern extracts**

(57) Drugs having curing and beneficial effects in pathological diseases which affect the osteolocomotive system of a human being e.g. arthritis originate from ferns of the Polypodaceae family, both from the leaves as well as the rhyzomes thereof.

GB 2 075 834 A

SPECIFICATION

Process for obtaining a drug useful in the treatment of arthritis and other diseases of the locomotive system

Background of the Invention

The pathological diseases which affect the osteolocomotive system, and particularly the diverse forms of arthritis, have long been known, and signs of denegerative arthritic processes suffered during life have been observed in mummified skeletons. During the last quarter of the twentieth century no effective therapy for treating these diseases has been found and those drugs which are more effective therapeutically are unfortunately also those which produce greater toxic effects and secondary reactions, whereby administration thereof should be suspended in the majority of patients, these patients consequently being in a difficult position since to their initial pathological state should be added an acquired ioatrogenic disease.

All the drugs used in disorders of the osteolocomotive system are known to produce secondary effects and even the aspirin itself, considered innocuous at other times, when administered in larger dosages, produces, besides gastric irritation, general organic alterations, such as clotting of the blood, blood vessels, etc. As the strength of the effect of the drugs used increases, the secondary toxic effects also increase.

The present invention, besides being an effective drug, does not produce any secondary effect nor does it present toxicity.

The results are much more favourable in pure diseases with respect to the etiopathogeny thereof, having the advantage that it can be associated with other drugs to cure and recover those pathological conditions wherein the etiopathogeny is more obscure or uncertain, a symptomatic treatment having, therefore, to be carried out to free the patient of his sufferings.

This invention has the advantage that the high metabolic power thereof is physiological, since it activates organic systems which are depressed due to the pathological state, thereby resulting promising in geriatrics.

As will subsequently be mentioned, it has been used in all pathological diseases affecting the osteolocomotive system.

Summary of the Invention

The chemical compounds of this invention, obtained from the rhyzomes and leaves of ferns of the Polypodiaceae family, having curing and improving effects in many diseases affecting the osteolocomotive system of the human being. The main ingredients of this invention are found in the following species pertaining to the Polypodiaceae family:

Poypodiaceae family

Grammitideaceae
Nevrodium
Dicranoglossum
Microgramma
Pleopeltis
Niphidium
Campyloneuron
Polypodium
Plebodium

A methodology, which will subsequently be described, has been devised to obtain these active ingredients, permitting sufficient amounts of industrial yields to be obtained for the carrying out of a number of pharmaceutical formulae. Since these investigations date back to 15 years, cutting of the leaf or use of the rhyzome is perfectly controlled so that if this plant generates at any moment during its vital cycle a toxic substance (which is very improbable), it has never appeared in the industrial obtention.

The method of this invention essentially consists of obtaining an extract from the leaves and rhyzomes of the mentioned ferns and purifying this extract to isolate the active ingredients useful in the treatment of osteolocomotive diseases.

Extraction can be carried out on the cut and dried leaves and rhyzomes of the ferns, on the fresh leaves and rhyzomes, or on the wet cake resulting from a pressing operation to extract the juice or sap from the fresh leaves and rhyzomes. In this latter case, the extract of the wet cake can be mixed with the fresh sap for the subsequent elaboration thereof.

The process of this invention shall now be described in more detail.

Drying

Both the rhyzomes and the leaves are dried at a temperature of 65°C (150°F) approximately.

The rhyzomes are previously cut into strips of 2-3 cms.; the leaves are dried and they are collected; drying takes place continuously, discontinuously or naturally. For example, the material can be introduced in a drying machine comprising a metallic wirecloth having approximately 5 m. in width and 25 m. in length, which is moved within a hot compartment through which a stream of hot air is passed. By adjusting the speed of the metallic wirecloth, approximately half a ton of moist material can be dried per hour.

Drying can also be carried out by convection and radiation, using solar collectors. The solar collector is

made of a light material, the dimensions of which depend on the volume in question. The drying time ranges of from 24 to 36 hours, until a residual moisture of from 12% to 8% is reached to enable granulating. The maximum inner temperature reached in this case is of 70°C.

Granulating

To facilitate extraction, the leaves and rhyzomes can be subjected to granulation.

In the case of the rhyzome, a disc mill is used. Dry or fresh rhyzomes, carefully selected and washed, are used. The size of the granultated rhyzome can vary of from 4 to 6 mm. This size is determined by a metallic wirecloth against which it is pressed by the discs.

The dry leaf is granulated in a hammer mill until it is finely divided. The yield and ease with which the active ingredient is extracted depends on the size of the granulated lead.

Extraction

As previously indicated, extraction can be carried out on the fresh or dried parts of the plant.

Fresh extraction

1. The fresh rhyzomes cut in strips and the fresh leaves packed in separate batches are subjected to the action of a press to extract, by pressing, the inter and intracellular juice.
2. The residual cake is subjected to an alcohol wash (water containing 10% alcohol), using a residual cake: hydroalcoholic mixture ratio of from 1:2 to 1:10. This mother liquor can be added to the juice or sap obtained in the preceding step to be treated jointly, or it can be treated separately.

2'. The fresh leaves and rhyzomes duly cut are extracted with a polar solvent, the dielectric constant of which ranges of from 1.8890 to 80.37, at temperatures of from that of ambient to that of boiling point.

3. The juice or sap of step 1, the mother liquor of step 2, the extract of step 2', or any mixture of these substances is extracted with a like volume of non-polar solvent, for example, hexane, to remove the lipidic substances.
4. The mixture is stirred for 15 minutes, it is then allowed to stand for about 2 hours, and finally the non-polar solvent phase is removed, which is discarded, obtaining a final extract in the polar solvent.

Dry extraction

The dry and granulated leaves and rhyzomes are extracted with a solvent, the dielectric constant of which ranges of from 1.8890 to 80.37.

The weight:volume ratio (Kg:liter) of leaves or rhyzomes to solvent can range of from 1:1 to 1:10, depending on the capacity of the containers used. Extraction can take place at temperatures of from that of ambient to that of boiling point, consequently varying the times.

Subsequently the extract obtained in the polar liquid is subjected to another liquid-liquid extraction with a non-polar solvent, for example, hexane, to eliminate the lipidic substances. Finally, the non-polar phase, which is discarded, is removed, obtaining an extract in the polar solvent.

Evaporation

The final extract obtained in the fresh or dry operations is evaporated, using steam-heated continuous, semicontinuous or discontinuous evaporators. The operation continues until about 10% of the original volume of the solvent used is obtained in the solid-liquid extract.

Purification

The method followed to carry out purification determines the composition of the final product obtained and, therefore, the therapeutic activity thereof.

The concentrated liquid of the preceding step is filtered and is then passed through an ion-interchanging resin column, having an anionic nature. In this column it is subjected to a counter-current extraction with a non-polar solvent, preferably hexane.

It is then passed through a cationic interchanging column. To the eluate obtained is added a weak base to neutralise it and then the neutral solution is passed through another anionic interchanging resin column. Activated carbon is added to this solution obtained to clarify same.

The solution obtained is concentrated by evaporation until about 20% of the original volume thereof, thus obtaining a viscous liquid having a bitter-sweet taste and amber in colour.

This viscous liquid, object of the invention, can be lyophilized, obtaining a rather hygroscopic whitish powder which changes colour rapidly when subjected to the humidity of the air.

The following examples are given by way of illustration and non-limiting of the invention.

Example 1

100 Kg. of rhyzomes were introduced in a stainless steel container provided with heating coils, and steam was applied until it was heated to reflux using, as the solvent, 600 liters of methanol at 64°C. After heating for 1 hour, the rhyzome was separated from the methanol. The rhyzome was mechanically separated and then pressed to completely extract the active ingredient.

The extract was concentrated to 1/3 of the original volume so that the interchanging columns (mixed-bed

resins) can be pump-operated; then it is neutralized with sodium hydroxide to pass it through a movable carbon phase to whiten same. Finally it is filtered and concentrated to the permitted levels at a temperature of from 50°C and 600 mm. of Hg., giving approximately 6 liters of the product.

Example 2

100 Kg. of leaves were introduced in a stainless steel container, completely covering same with 500 liters of ethanol. After heating at 72°C for 1 hour, it was concentrated to a volume of 50 liters and then passed through ionic interchanging columns (mixed-bed resins) and then neutralised with calcium hydroxide. The neutralised product was passed through activated carbon columns until the complete decolorisation thereof. Finally, it was concentrated to 4 liters. giving a product very similar to bee honey. The yield obtained depended on the leaf or rhyzome, on the harvesting time, and on the processing conditions.

Example 3

100 Kg. of leaves and 500 liters of water were introduced in a container and heated to 90°C for 1 hour. The extract was allowed to stand for 24 hours to completely separate the resins; it was then filtered to obtain a clean filtrate. It was passed through the ionic interchanging column (mixed-bed resins) and neutralised with calcium hydroxide. It was then decoloured and filtered to finally evaporate until a volume of 5 liters of a pleasant smelling liquid were obtained, the colour whereof varied with the duration of the final evaporation.

The pharmaceutical compositions of this invention are prepared in conventional dosage forms by incorporating the sirupy product obtained with the process of the invention to a pharmaceutical carrier. The resultant pharmaceutical compositions also constitute an object of this invention. The active ingredients should be used in the compositions in a sufficient amount to produce an anti-arthritic activity.

The compositions of the invention preferably contain from about 10 to about 100 mg of the active ingredient per unit dosage. The pharmaceutical carrier can, for example, be solid or liquid. Starch, lactose, magnesium stearate, alba terra, saccharose, talc, stearic acid, gelatin, agar, pectin, gum arabic, aerosil and the like are examples of solid carriers. Examples of liquid carriers are alcohols (such as ethanol or propyleneglycol) water containing a solubilizing agent such as polyethyleneglycol, peanut oil, olive oil and the like. The carrier or diluent can include a retarding agent such as glyceryl monostearate or glyceryl distearate, on its own or with a wax.

A wide variety of pharmaceutical forms can be used. Thus, if a solid carrier is used, the preparation can take the form of tablets, introduced into a hard gelatin capsule in the form of a powder or granules, or it can adopt the trochiscus form. The amount of solid carrier can vary between wide ranges, but preferably from about 25 mg. to about 300 mg.

If a liquid carrier is used, the preparation can adopt the form of a syrup, emulsion, soft gelatin capsule, suspension or liquid solution, or a sterile injectable form for parenteral use, for example, in an ampule. The lyophilized powder can be used in individual dosages to be reconstituted when administered.

The pharmaceutical compositions of this invention in liquid suspensions or solution, do not include the simple liquid suspensions or solutions of the active ingredient in the common unsuitable solvent for the internal administration to produce the desired pharmacological activity.

The unit dosages in the form of tablets, capsules, trochiscus, suppositories, liquid suspension or sterile injectable liquid for internal administration which can produce the anti-arthritic activity thereof, also form part of this invention.

The mode of administration can be oral or parenteral, rectal, preferably oral. The active ingredient will preferably be administered in a daily dosage amount of from about 100 mg. to about 1,500 mg., and even better of from 200 to 600 mg. It is advantageous to administer dosages equivalent to 1 to 4 times per day. When administration is as previously described, an anti-arthritic activity is obtained.

Since arthritis and the like are chronic insiduous diseases having a genetic etiopathogeny, it is preferible to use the form of soft gelatin capsules or tablets, to maintain stable blood concentrations and which permit a continued action of the medicament. Undoubtedly, when treating serious attacks of arthritis the intravenous injection or the continuous dropwise feeding of serum can be used to prevent the crisis.

It should be pointed out that the form of drops or emulsion is used in pediatrics for obvious reasons, and in the case of adults having problems of the upper digestive canal and children, suppositories can be used.

The pharmaceutical compositions are prepared by conventional techniques such as mixing, granulation and compression when necessary, or by mixing and dissolving the suitable ingredients for the desired compositions.

The following examples illustrate the pharmaceutical compositions of the invention:

Example A

Polar fraction of this invention	100 mg
Starch	250 mg
Lactose	60 mg
Magnesium stearate	5 mg

The ingredients are mixed, sifted, and introduced in hard gelatin capsules. The dosage can vary from 3 to 6 capsules per day.

Example B

Polar fraction of this invention	60 mg
Aerosil	60 mg
Starch	40 mg
Polyvinylpyrrolidone	5 mg

The polar fraction is mixed with the aerosil, granulated with a wet strach dough and dried for 8 to 12 hours. The granules are sifted and converted into tablets.

During the first part of the clinical test about 900 patients suffering from rheumatoid arthritis, ankylopoitic spondylitis, psoriatic arthritis, diseases of the connective tissue, rheumatic fevers, articular degenerative diseases (osteoarthritis, osteoarthrosis), non-articular rheumatism, infectious arthritis, traumatic and neurogenetic arthritis, arthritis due to biochemical or endocrinal disorders, ioatrogenic arthritis, as well as diseases affecting parts of some organisms forming the articulation as a whole, for example, the tendons, were treated.

The chemical compounds of this invention were administered in the form of natural extracts, as well as in the form of derivatives. Prior to treatment of the human patients, toxicological studies were made on test animals following the rules of "Guidelines for Human Use" and following the rules of the F.D.A. of the U.S.A. on three animal species, one of which was not a mouse but, in our case, a dog.

The results were completely satisfactory. The DL_{50} found in our experiments for these chemical compounds were 60 times greater than the maximum therapeutic dosage used. The studies concerning fertility, teratogenesis and carcinogenesis made with these animal species showed no alteration or production of pathologies of this nature.

The dosage administered to these animals was of 100 mg/kg per body weight. The animals were sacrificed 48 hours, 6 weeks, 36 weeks and 52 weeks after administration, during which periods they were clearly under the effects of these drugs, and no organic alteration was observed when carrying out an autopsy. The methodology followed was that described by Jull, J.W. Brit. J. Cancer *5*, 328 (1951) and the statistical evaluation used was that described by Arcos y Col., Chemical Induction of Cancer, Academic Press Ing., New York (1963).

The patients were observed for 2 years and the daily dosage administered ranged from 200 to 1500 mg, depending on age, sex, weight, and osteolocomotive disease of the patient.

The average dosage of 3 mg/kg per body weight was used. The results were very satisfactory in those cases where the pathological disease attacked the cartilages (as in osteoarthrosis) or the collagen fibers (as in connectivitis) and in those diseases which mainly affect the synovial membranes (as in rheumatoid arthritis), the results were sufficiently favourable, although less intense.

Clinical tests were made using the double blind test system and as the control the placebo of an inert substance and two comparative drugs, asprin and phenylbutazone. Patients suffering from different degrees of seriousness of the osteolocomotive disease were used and in all of them an improvement was obtained.

Without being bound to any theory, presently the action mechanism is assumed to be the following:

A) increase in the activation process of growth and maturity of the collagen, thereby producing a firmer and more resistant collagen.

B) increase in the permeability to water and to ions through the lysosome membrane of the cells, both normal and pathological, of the affected area. This increase in the permeability of the membranes explains the possible increase in the regenerative capacity of the cartilage and of the cells (chondrocyte) which have been observed using marked substances.

C) likewise, there is produced a positive interchange towards the interior of the nutrient cells, such as glucose, fructose and aminoacids of valine, lysine and others which would favour the cellular nutrition which is in a precarious position in this pathology. At the same time, we have observed a greater elimination of CO_2 as a result of the high metabolism developed in the cell.

D) initial studies have demonstrated that this drug produces an increase in the activity of the carriers, such as lipoproteins, which result also implies a probable action mechanism of the drug.

E) likewise the activity of the hepatic cells (hepatocytes) is increased and a good number of enzymatic systems inherent to the cells is activated, which explains the improvement experienced by patients suffering from uric type arthritis.

F) inflammation and pain diminish due to the high degree of metabolism produced by a greater supply of oxygen and blood to the affected tissues.

The product of this invention does not produce any effect or activity of the steroidal type. Its activity on the cellular membranes of the myocardium produces a very beneficial bradycardiac effect in older patients on whom the use of digitalis was suspended, since the product of this invention produces an effect similar thereto.

CLAIMS

1. Process for obtaining a drug useful in the treatment of arthritis and other diseases of the locomotive system, said drug comprising natural polar fractions of rhyzomes and leaves of ferns of the Polypodiaceae family, species: Gammitideaceae, Nevrodium, Dicranoglossum, Microgramma, Pelopeltis, Piphidium, Campyleneorum, Polypodium and Phlebodium, which process comprises:

1. Extracting, with a polar solvent, a material selected from the group consisting of dry, granulated leaves and rhyzomes of the aforementioned ferns, fresh, cut leaves and rhyzomes of said ferns, and the cake resulting from the pressing of said fresh leaves and rhyzomes, in which case the extract obtained is added to the juice or sap obtained from said pressing operation, the weight:volume ratio of solid material to solvent extract being of from 1:2 to 1:10;

2. Mixing the extract of the preceding step with a non-polar solvent, allowing the layers to be separated and removing the non-polar phase;

3. Evaporating the partially purified polar extract obtained in the preceding step until at least 10% of the original volume is reached; and

4. Purifying the concentrated extract and subjecting it to the following operations:

a) Filtering the concentrated liquid;

b) Passing it through an ion interchanging resin column having an anionic nature where it is subjected to a counter-current extraction with a non-polar solvent;

c) Passing it through a cationic interchanging column;

d) Adding a weak base to the eluate obtained to neutralise it;

e) Passing the neutral solution through another anionic interchanging resin column;

f) adding activated carbon to the solution obtained to clarify it;

g) concentrating the solution obtained by evaporation until approximately 20% of its original volume to obtain a viscous liquid having a bitter-sweet taste and amber in colour; and

h) optionally, lyophilizing the viscous liquid of the preceding step to obtain a white crystalline powder.

2. Process according to claim 1, wherein the dielectric constant of the polar solvent is of from 1.8890 to 80.37.

3. Process according to claims 1 and 2, wherein the polar solvent is a hydroalcoholic mixture.

4. Process according to claims 1 to 3, wherein the hydroalcoholic mixture is a hydroethanol mixture, the non-polar solvent is hexane, and the weak base used to neutralise is calcium hydroxide.

5. Process according to the preceding claims, wherein the extraction is carried out at a temperature of from that of ambient to that of boiling point of the solvent.

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1981.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.