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(54) Title: USE OF CARYOPHYLLENES IN THE MANUFACTURE OF MEDICAMENTS AND TREATMENT OF BODILY CONDITIONS OF INFLAMMATION AND INFLAMMATORY PAIN

(57) Abstract: The invention concerns the use of caryophyllenes related to medicaments and to the treatment of bodily conditions of inflammation and inflammatory pain.

USE OF CARYOPHYLLENES IN THE MANUFACTURE OF MEDICAMENTS, AND TREATMENT OF BODILY CONDITIONS OF INFLAMMATION AND INFLAMMATORY PAIN.

The present invention concerns the use of caryophyllenes related to medicaments and to the treatment of bodily conditions of inflammation and inflammatory pain. It relates particularly to the use of caryophyllenes in the manufacture of medicaments for the treatment of inflammatory conditions of the animal body, including the human body. The invention also concerns the use of caryophyllenes for the treatment of inflammatory conditions of the body, including inflammatory pain.

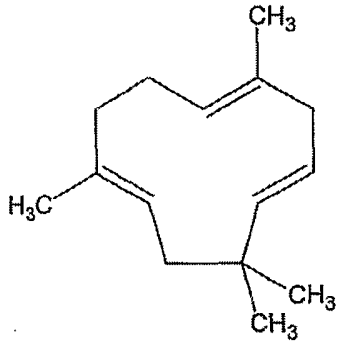
Caryophyllenes are known chemical compounds, useful in various applications. For instance patent document US 3,987,008 reveals sesquiterpenic derivatives as odor and taste-modifying agents; In *J. Nat. Prod.* 1992 Jul;55(7):999-1003, beta-caryophyllene and alpha-humulene as cited as potential anticarcinogenic agents; in patent application WO 9218001 alpha and beta-humulene and (-)-beta-caryophyllene are cited in the control of whitefly species; in patent US5,314,693 alpha-humulene is cited as a repellent for pine wood nematodes; in patent application WO 02078719 alpha and beta-caryophyllene are comprised in antitumor compositions.

The present invention concerns, in one particular aspect, a new and useful use for caryophyllenes, more particularly alpha-humulene or beta-caryophyllene, as anti-inflammatory and as analgesic, in a broad sense. In a more specific sense, the compounds were found to be useful inhibitors of entities that are known to be involved in the inflammatory process :

- pro-inflammatory cytokines IL-1β (interleukin 1β) and TNFα (tumor necrosis factor α);
- PGE2 (prostaglandin-E2),
- Expression of COX-2 (cycloxigenase–2) and iNOS (inducible nitric oxide

synthase) enzymes

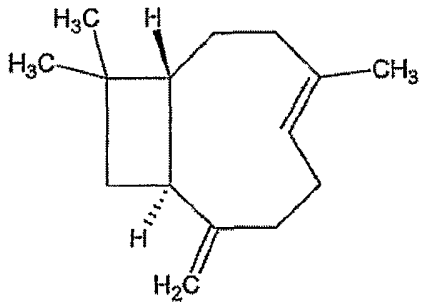
One particular example of caryophyllene of the invention is alpha-humulene, a sesquiterpene identified with the CAS (Chemical Abstracts Service) registry number 6753-98-6, also known as alpha-caryophyllene, represented by the following structure :



Another particular example of caryophyllene of the invention is the trans–caryophyllene (or beta-caryophyllene), also a sesquiterpene, identified with the CAS (Chemical Abstracts Service) registry number 87-44-5, represented by the following alternative structures A and B:

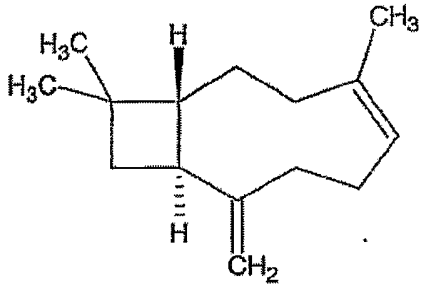
Trans- caryophyllene

Structure A



Trans -caryophyllene

Structure B



According to the meaning employed herein, mention to caryophyllenes, object of the invention, includes the molecules as such, their salts, isomers, metabolites, pro-drugs, solvates (including hydrates) and adducts.

Terpenes, including sesquiterpenes, are often mentioned as components of complex mixtures extracted from plants, where – as known to a

persons skilled in the art - it is undetermined what compound or compounds are effective, how much effective they are, and whether they are active by themselves, by way of the vehicle/solvent the composition contains (water, alcohol, other solvents, mixtures of those, etc), or by way of their interaction with other components within the mixtures. Individual terpenes per se, of natural origin or products of synthesis (for instance *J. Am. Chem. Soc.*, **99**, 3864 (1977)), are rarely mentioned as effective pharmaceutical agents. Alpha-humulene has even been mentioned to be virtually inactive concerning anti-inflammatory or chemo-therapic effects (reference: Carcinogenesis (2002), 23(5), 795-802).

The applicant has now found out that caryophyllenes, particularly alpha-humulene and trans-caryophyllene, have marked anti-inflammatory effects, including inflammatory pain, comprised therein the inhibitory effect upon the production of pro-inflammatory cytokines IL-1β and TNFα, prostaglandin PGE2, or the expression of enzymes COX-2 and iNOS.

The applicant has also found out that caryophyllenes, particularly alpha-humulene and trans-caryophyllene, have anti-allergic, particularly anti-histaminic effects.

The caryophyllenes of the invention are part of the ongoing search for drugs with direct of indirect inflammatory activity, which inhibit the physiopathology processes involved in inflammation. They are used in the control of chronic-degenerative diseases as rheumatoid arthritis, osteoarthritis, systemic lupus eritematosus, ulcerative colitis, psoriasis, atopic eczema, atherosclerosis, and other non degenerative diseases as depression, and cellulites, and allergies.

Therefore, one of the objects of the present invention is the use of caryophyllenes, particularly alpha-humulene and/or trans-caryophyllene, or compositions comprising caryophyllenes, in the manufacture of a medicament for the treatment of inflammatory conditions of the animal body, particularly the

human body.

Another object of the present invention is the use of caryophyllenes, particularly alpha-humulene and trans-caryophyllene, or compositions containing caryophyllenes, in the treatment of inflammatory conditions of the animal body, particularly the human body.

Another object of the present invention is a method of treatment of an inflammatory condition of the animal body, particularly the human body, comprising the administration of a therapeutically effective amount of caryophyllenes, particularly alpha-humulene or trans-caryophyllene, to a patient.

Another object of the present invention is the use of caryophyllenes, particularly alpha-humulene and trans-caryophyllene, or compositions containing caryophyllenes, for the inhibition of the bodily production of one or more of cytokine IL-1β, cytokine TNFα, prostaglandin PGE2, expression of enzymes COX-2 and iNOS.

The caryophyllene of the invention, as well as compositions comprising the caryophyllene according to the invention, can be administered to the subject in need of treatment in any adequate way, enteral or parenteral, including oral, topical, transdermal, subcutaneous, intraperitonial, intravenous, by infiltration, by inhalation, transdermal, transmucosal, intramuscular, intrapulmonary, vaginal, rectal, intraocular, and sublingual. Particularly adequate ways of administration in the present invention are systemically (infiltration, oral, inhalation by spray, transdermal) and topically. The caryophyllene of the invention can be comprised in a slow or controlled release composition. Known adjuvants and excipients can be utilized in the compositions. A reference for pharmaceutical dosage forms useful for the compositions related to the inventions can be found in the publication *Remington's Pharmaceutical Sciences*, Mack Publishing.

The compositions comprising caryophyllene can be administered to

patients as solids, liquids or semi-liquids, tablets, capsules, pills, powder, granules, suspensions, emulsions, dispersions and any other useful known form.

The compositions might contain further active agents, for instance antibiotics, depending on the desired effect.

For oral administration as tablets or capsules (both soft and hard capsules), the caryophyllene can be combined with pharmaceutically acceptable inert vehicles, such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium phosphate, manitol, sorbitol, and similars; for oral administration in the liquid form, the caryophyllenes can be combined with ethanol, glycerol, water, and similars. When desired or necessary, agglomerating agents, lubricant agents, disintegrating agents, color and fragrance can be added to the mixture. Common agglomerating agents are glucose, β-lactose, corn sweeteners, natural or synthetic gums such as gum arabica, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, wax and similars. Lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride. Disintegrants include starch, methyl cellulose, agar, bentonite, xanthan gum, and similars.

The compositions concerned in the invention can also be administrated as liposomes or coupled with soluble polymers as vehicles.

Liquid dosage forms for oral administration may comprise colorants and edulcorants to increase acceptance by patients. Acceptable vehicles for water dosage forms are, water, an appropriate oil, a saline solution, aqueous dextrose, other sugar solutions and glycols as propylene glycol or polyethylene glycols, phosphate buffer.

Compositions related to the present invention typically comprise about 1mg to about 1000 mg of one or more caryophyllenes, particularly about 10 to 200mg and more particularly about 30 to 100 mg. In such compositions

the caryophyllene represents about 0.1 to 99% in weight, particularly about 1 to 70% and more particularly about 10 to 40%, optionally comprising at least one pharmaceutically acceptable vehicle.

EXAMPLES

The examples that follow represent particular embodiments of the invention, and do not impose any limitation to its extension, which is limited only by the claims attached hereto.

EXAMPLE 1

INFLAMMATORY NOCICEPTION INDUCED BY CARREGENIN.

The evaluation methodology used in this tests is described by Vaz *et al.* in J. Pharmacol. Exp. Ther. 278:304-312, 1996.

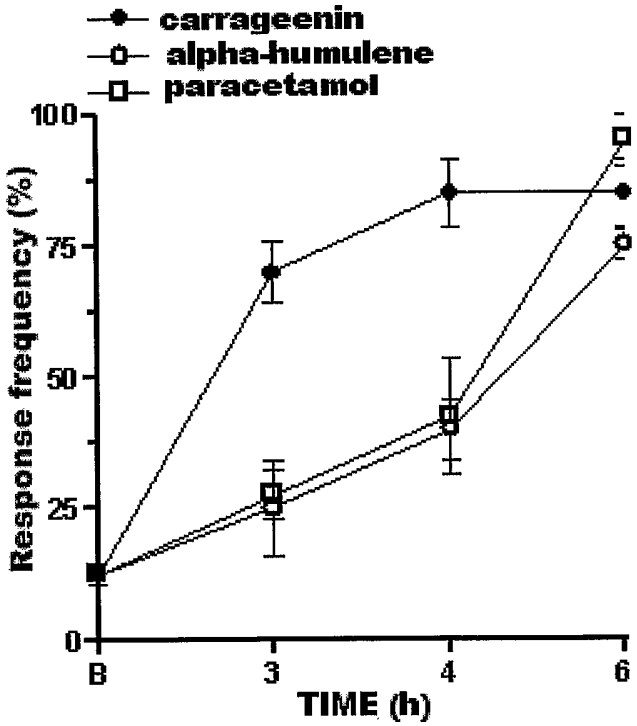
Male mice (25-35g) were systemically (orally) treated with alpha-humulene, 50 mg/kg, administered 1 hour before the experiment. Animals treated with 0.9% saline solution (0.1ml/10g) were used as the control. Another group of animals was treated with paracetamol (600 mg/kg, orally, administered 1h before the treatment), that was used as positive control. For the induction of inflammatory pain, the animals received an intraplantar injection of 0.05 ml of carrageenin (300 µg per paw) at the plantar surface of the right hind paw. This dosage causes oedema, nociception and substantial swelling of the injected paw.

The nociception was evaluated with a Von Frey filament (0.4g) after 3, 4 and 6 hours. To obtain a basal response, the animals were pre-tested the previous day with the 0.4g von Frey filament. Only animals with a response of about 20% were selected. The filament was applied to the right hind paw, complying with the criteria of (1) the application was perpendicular to the plantar surface, with enough pressure to cause the filament to bend, thus obtaining total pressure; (2) the animals were evaluated when the four paws were touching the screen; (3) the paw withdrawal response was considered when the animal removed the paw entirely from the support screen; (4) each animal

was stimulated 10 consecutive times, each stimulation lasting 1 second; (5) each paw withdrawal event was considered as 10% of the response, with 10 withdrawal events corresponding to 100% response.

Graph 1 below compares the pain inhibition obtained by alpha-humulene with the administration of paracetamol. Each point represents the average of 5 animals, and the vertical bars the mean standard error deviation.

Graph 1



The graph clearly shows that, according to the invention, a caryophyllene as alpha-humulene reduced the inflammatory nociception, as a result of reduction of inflammation, as much as a known analgesic, paracetamol.

EXAMPLE 2

CARRAGEENIN OEDEMA IN MOUSE PAW

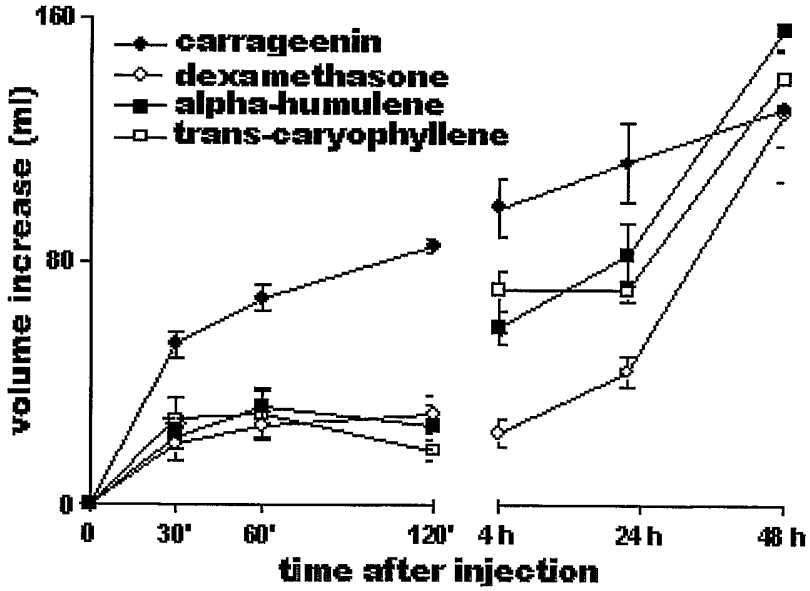
The test used below is described by Cunha *et. al.* in the publication Life Sci.70:159-169, 2001.

Male 25g-35g mice were slightly sedated with ether and were injected 50 µl saline containing carrageenin (300 µl/paw) in the right paw. The

left paw received the same volume of saline and was taken as a negative control. The swelling was measured with a plethysmometer (manufacturer: Ugo Basile, Italy) along various time intervals after the injection of the phlogistic agent. The difference between the volumes of the right and the left paw were quantified (in ml) and taken as an index of oedema. One hour before the test the animals were systemically treated (orally) with 50 mg/kg of alpha-humulene or trans-caryophyllene.

Graph 2 below compares the inhibition of the volume of the oedema by administration of either alpha-humulene or trans-caryophyllene with inhibition obtained with the administration of dexamethasone (0.5 mg/kg, injected subcutaneously 4h before test) and used as positive control. Oedema volume measurement time point intervals were 30, 60, 120 and 240 min, 24h and 48h. Each point represents the average of 5 animals, and the vertical bars the mean standard error deviation.

Graph 2



The graph clearly shows that, according to the invention, a caryophyllene as alpha-humulene reduced the inflammatory volume, as did dexamethasone.

EXAMPLE 3

BRADYKININ OEDEMA IN MOUSE PAW

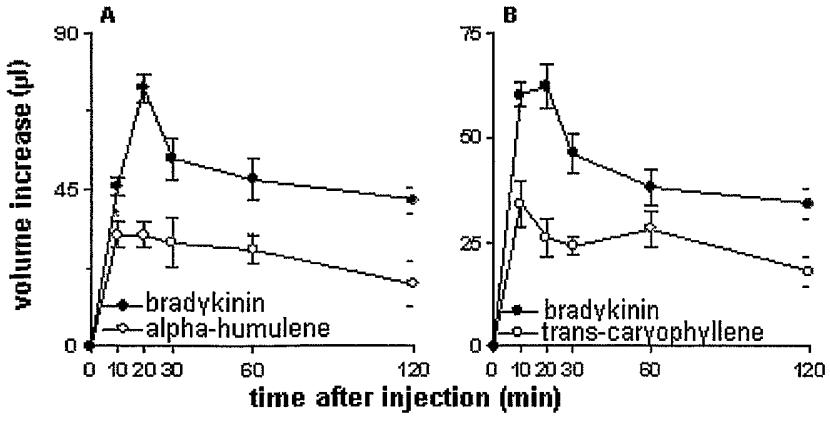
The test used below is described by Cunha *et. al.* in the publication Life Sci.70:159-169, 2001.

Male 25g-35g mice were slightly sedated with ether and were injected 50 µl saline containing bradykinin (BK, 3 nmol/paw), intraplantar, in the right paw. The left paw received the same volume of saline and was taken as negative control. The swelling was measured with a plethysmometer (manufacturer: Ugo Basile, Italy) along various time points intervals after the injection of the phlogistic agent. The difference between the volumes of the right and the left paw were quantified (in ml) and taken as an index of oedema. One hour before the test the animals were systemically treated (orally) with 50 mg/kg of alpha-humulene or with trans-caryophyllene.

The animals were pre-treated with 5 mg/kg of captopril, injected subcutaneously, 1 hour before the test, in order to avoid degradation of kinines.

Graphs 3A and 3B below compare the inhibition of the volume of the oedema by administration of alpha-humulene (3A) or trans-caryophyllene (3B). Oedema volume measurement time intervals were 10, 20, 30, 60, and 120 min, 24h and 48h. Each point represents the average of 5 animals, and the vertical bars the mean standard error deviation.

Graph 3



The graph clearly shows that, according to the invention, a caryophyllene as alpha-humulene or trans-caryophyllene markedly reduced bradykinin-induced paw oedema.

EXAMPLE 4

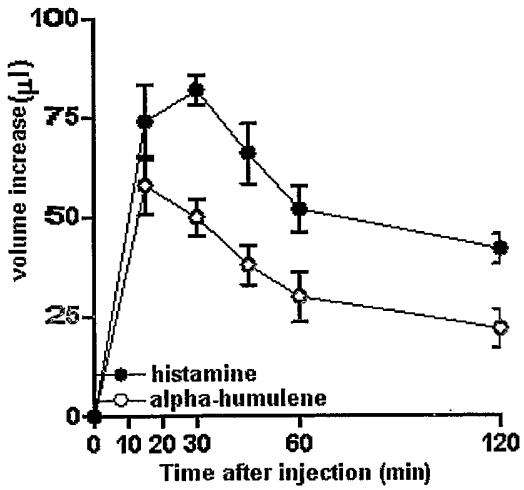
HISTAMINE OEDEMA IN MOUSE PAW

The test used below is described by Cunha *et. al.* in the publication Life Sci.70:159-169, 2001.

Male 25g-35g mice were slightly sedated with ether and were injected 50 µl saline containing histamine (100 nmol/paw), intraplantar, in the right paw. The left paw received the same volume of saline and was taken as negative control. The swelling was measured with a plethysmometer (manufacturer: Ugo Basile, Italy) along various time intervals after the injection of the phlogistic agent. The difference between the volumes of the right and the left paw were quantified (in ml) and taken as an index of oedema. One hour before the test the animals were systemically treated (orally) with 50 mg/kg of alpha-humulene.

Graph 4 below compares the inhibition of the volume of the oedema by administration of alpha-humulene. Oedema volume measurement time intervals were 10, 20, 30, 60, and 120 min, 24h and 48h. Each point represents the average of 5 animals, and the vertical bars the mean standard deviation.

GRAPH 4



The graph clearly shows that, according to the invention, a caryophyllene as alpha-humulene significantly reduced histamine-induced oedema formation. It also indirectly shows effect against allergy.

EXAMPLE 5

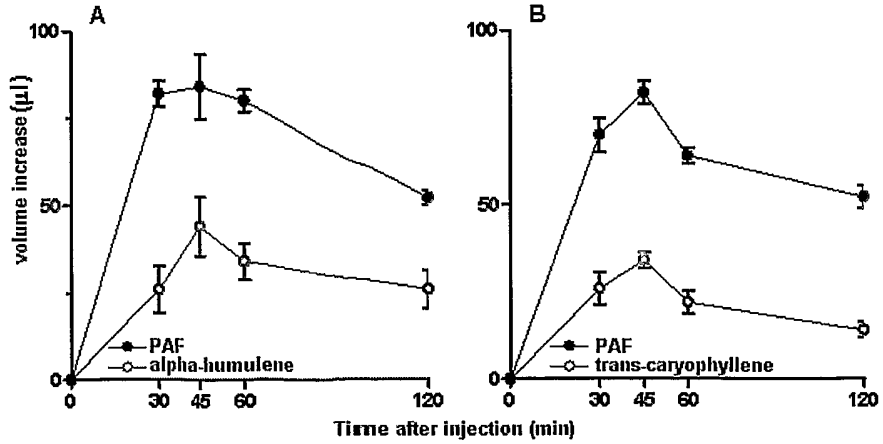
PLATELET AGGREGATION FACTOR (PAF) OEDEMA IN MOUSE PAW

The test used below is described by Cunha *et. al.* in the publication Life Sci.70:159-169, 2001.

Male 25g-35g mice were slightly sedated with ether and were injected 50 µl saline containing platelet aggregation factor (PAF, 3nmol/paw), intraplantar, in the right paw. The left paw received the same volume of saline and was taken as negative control. The swelling was measured with a plethysmometer (manufacturer: Ugo Basile, Italy) along various time intervals after the injection of the phlogistic agent. The difference between the volumes of the right and the left paw were quantified (in ml) and taken as an index of oedema. One hour before the test the animals were systemically treated (orally) with 50 mg/kg of alpha-humulene or trans-caryophyllene.

Graph 5 below compares the inhibition of the volume of the oedema by administration of alpha-humulene (5A) and trans-caryophyllene (5B). Oedema volume measurement time intervals were 30, 45, 60, and 120 min. Each point represents the average of 5 animals, and the vertical bars the mean standard deviation.

Graph5



The graph clearly shows that, according to the invention, a caryophyllene as alpha-humulene or trans-caryophyllene markedly reduced PAF-induced oedema formation. As PAF is also known to be involved in allergic processes, such data further reinforces the use of caryophyllenes in the management of allergic states.

EXAMPLE 6

ARACHIDONIC ACID OEDEMA IN MOUSE EAR

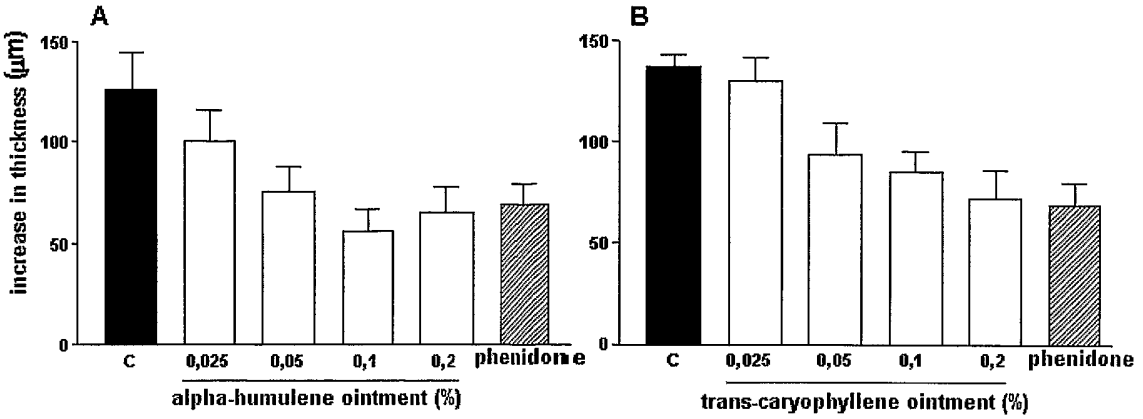
The ear oedema in the test below was measured according to Calixto *et. al.* in the publication *Prostaglandins*, **5**: 515 – 526, 1991, with minor modifications.

Male 25g-35g mice, in a first group, were topically applied, in the inner surface of the ears, an ointment comprising a range from 0.025 to 0.2% alpha-humulene or trans-caryophyllene. In the positive control group, the animals were topically applied 0.05 mg of phenidone per ear. After 60 minutes, the animals received 20 µl of arachidonic acid (2 mg/ear), dissolved in acetone, in the inner surface of the right ear. The oedema was measured using a digital micrometer, and the responses were expressed as µm, the difference between the ear thickness before and after the application of arachidonic acid. The responses of the animals treated with caryophyllenes were compared to those observed in the control group animals, treated with base ointment.

Graphs 6A and 6B below compare the inhibition of the volume of the oedema by topic administration of alpha-humulene (6A) and trans-caryophyllene (6B). Oedema volume measurement was performed after application of 0.025%, 0.05%, 0.1, and 0.2% caryophyllene content ointment, compared to the oedema volume caused by the application of phenidone and arachidonic acid (C). Each point represents the average of 5 animals, and the vertical bars the mean standard error

deviation.

Graph 6



The graphs clearly show that, according to the invention, the topic application of a caryophyllene as alpha-humulene or trans-caryophyllene markedly reduced oedema formation, in a dose-dependent manner.

EXAMPLE 7

LEVELS OF PRO-INFLAMMATORY CYTOKINE IL-1B

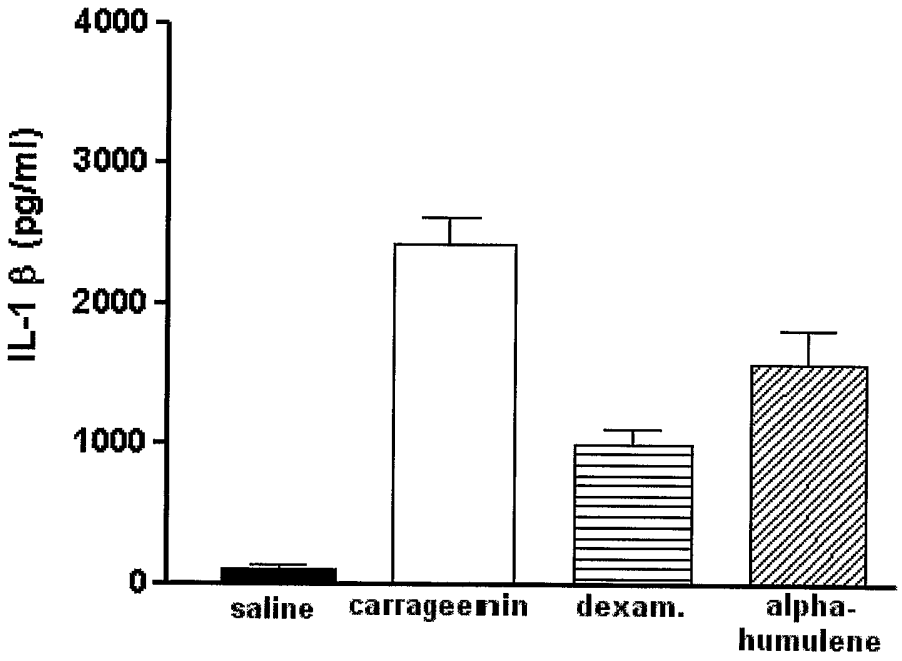
The test used below is described by Campos *et. al.* in the publication *Br. J. Pharmacol.* **135**: 1107-1114, 2002, with minor modifications.

Male 160-180g rats were orally given 50 mg/kg of alpha-humulene. Animals treated with 0.9% (0.1 ml/10g) saline were used as control. Another group of animals was treated with 0.5 mg/kg dexamethasone, subcutaneously, 4 hours before the test, and used as positive control. After 60 minutes, the animals received intraplantar injections of 100 µl of carrageenin (300 µg/paw) and were sacrificed after 180 minutes. Control animals received saline. The subcutaneous tissue of the injected paws was removed and put in a phosphate buffer containing 0.5% tween 20, 0.1 mM benzametonium chloride, 10 mM EDTA, 2µg/m aprotinin, 0,1 mM PMSF (phenyl methyl sulfonyl fluoride) and 0,5 % BSA (bovine serum albumin). The tissues were homogenized and centrifuged at 3000 g, for 10 min, at - 4 °C. The supernatant was used in the test. The levels of IL-1β were measures with an Elisa kit,

according to the manufacturer's instructions (R & D Systems ®, USA). The tests were performed in duplicate, and repeated three times. The answers are expressed in pg/mg of tissue.

Graph 7 below (each result represents the average of 5 animals, and the vertical bars the mean standard error deviation) compares the inhibition of production of inflammatory cytokine IL-1β induced by carrageenin in the paws of rats.

Graph 7



The graph clearly shows that, according to the invention, the administration of a caryophyllene, such as alpha-humulene, markedly inhibited the production of pro-inflammatory cytokine IL-1β induced by carrageenin in the paws of rats.

EXAMPLE 8

LEVELS OF PRO-INFLAMMATORY CYTOKINE TNFα

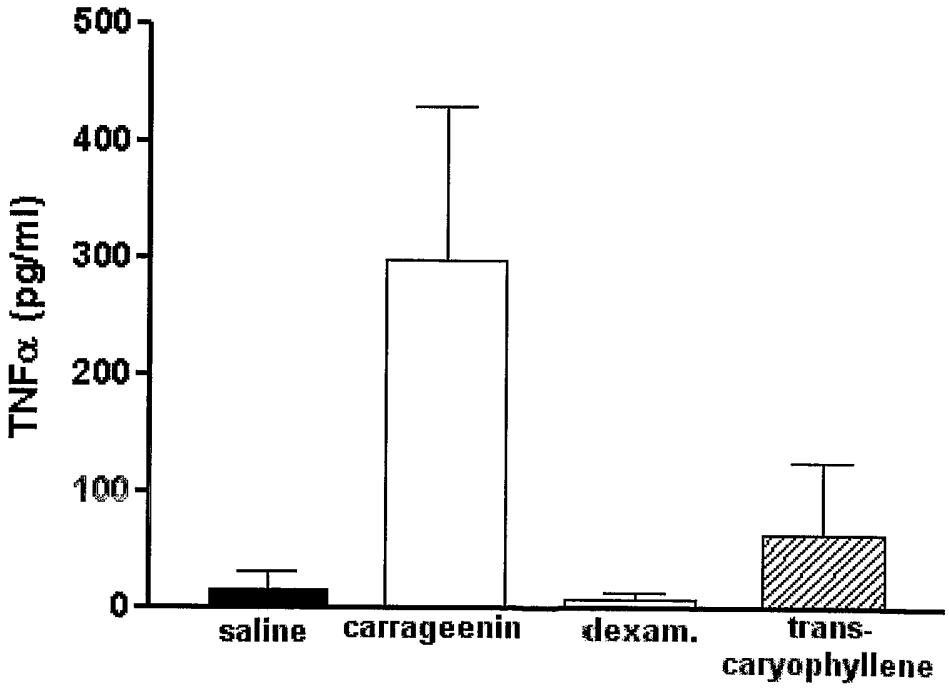
The test used below is described by Campos *et. al.* in the publication *Br. J. Pharmacol.* **135**: 1107-1114, 2002, with minor modifications.

Male 160-180g rats were orally given 50 mg/kg of trans-caryophyllene.

Animals treated with 0.9% (0.1 ml/10g) saline were used as control. Another group of animals was treated with 0.5 mg/kg dexamethasone, subcutaneously, 4 hours before the test, and used as positive control. After 60 minutes, the animals received intraplantar injections of 100 µl of carrageenin (300 µg/paw) and were sacrificed after 180 minutes. Control animals received saline. The subcutaneous tissue of the injected paws was removed and put in a phosphate buffer containing 0.5% tween 20, 0.1 mM benzametonium chloride, 10 mM EDTA, 2µg/m aprotinin, 0,1 mM PMSF (phenyl methyl sulfonyl fluoride) and 0,5 % BSA (bovine serum albumin). The tissues were homogenized and centrifuged at 3000 g, for 10 min, at - 4 °C. The supernatant was used in the test. The levels of TNFα were measures with an Elisa kit, according to the manufacturer's instructions (R & D Systems ®, USA). The tests were performed in duplicate, and repeated three times. The answers are expressed in pg/mg of tissue.

Graph 8 below (each result represents the average of 5 animals, and the vertical bars the mean standard error deviation) compares the inhibition of production of inflammatory cytokine TNFα induced by carrageenin in the paws of rats.

Graph 8



The graph clearly shows that, according to the invention, the administration of a caryophyllene, such as trans-caryophyllene, markedly inhibited the production of the pro-inflammatory cytokine TNFα induced by carrageenin in the paws of rats.

EXAMPLE 9

LEVELS OF PGE2

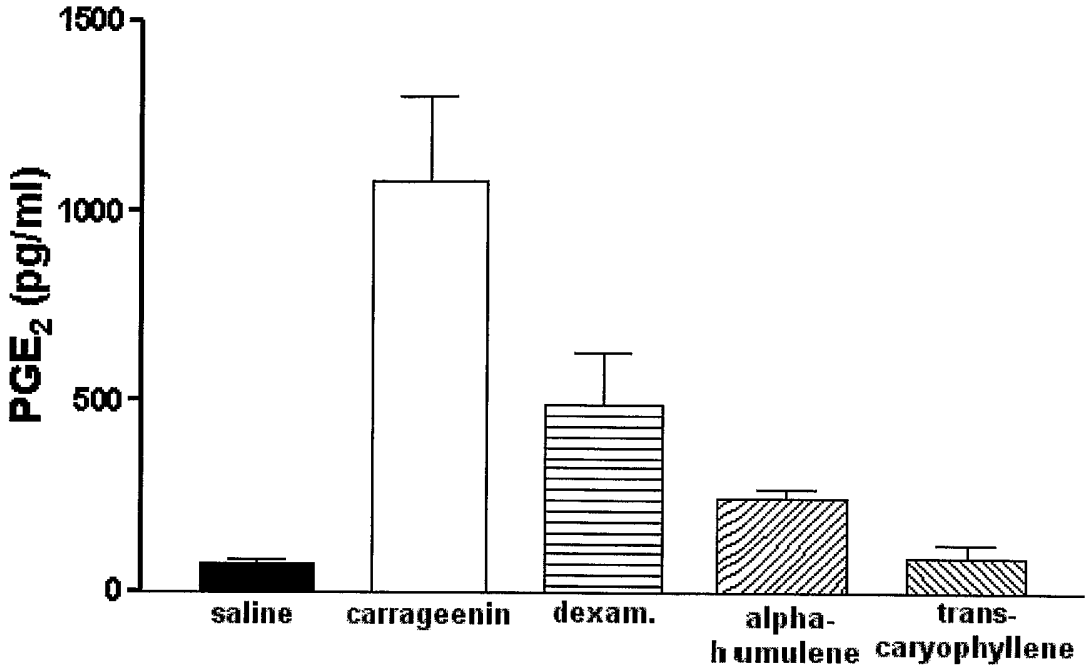
The test used below is described by Pinheiro *et. al.* in the publication *Inflamm. Res.* **51**: 603- 610, 2002, with minor modifications.

Male 160-180g rats were orally given 50 mg/kg of trans-caryophyllene. Animals treated with 0.9% (0.1 ml/10g) saline were used as control. Another group of animals was treated with 0.5 mg/kg dexamethasone, subcutaneously, 4 hours before the test, and used as positive control. After 60 minutes, the animals received intraplantar injections of 100 µl of carrageenin (300 µg/paw) and were sacrificed after 180 minutes.

The exsudate of the paws was collected by dialysis with the help of two polyethylene canulas, and was utilized for the quantification of PGE2, with an Elisa kit, according to the manufacturer's instructions (R & D Systems ®, USA). The tests were performed in duplicate, and repeated three times. The answers are expressed in pg/mg of tissue.

Graph 9 below (each result represents the average of 5 animals, and the vertical bars the mean standard error deviation) compares inhibition of the PGE2 level growth induced by carrageenin in the paws of rats, by administration of alpha-humulene and trans-caryophyllene, compared to the effect obtained by treatment with dexomethasone.

Graph 9



The graph clearly shows that, according to the invention, the administration of a caryophyllene, such as alpha humulene or trans-caryophyllene, markedly inhibited the growth of PGE2 levels induced by carrageenin in the paws of rats.

Example 10

Inhibition of the expression of enzymes COX-2 and iNOS

The expression of enzymes COX-2 and iNOS were determined by Western blot according to the methodology described by Medeiros *et. al.* in the publication *Circ Res.* **28**:1375 - 1382, 2004.

Male 160-180g rats were orally given 50 mg/kg of trans-caryophyllene. Animals treated with 0.9% (0.1 ml/10g) saline were used as control. Another group of animals was treated with 0.5 mg/kg dexamethasone, subcutaneously, 4 hours before the test, and used as positive control. After 60 minutes, the animals received intraplantar injections of 100 µl of carrageenin (300 µg/paw) and were sacrificed after 180 minutes, and subcutaneous paw tissue was removed 240 minutes after

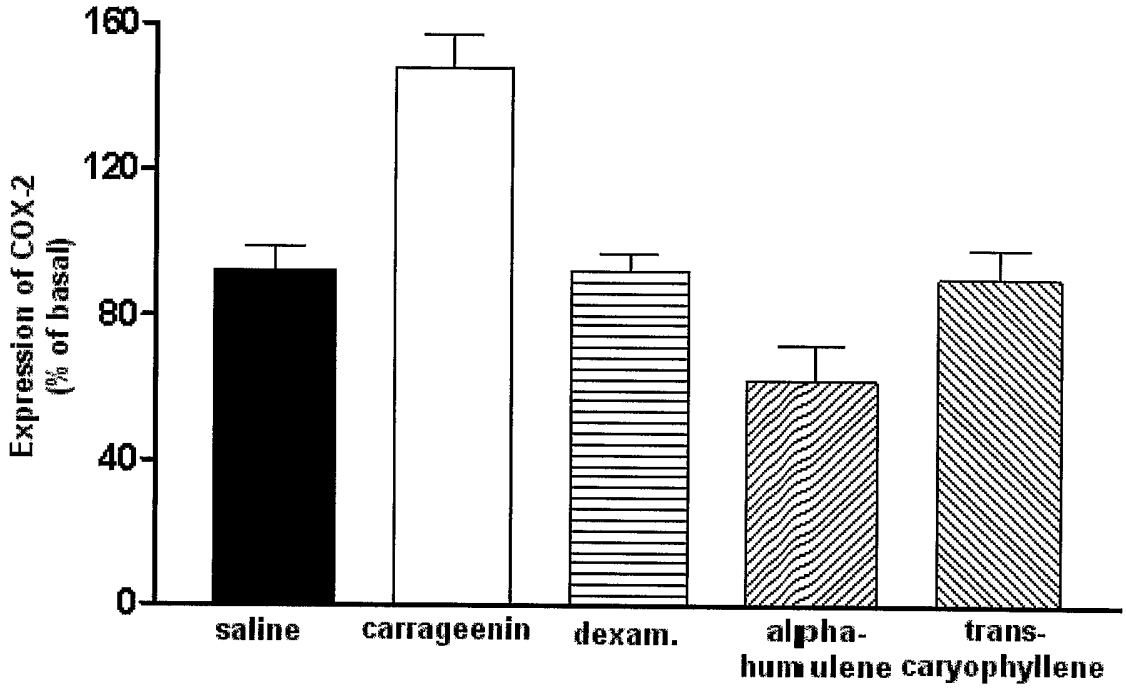
the carrageenin injection.

The collected tissue was immediately frozen in liquid nitrogen and re-suspended in a buffer of hypotonic lysis (10mM HEPES N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 1.5mM MgCl2, 10mM KCl, 0.5mM PMSF phenylmethylsulphonyl fluoride, 1.5 μg/ml trypsin inhibitor, 7 μg/ml pepstatin A, 5 μg/ml leupeptin, 0.1 mM benzamidine 0,1 mM and 0.5 mM dithiothreitol) and homogenized. The homogenate was divided in three 2ml aliquots, cooled in ice for 15 minutes, vigorously agitated and once again cooled in ice, in the presence of 20 μl 10% non-ionic detergent Nonidet P-40 (Roche Diagnostics, USA).The nuclear fraction was precipitated by centrifugation (1,500 g, 5 minutes) and the supernatant containing the cytosolic extract was stored at -70°C for the Western blot tests. The protein concentration was determined by the Bradford method (BioRad Laboratories Inc. kit, Milan, Italy). The extracts were boiled with v/v equivalent amounts of Laemmly buffer (125 mM of Tris-HCl, 2 mM of EDTA, 4 % of dodecyl sodium sulphate, 20 % of glycerol, 10 % of 2-mercaptoethanol and 0,1 % of Comassie brilliant blue, pH 6.8). The proteins were transferred to nitrocellulose membranes (100 μg/well) and separated by electrophoresis. The membranes were later blocked by *overnight* incubation (4 °C) with skimmed powder milk (10 % PBS), and then incubated with the anti-iNOS or anti-COX-2 antibodies for 1 h at room temperature. The membranes were washed three times with 10% Triton-X in PBS with the antibody peroxidase conjugated (anti-rabbit). The bands thus obtained were quantified using a chemoluminescence kit and densitometry analysis (relative units) in radiographic films.

Graph 10 below (each result represents the average of 5 animals, and the vertical bars the mean standard error deviation) compares inhibition of expression of the COX2 enzymes, obtained by the

administration of alpha-humulene and trans-caryophyllene, when evaluated in the subcutaneous tissue or the paw injected with carrageenin, compared to the expression of COX2 induced by carrageenin obtained by treatment with dexomethasone.

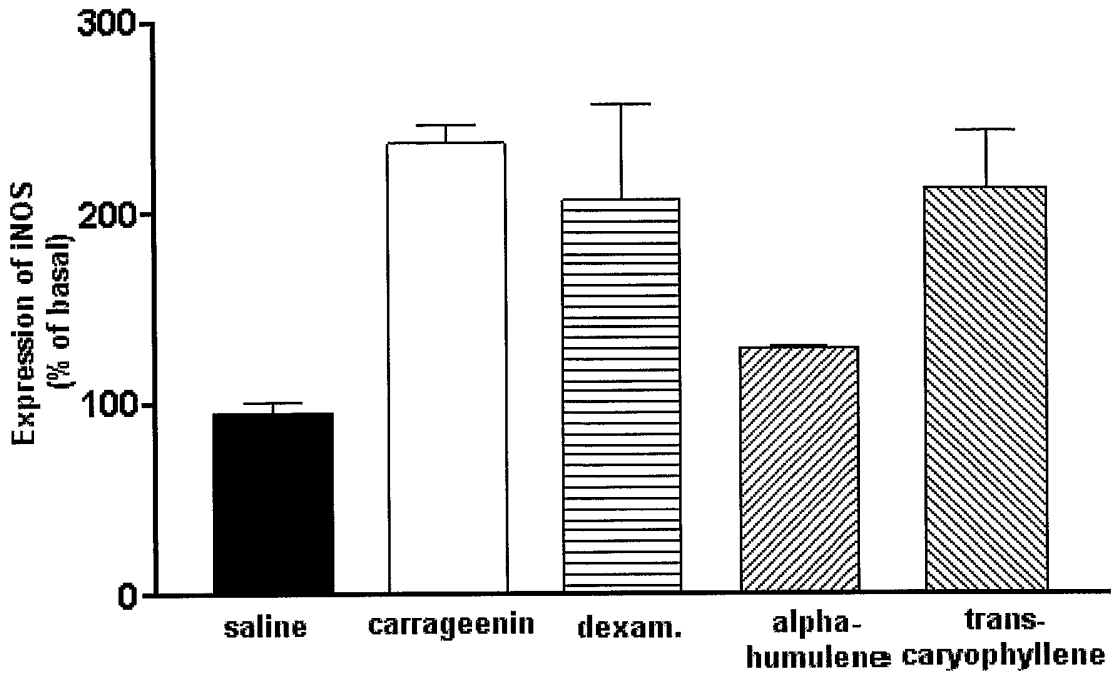
Graph 10



The graph clearly shows that, according to the invention, the administration of a caryophyllene, such as alpha humulene or trans-caryophyllene, markedly inhibited the expression of enzymes COX2 induced by carrageenin in the paws of rats.

Graph 11 below (each result represents the average of 5 animals, and the vertical bars the mean standard error deviation) compares inhibition of expression of the iNOS enzymes, obtained by the administration of alpha-humulene, when evaluated in the subcutaneous tissue or the paw injected with carrageenin, compared to the expression of iNOS induced by carrageenin obtained by treatment with dexomethasone.

Graph 11



The graph clearly shows that, according to the invention, the administration of a caryophyllene, such as alpha humulene, markedly inhibited the expression of enzymes iNOS induced by carrageenin in the paws of rats.

The examples and the information provided herein concern particular embodiments of the present invention, which is only limited by the breath of the claims attached hereto.

CLAIMS

1. Use of caryophyllenes, or compositions comprising caryophyllenes, characterized by the fact that it is in the manufacture of a medicament for the treatment of inflammatory conditions and inflammatory pain of the animal body, particularly the human body.

2. Use of caryophyllenes, or compositions comprising caryophyllenes, characterized by the fact that it is in the treatment of inflammatory conditions and inflammatory pain of the animal body, particularly the human body.

3. Use of caryophyllenes, or compositions comprising caryophyllenes, characterized by the fact that it is for the inhibition of the bodily production of one or more of the group comprising cytokine IL-1β, cytokine TNFα, prostaglandin PGE2, or expression of enzymes COX-2 and iNOS.

4. Use of caryophyllenes, or compositions comprising caryophyllenes, characterized by the fact that it is in the manufacture of a medicament for the inhibition of the bodily production of one or more of the group comprising cytokine IL-1β, cytokine TNFα, prostaglandin PGE2, or expression of enzymes COX-2 and iNOS.

5. Use of caryophyllenes according to one of claims 1-4 characterized by the fact that is in the treatment of chronic-degenerative diseases comprised in the group of rheumatoid arthritis, osteoarthritis, systemic lupus eritematosus, ulcerative colitis, psoriasis, atopic eczema, atherosclerosis, or in the treatment of non degenerative diseases comprised in the group of depression, and cellulites, and allergies.

6. Use of caryophyllenes according to one of claims 1-4 characterized by the fact that it is in the manufacture of a medicament for the treatment of chronic-degenerative diseases comprised in the group of rheumatoid arthritis, osteoarthritis, systemic lupus eritematosus, ulcerative

colitis, psoriasis, atopic eczema, atherosclerosis, or in the treatment of non degenerative diseases comprised in the group of depression, and cellulites, or allergies.

7. Use of caryophyllenes, or compositions comprising caryophyllenes, according to one of claims 1 to 4, characterized by the fact that it is administered via enteral or parenteral, including oral, topical, transdermal, subcutaneous, intraperitonial, intravenous, by infiltration, by inhalation, transdermal, transmucosal, intramuscular, intrapulmonary, vaginal, rectal, intraocular, and sublingual.

8. Use according to claim 7, characterized that said administration is topical or systemical, particularly chosen among infiltration, oral, inhalation or transdermal.

9. Use of caryophyllenes, or compositions comprising caryophyllenes, according to one of claims 1 to 4 characterized by the fact that said caryophyllenes are one or more of alpha-humulene and trans-caryophyllene.

10. A method of treatment of inflammatory conditions and inflammatory pain of the animal body, particularly the human body, characterized by the fact that it comprises the administration of a therapeutically effective amount of caryophyllenes, to a patient.

11. A method according to claim 10 characterized by the fact that said inflammatory conditions and inflammatory pain are present in chronic-degenerative diseases comprised in the group of rheumatoid arthritis, osteoarthritis, systemic lupus eritematosus, ulcerative colitis, psoriasis, atopic eczema, atherosclerosis, or in the non degenerative diseases comprised in the group of depression, and cellulites, or in allergies.

12. A method of inhibiting the bodily production of one or more of cytokine IL-1β, cytokine TNFα, prostaglandin PGE2, or expression of

enzymes COX-2 and iNOS, characterized by the fact that it comprises the administration of a therapeutically effective amount of caryophyllenes, to a patient.

13. A method according to one of claims 10 to 12 characterized by the fact that said caryophyllene is one or more of alpha-humulene and trans-caryophyllene.

14. A composition comprising caryophyllene characterized by the fact that the amount of said caryophyllene is about 1 to about 1000mg.

15. A composition according to claim 14 characterized by the fact that said amount is about 10 to about 200 mg.

16. A composition according to claim 14 characterized by the fact that said amount is about 30 to about 100 mg.

17. A composition according to claim 14 characterized by the fact that said caryophyllene comprises about 0.1 to about 99% in weight of said composition.

18. A composition according to claim 14 characterized by the fact that said caryophyllene comprises about 1 to about 70% in weight of said composition.

19. A composition according to claim 14 characterized by the fact that said caryophyllene comprises about 10 to about 40% in weight of said composition.

20. A composition according to one of claims 15 to 19 characterized by the fact that said caryophyllene is one or more of alpha-humulene and trans-caryophyllene.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR 2004/000189

A. CLASSIFICATION OF SUBJECT MATTER
IPC[7]: A61K 31/015, 35/78
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)
IPC[7]: A61K

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)
WPI, EPODOC, TXTE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 7215846 A (TAKEDA CHEM IND LTD) 15 August 1995 (15.08.1995) *abstract (WPI; Acc.No.: 1995-317402).* --	5, 6, 9, 11, 13-20
X	US 2003/0008021 A1 (BABIAH et al.) 9 January 2003 (09.01.2003) *paragraph [0005],[0045], claims 1, 2, 5, 11, 26, 27, 30.* --	1, 2, 5-11, 13-20
X	WO 2004/066912 A2 (TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.) 12 August 2004 (12.08.2004) *claims 1, 2, 12, 23, 24, 26, 28, 52-57.* --	1, 2, 5-11, 13-20

☒ 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 but 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
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 2005 (02.06.2005)	28 June 2005 (28.06.2005)
Name and mailing address of the ISA/ AT **Austrian Patent Office** Dresdner Straße 87, A-1200 Vienna Facsimile No. +43 / 1 / 534 24 / 535	Authorized officer KRENN M. Telephone No. +43 / 1 / 534 24 / 435

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR 2004/000189

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/020371 A2 (F.P.L. PHARMA INC.) 13 March 2003 (13.03.2003) *claims 1, 3.* ---	14-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR 2004/000189

Continuation of first sheet

Continuation No. II:

Observations where certain claims were found unsearchable

(Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reason:

Claims Nos.: 2, 5, 10, 11 because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 2, 5, 10 and 11 refer to a method of treatment of the human/animal body, a search has been carried out and bases on the alleged effects of the compound/composition.

Claims Nos.: 3, 4, 12 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

A second medical use claim (claim 4) has to refer to a concrete disease state and not to physiological processes, e.g. inhibition of an enzyme.
Although claims 3 and 12 refer to a method of treatment of the human/animal body by therapy (see above), no search report has been established for them, because they refer to methods of inhibiting physiological processes and not to the treatment of concrete disease states.

INTERNATIONAL SEARCH REPORT

Information on patent family members

mational application No.
PCT/BR 2004/000189

Patent document cited in search report			Publication date	Patent family member(s)	Publication date
JP	A	7215846A 2		none	
US	A	20030008 021		none	
WO	A	20030203 71		none	
WO	A2	20040669 12	2004-08-12	none	