COMMONWEALTH of AUSTRALIA
Patents Act 1952
APPLICATION FOR A STANDARD PATENT

I/We
Basootherm GmbH
of
Biberach an der Riss, D-7950, Federal Republic of Germany

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

Ophthalmic solution for intraocular pressure adjustment

which is described in the accompanying complete specification.

Details of basic application(s):-

<table>
<thead>
<tr>
<th>Number</th>
<th>Convention Country</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>99128/88</td>
<td>Japan</td>
<td>21 April 1988</td>
</tr>
<tr>
<td>278317/88</td>
<td>Japan</td>
<td>2 November 1988</td>
</tr>
</tbody>
</table>

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

DATED this TWENTY FIRST day of APRIL 1989

To: THE COMMISSIONER OF PATENTS

[Signature]

a member of the firm of DAVIES & COLLISON for and on behalf of the applicant(s)

Davies & Collison, Melbourne
COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952

DECLARATION IN SUPPORT OF CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT

In support of the Application made for a patent for an invention entitled: "OPHTHALMIC SOLUTION FOR INTRAOCULAR PRESSURE ADJUSTMENT"

We, Mr. Ulrich Pitkamin and Prof. Tibor Rozman of Basotherm GmbH of D-7950 Biberach an der Riss Federal Republic of Germany do solemnly and sincerely declare as follows:

1. (a) We are or (b) We are authorized by Basotherm GmbH the applicant for the patent to make this declaration on its behalf.

2. (a) XX or (b) XX

1. Taira OKAMOTO of 10-40, Kojogaoka, Otsu-shi, Shiga-ken, Japan.


The actual inventors of the invention and the facts upon which the applicant is entitled to make the application are as follows:

The applicant would, if a patent were granted on an application made by the said actual inventors, be entitled to have the patent assigned to it. Priority is claimed from the basic applications with the consent of Kaken Pharmaceutical Co., Ltd.

3. The basic application as defined by Section 141 of the Act was made in Japan on the 21 April 1988 by Kaken Pharmaceutical Co., Ltd.

4. The basic application referred to in paragraph 3 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

Declared at Biberach this 3 May 1989

Pitkamin Prof. Rozman

Note: Initial all alterations

DAVIES & COLLISON, MELBOURNE and CANBERRA.
OPHTHALMIC SOLUTION FOR INTRAOCULAR PRESSURE ADJUSTMENT

Title

International Patent Classification(s)

A61K 031/40 A61K 031/135

Application No.: 33274/89

Application Date: 21.04.89

Priority Data

63-99128 21.04.88 JP JAPAN
63-278317 02.11.88 JP JAPAN

Publication Date:

Claim

1. An ophthalmic solution for intraocular pressure adjust-
which comprises racemic mixtures or optically active isomers of

a compound of the formula I

\[
\text{Hal} \quad \text{Hal} \\
\text{H}_2\text{N} \quad \text{Hal} \\
\text{CH-C-N} \\
\text{R}_1 \quad \text{R}_2 \quad \text{R}_3 \quad \text{R}_4 \\
\text{R}_5 \\
\text{Hal} \quad \text{Hal}
\]

wherein each Hal is chlorine or bromine,

\( R_1 \) is hydrogen or hydroxyl,

\( R_2 \) and \( R_3 \) are each hydrogen or alkyl of 1 to 4 carbon
atoms, and

\( R_4 \) and \( R_5 \) are each hydrogen, lower alkyl, lower alkenyl,
lower alkinyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl,
di-lower alkyl-alkyl, lower alkyl-aminolower alkyl, cycloalkyl, phenyl,
benzyl or adamantyl; or, together with each other and the
nitrogen atom to which they are attached, pyrrolidino, lower
alkyl-pyrrolidino, piperidino, lower alkyl-piperidino, piperazino, N'-lower alkyl-piperazino, morpholino, lower alkyl-morpholino, hexamethyleneimino, lower alkyl-hexamethyleneimino, camphidino or lower alkyl-camphidino; or a non-toxic, pharmacologically acceptable acid addition salt thereof,

or racemic mixtures or optically active isomers of a compound of the formula II

wherein

$R_1$ is hydrogen, fluorine, chlorine, bromine, iodine or cyano,

$R_2$ is fluorine, trifluoromethyl, nitro or cyano, and

$R_3$ is alkyl of 3 to 5 carbon atoms, hydroxyalkyl of 3 to 5 carbon atoms, cycloalkyl of 3 to 5 carbon atoms, 1-(3,4-methyleneoxy-phenyl)-2-propyl or 1-(p-hydroxy-phenyl)-2-propyl,

or racemic mixtures or optically active isomers a compound of the formula III

wherein

$R_1$ is hydrogen, halogen or cyano,

$R_2$ is fluorine, cyano, trifluoromethyl, nitro or alkyl of 1 to 4 carbon atoms,
R₃ is alkoxy of 1 to 5 carbon atoms, alkenyloxy of 2 to 5 
carbon atoms, aryloxy of 6 to 10 carbon atoms, aralkoxy of 7 
to 11 carbon atoms or -NR₅R₆ where R₅ and R₆ are 
each hydrogen, alkyl of 1 to 5 carbon atoms, alkenyl of 2 
to 5 carbon atoms, aryl of 6 to 10 carbon atoms or aralkyl 
of 7 to 11 carbon atoms, and

R₄ is cycloalkyl of 3 to 5 carbon atoms or alkyl of 3 to 5 
carbon atoms,

or non-toxic, pharmacologically acceptable acid addition 
salts of the compounds of formulae I, II and III in association 
with ophthalmologically acceptable carriers, diluents and 
excipients.
COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952
COMPLETE SPECIFICATION

NAME & ADDRESS
OF APPLICANT:

Basotherm GmbH
Biberach an der Riss D-7950
Federal Republic of Germany

NAME(S) OF INVENTOR(S):

Taira OKAMOTO
Motoyuki YAJIMA

ADDRESS FOR SERVICE:

DAVIES & COLLISON
Patent Attorneys
1 Little Collins Street, Melbourne, 3000.

COMPLETE SPECIFICATION FOR THE INVENTION ENTITLED:

Ophthalmic solution for intraocular pressure adjustment

The following statement is a full description of this invention, including the best method of performing it known to me/us:-
The present invention relates to a novel ophthalmic solution for intraocular pressure adjustment, and more particularly to an ophthalmic solution for intraocular pressure adjustment useful in the treatment of ocular hypertension and glaucoma.

Prior art and problems to be solved by the invention:

Hitherto, a pilocarpine ophthalmic solution has been employed as an intraocular pressure adjusting agent for use in the treatment of ocular hypertension and glaucoma. It is known however, that whilst the pilocarpine ophthalmic solution decreases the intraocular pressure, it also acts on the sphincter of the pupil and ciliary body and has side effects such as visual darkness due to miosis, accommodation disorders and conjunctival injection. Such side effects give rise to serious problems, particularly for people working in communication and transportation. Also, in case of a middle-aged cataract patient, the side effects increase visual
problems due to miosis. Thus there is a need for the development of intraocular pressure adjusting agents for treating ocular hypertension and glaucoma to replace the pilocarpine ophthalmic solution.

An epinephrine ophthalmic solution was developed on the basis of such a need, but it has side effects such as conjunctival congestion, pain in the eye-brow region or allergic blepharoconjunctivitis and in some cases, it brings about an intraocular pressure rise due to mydriasis. Therefore, the epinephrine ophthalmic solution is not widely used. Also, it has been attempted clinically to use surface anesthetics and psychotropic drugs to produce a decrease of intraocular pressure of the glaucomatous eye, but such drugs have not been put into practical use.

It has recently been observed that various β-receptor blocking agents decrease intraocular pressure when orally administered; subsequently it was found, that bupranolol, timolol, and the like among the β-receptor blocking agents show the above effect also in topical solutions.

However, it is difficult to use them clinically as ophthalmic solutions due to their strong irritating effects. Also, ophthalmic solutions containing β-receptor blocking agents are contraindicated, since asthma is induced when they are applied to asthmatic patients. In some cases, bradycardia is caused.

It is an object of the present invention to provide an ophthalmic solution for intraocular pressure adjustment, which shows no miosis action as is the case for the pilocarpine ophthalmic solutions, and which is not accompanied by a risk that they are contraindicated for asthmatic patients or that bradycardia is caused as is the case when ophthalmic solutions containing β-receptor blocking agents are invol-
ved; and which has a curative effect when applied in small quantities due to their great intraocular pressure reducing activity.

The present invention provides an ophthalmic solution for intraocular pressure adjustment which comprises racemic mixtures or optically active isomers of a compound of the formula I

\[
\text{Hal} \quad \text{Hal} \quad \text{H}_2\text{N} \quad \text{Hal} \\
\text{CH-\text{O-N}} \quad \text{R}_1 \quad \text{R}_2 \quad \text{R}_3 \quad \text{R}_4 \quad \text{R}_5
\]

(I)

wherein each Hal is chlorine or bromine,

\( R_1 \) is hydrogen or hydroxyl,

\( R_2 \) and \( R_3 \) are each hydrogen or alkyl of 1 to 4 carbon atoms, and

\( R_4 \) and \( R_5 \) are each hydrogen, lower alkyl, lower alkenyl, lower alkinyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl, di-lower alkyl-amino-lower alkyl, cycloalkyl, phenyl, benzyl or adamantyl; or, together with each other and the nitrogen atom to which they are attached, pyrrolidino, lower alkyl-pyrrolidino, piperidino, lower alkyl-piperidino, piperazino, \( \text{N'} \)-lower alkyl-piperazino, morpholino, lower alkyl-morpholino, hexamethyleneimino, lower alkyl-hexamethyleneimino, camphidino or lower alkyl-camphidino; or a non-toxic, pharmacologically acceptable acid addition salt thereof,

or racemic mixtures or optically active isomers of a compound of the formula II
wherein

$R_1$ is hydrogen, fluorine, chlorine, bromine, iodine or cyano,

$R_2$ is fluorine, trifluoromethyl, nitro or cyano, and

$R_3$ is alkyl of 3 to 5 carbon atoms, hydroxyalkyl of 3 to 5 carbon atoms, cycloalkyl of 3 to 5 carbon atoms, 1-(3,4-methylenedioxy-phenyl)-2-propyl or 1-(p-hydroxy-phenyl)-2-propyl,

or racemic mixtures or optically active isomers of a compound of the formula III
R₃ is alkoxy of 1 to 5 carbon atoms, alkenyloxy of 2 to 5 carbon atoms, aryloxy of 6 to 10 carbon atoms, aralkoxy of 7 to 11 carbon atoms or -NR₅R₆ where R₅ and R₆ are each hydrogen, alkyl of 1 to 5 carbon atoms, alkenyl of 2 to 5 carbon atoms, aryl of 6 to 10 carbon atoms or aralkyl of 7 to 11 carbon atoms, and

R₄ is cycloalkyl of 3 to 5 carbon atoms or alkyl of 3 to 5 carbon atoms, or non-toxic, pharmacologically acceptable acid addition salts of the compounds of formulae I, II and III in association with ophthalmologically acceptable carriers, diluents and excipients.

Preferably compounds of formula III are selected from 1-(4'-amino-3',5'-dichloro-phenyl)-2-(tert.butylamino)-ethanol-(1), especially preferably 1-(4'-amino-3'-chloro-5'-trifluoromethyl-phenyl)-2-tert.butylamino-ethanol (hereinafter referred to as "mabuterol") or their non-toxic, pharmaceutically acceptable acid addition salts.

The compounds of formula I exhibit bronchodilating, analgesic, sedative, antipyretic, antiphlogistic and anti-tussive activities as well as enhance blood circulation in warm-blooded animals; such compounds have been disclosed in U.S. Patent No. 3 536 712. For instance, the compound 1-(4'-amino-3',5'-dichloro-phenyl)-2-(tert.butylamino)-ethanol-(1), known as clenbuterol, exhibits bronchodilating properties.

The compounds of formula II exert bronchospasmolytic activities and are described by U.S. Patent No. 4 119 710. The compounds of formula III show bronchospasmolytic and anti-asthmatic activities and are described by U.S. Patent No. 4 214 001. However, there has, hitherto, been no literature suggesting that these compounds of formulae I to III exert the pharmacological action as disclosed in the present invention.
Mabuterol, which is 1-(4'-amino-3'-chloro-5'-trifluoromethyl-phenyl)-2-tert.butylamino-ethanol and its non-toxic, pharmacologically acceptable acid addition salts, which is disclosed in U.S. Patent No. 4 119 710, shows excellent results with regard to the new activity which is disclosed in the present specification.

In the ophthalmic solution for intraocular pressure adjustment (hereinafter referred to as "ophthalmic solution") of the present invention, the compounds of formulae I to III, especially mabuterol, may be used in their free form or as a salt thereof. It is preferable to use the compounds, e.g. mabuterol, as ophthalmologically acceptable salts in aqueous solution, since such formulations offer least pain and inconvenience.

The formulations are not limited to an aqueous solution, and the ophthalmic solution can be employed in any form such as an oily collyrium, a sustained release collyrium or a suspension. Moreover, the ophthalmic solution can be in the form of crystals capable of dissolving or being suspended in a suitable solvent before application.

Examples for the salts of the compounds of formulae I to III, e.g. of mabuterol, are, for instance, inorganic acid salts such as phosphoric acid salt, hydrochloric acid salt, sulfuric acid salt and hydrobromic acid salt, organic acid salts such as citric acid salt, maleic acid salt, fumaric acid salt, tartaric acid salt and malic acid salt, and the like. The hydrochlorid acid salt is particularly preferred from viewpoints of economy and stability of the ophthalmic solution.
When an aqueous solution is prepared by employing the above mentioned salts, e.g. of mabuterol, as the active ingredients, it is preferable and convenient that the salts are dissolved in an aqueous solvent such as water, physiological saline or phosphate buffer solution. Also non-aqueous solvents for the preparation of an ophthalmic solution can be used.

In the case of preparing a non-aqueous ophthalmic solution this can be prepared in a manner known per se employing the compounds of formulae I to III, e.g. mabuterol, or the salts thereof as the active ingredient.

It is preferred that the ophthalmic solution of the present invention is administered once or twice daily in a dosage of 1 picogram to 2.0 mg of the active ingredient per day, to adjust intraocular pressure. It is especially preferred that the ophthalmic solution is administered once or twice daily in a dosage of 50 picograms to 5 micrograms per day, especially when mabuterol is used, from the viewpoints of intraocular pressure reduction and the duration of the effect. Further, it is preferred that the concentration of the active ingredient in the ophthalmic solution is from 0.001 to 4.0 % (w/v) by weight, hereinafter the same) from the viewpoint of adjusting the intraocular pressure, more preferably from 0.0002 to 2.0 %; this applies especially to mabuterol.

The ophthalmic solution of the present invention is usually used in a dose of about 1 to 2 drops (35 to 70 µl) to give satisfactory duration of activity and at this dosage shows a remarkable effect in decreasing intraocular pressure. In addition to the above mentioned active ingredients, the ophthalmic solution of the present invention suitably may contain additives usually used in the ophthalmic solutions such as preservative agents e.g. chlorobutanol, sodium dehydroacetic acid, benzalkonium chloride and methyl p-hydroxyben-
zoate, buffering agents e.g. boric acid and borax, viscosity-inducing agents e.g. methyl cellulose (MC), sodium carboxymethyl cellulose (CMC-Na) and chondroitin sulfuric acid and other additives e.g. sodium chloride and polyvinyl alcohol.

Preferably, an ophthalmic solution of the present invention is stored in a refrigerator shielding it from light or in a dark cold place.

The ophthalmic solution of the present invention is more specifically described by means of the following examples. It is to be understood that the present invention is not limited to the examples.
Example 1

Mabuterol hydrochloride was dissolved in phosphate buffer solution of pH 6,7 to give an ophthalmic solution containing 0,0002 %, 0,002 %, 0,02 %, 0,2 % or 2 % of mabuterol hydrochloride (hereinafter referred to as "0,0002 % ophthalmic solution", "0,002 % ophthalmic solution", "0,02 % ophthalmic solution", "0,2 % ophthalmic solution" and 2 % ophthalmic solution" respectively).

Each of the obtained ophthalmic solutions was then subjected to the following test:

Test Example 1 (effect on the decrease of intraocular pressure of normal eyes of the rabbit)

Experimental animals

Thirty male normal adult white rabbits having a body weight of 2,5 to 3,5 kg (Japanese white native species, 6 to 13 months old) were divided into 6 equal groups. In each group, both eyes of each rabbit were used.

Apparatus for measurement

As the apparatus for measurement, an air tonometer (Pneumatic tonometer made by Alcon Laboratories, INC., hereinafter referred to as "PTG") was used. Calibration was carried out in every measurement, and the measurements were carried out every 1 hour by the same man at the same time.

Method to administer using agents

50 µl of 0,0002 %, 0,002 %, 0,02 %, 0,2 % and 2 % ophthalmic solution were measured with a micropipette and each solution was applied to both eyes of each of 5 rab-
bits in each respective group. A phosphate buffer solution having pH 6.7 was applied to both eyes of each rabbit constituting a control group in the same manner as above.

**Test method**

After anesthetising the rabbits by instilling 0.4% of oxyprocaine hydrochloride, intraocular pressures of both eyes of the rabbit were measured three times by using PTG, before the application of the ophthalmic solution and at intervals of 1 hour during 5 hours after application.

**Measurement results**

Effect on intraocular pressure in relation to the dosage of mabuterol

By the above-mentioned measurements a decrease of intraocular pressure was found for ophthalmic solutions containing not less than 0.0002% mabuterol hydrochloride. The decrease in intraocular pressures found after 1 hour after application were 7.0 ± 0.63 mmHg for the 2% ophthalmic solution, 5.2 ± 0.5 mmHg for the 0.2% ophthalmic solution, 3.5 ± 1.06 mmHg for the 0.02% ophthalmic solution, 2.2 ± 1.05 mmHg for the 0.002% ophthalmic solution and 1.13 ± 0.48 mmHg for the 0.0002% ophthalmic solution.

Fig. 1 is a graph showing a relation between the concentrations of mabuterol in the ophthalmic solution (the concentration of the ophthalmic solution) and the intraocular pressure change 1 hour after the application.
Effect on intraocular pressure of mabuterol with the lapse of time

When the 0.0002% ophthalmic solution was used, the intraocular pressure 1 hour after the application had decreased by about 1.0 mmHg, thereafter the intraocular pressure gradually increased to the value existing before the treatment. It was found that the intraocular pressure at 4 hours after the application showed the same pressure as the intraocular pressure of the control group. The remarkable effect on decrease of intraocular pressure by mabuterol disappeared after 4 hours from the instillation when using mabuterol hydrochloride solutions in low concentrations (0.0002% and 0.002%), and it disappeared after 5 hours from application when using mabuterol hydrochloride solutions in middle and high concentrations (0.02%, 0.2% and 2.0%).

Fig. 2a and Fig. 2b are graphs showing a relation between time after instillation and intraocular pressure.

Example 2

Mabuterol hydrochloride was dissolved in phosphate buffer solution having a pH of 6.7 to adjust a concentration thereof to $10^{-4}$ g/ml. The obtained mabuterol hydrochloride solution was used in the following test:

Test Example 2 (influence on dog's heart vessel system)

The mabuterol hydrochloride solution obtained according to Example 2, having a concentration of $10^{-4}$ g/ml, was intravenously administered into the cervical vein of a male dog having a body weight of about 15 kg (beagle, about 13 months old), and the carotid artery pressure was measured using a pressure transducer. Fig. 3 is a sketch of a part of a chart
showing the change in carotid artery pressure by time after administration, recorded in a polygraph by the transducer.

The arrow A shows the point of time when the mabuterol hydrochloride solution was administered.

About 30 seconds after the administration of the mabuterol hydrochloride, the mean blood pressure decreased by 15 mmHg. It can be seen that, although the ophthalmic solution of the present invention decreases the intraocular pressure to a remarkable degree, blood pressure is affected only slightly.

Example 3

An ophthalmic solution having a pH of 6.7 was prepared by dissolving 0.44 g of sodium chloride in 80 ml of a solution prepared by dissolving of 0.303 g of anhydrous potassium dihydrogenphosphate and 0.794 g of disodium hydrogenphosphate (dodecahydrate) in sterile purified water, adding 2 mg of mabuterol hydrochloride and 0.019 g of propyl p-hydroxybenzoate to the solution, adding sterile purified water thereto to adjust the total amount to 100 ml, and sterilising through a filter. The solution contains 0.002 % mabuterol-hydrochloride (w/v). Ophthalmic solutions containing various amounts of mabuterol-hydrochloride and 0.007% benzalkonium chloride, instead of the propyl p-hydroxybenzoate, were prepared similarly (see following Table).
### Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>0,0002 %</th>
<th>0,002 %</th>
<th>0,02 %</th>
<th>2 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ophthalmic solution</td>
<td>0,0002</td>
<td>0,002</td>
<td>0,02</td>
<td>2</td>
</tr>
<tr>
<td>ophthalmic solution</td>
<td>0,002</td>
<td>0,02</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ophthalmic solution</td>
<td>0,61</td>
<td>0,61</td>
<td>0,61</td>
<td>0,61</td>
</tr>
<tr>
<td>ophthalmic solution</td>
<td>0,007</td>
<td>0,007</td>
<td>0,007</td>
<td>0,007</td>
</tr>
<tr>
<td>proper quantity</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
</tr>
<tr>
<td>proper quantity</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
</tr>
<tr>
<td>proper quantity</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
</tr>
<tr>
<td>proper quantity</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
</tr>
</tbody>
</table>

**Note**

*1: mabuterol-hydrochloride  
*2: sodium hydrogenphosphate  
*3: anhydrous sodium dihydrogenphosphate  
*4: benzalkoniumchloride  
*5: sterile purified water ad 100 ml

According to the prescriptions in Table 2, 0,0002 %, 0,002 % and 0,02 % ophthalmic solutions containing β-cyclodextrin were prepared (pH 7).
Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>0.0002 %</th>
<th>0.002 %</th>
<th>0.02 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>ophthalmic</td>
<td>ophthalmic</td>
<td>ophthalmic</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td>solution</td>
<td>solution</td>
</tr>
<tr>
<td>*1</td>
<td>0.0002</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>*2</td>
<td>0.735</td>
<td>0.735</td>
<td>0.735</td>
</tr>
<tr>
<td>*3</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>*4</td>
<td>0.61</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>*5</td>
<td>proper</td>
<td>proper</td>
<td>proper</td>
</tr>
<tr>
<td></td>
<td>quantity</td>
<td>quantity</td>
<td>quantity</td>
</tr>
</tbody>
</table>

Note
*1: mabuterol-hydrochloride
*2: β-cyclodextrin
*3: sodium hydrogenphosphate
*4: anhydrous sodium dihydrogenphosphate
*5: sterile purified water ad 100 ml

Example 4

Stability tests of pharmaceutical preparations

Phosphate buffer solutions (pH 6.7), containing 0.0002 %, 0.002 %, 0.02 % or 2 % mabuterol-hydrochloride according to Example 1 were filled in 5 ml-vessels made of polypropylene and were stored at room temperature, 5°C and 40°C.

With the samples stored at room temperature and at 5°C on the fourteenth day, at 40°C on the twentieth day, the following measurements of pH-value, of the osmotic pressure and of content, besides observations of appearance and of forma-
tion of cleavage products were carried out according to the following conditions:

pH: pH meter (P-7 type, made by Horibasha),
osmotic pressure (mOsm/kg): osmometer OSMSTAT-OM-6020
(made by Kyoto Daiichi Kagakusha),
appearance: visual observation
content (%): spectrophotometer (200-10 types spectrophotometer, made by Hitachi, Ltd.),
confirmation: TLC
  - thin-layer plate (Kieselgel 60F254, made by Merck, 20 cm)
  - solvent for development (chloroform/ethanol/glacial acetic acid (8:1:1)).

In an ophthalmic solution the concentration of which is not more than 0.02 % mabuterol-hydrochloride, no change was observed in each value measured when stored at room temperature, 5°C and 40°C. In a 2 % ophthalmic solution, a change of appearance was observed at room temperature, 5°C and 40°C. After storing at 40°C, a decrease of content of the active substance and cleavage products were observed.

In Table 3, the results of stability tests are shown being carried out at the fourteenth day with ophthalmic solutions according to Example 1 when stored at 5°C and at room temperature. In Table 4, the results of stability tests are shown being carried out on the twentieth day with ophthalmic solutions according to Example 1 when stored at 40°C.
<table>
<thead>
<tr>
<th>Test item</th>
<th>Starting point of the stability test</th>
<th>pH</th>
<th>Osmotic pressure</th>
<th>Appearance</th>
<th>Content</th>
<th>Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mabuterol-hydrochloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 %</td>
<td></td>
<td>6,89</td>
<td>242</td>
<td>clear colorless</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0,0002 %</td>
<td></td>
<td>6,89</td>
<td>241</td>
<td></td>
<td>98,0</td>
<td>single spot</td>
</tr>
<tr>
<td>0,002 %</td>
<td></td>
<td>6,88</td>
<td>240</td>
<td></td>
<td>106,0</td>
<td></td>
</tr>
<tr>
<td>0,02 %</td>
<td></td>
<td>6,88</td>
<td>237</td>
<td></td>
<td>97,6</td>
<td></td>
</tr>
<tr>
<td>sample solution</td>
<td></td>
<td>6,81</td>
<td>356</td>
<td></td>
<td>98,5</td>
<td></td>
</tr>
</tbody>
</table>
After 40 days
5°C

<table>
<thead>
<tr>
<th>Test item</th>
<th>pH</th>
<th>Osmotic pressure</th>
<th>Appearance</th>
<th>Content</th>
<th>Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mabutrol-hydrochloride</td>
<td>6.90</td>
<td>245</td>
<td>no change</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.002 %</td>
<td>6.88</td>
<td>243</td>
<td>&quot;</td>
<td>101.5</td>
<td>no change</td>
</tr>
<tr>
<td>content in</td>
<td>6.87</td>
<td>241</td>
<td>&quot;</td>
<td>102.8</td>
<td>&quot;</td>
</tr>
<tr>
<td>sample solution</td>
<td>6.79</td>
<td>359</td>
<td>*1</td>
<td>101.5</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
After 40 days
Room temperature

<table>
<thead>
<tr>
<th>Test item</th>
<th>pH</th>
<th>Osmotic pressure</th>
<th>Appearance</th>
<th>Content</th>
<th>Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>6.87</td>
<td>246</td>
<td>no change</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mabuterol- 0.0002 %</td>
<td>6.87</td>
<td>242</td>
<td>&quot;</td>
<td>103.1</td>
<td>no change</td>
</tr>
<tr>
<td>hydrochloride 0.002 %</td>
<td>6.87</td>
<td>243</td>
<td>&quot;</td>
<td>105.7</td>
<td>&quot;</td>
</tr>
<tr>
<td>content in 0.02 %</td>
<td>6.87</td>
<td>241</td>
<td>&quot;</td>
<td>99.6</td>
<td>&quot;</td>
</tr>
<tr>
<td>sample solution 2.0 %</td>
<td>6.80</td>
<td>360</td>
<td>*1</td>
<td>101.5</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Note: *1: Slight white muddiness is found when a sample solution is stored at 5°C or at room temperature.
Table 4

<table>
<thead>
<tr>
<th>Storage condition and term</th>
<th>After 20 days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
<td>pH</td>
<td>Osmotic pressure</td>
<td>Appearance</td>
<td>Content</td>
<td>Confirmation</td>
</tr>
<tr>
<td>0 %</td>
<td>6.89</td>
<td>246</td>
<td>clear colorless</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mabuterol - 0,0002 %</td>
<td>6.88</td>
<td>244</td>
<td>&quot;</td>
<td>101.5</td>
<td>single spot</td>
</tr>
<tr>
<td>hydrochloride 0,002 %</td>
<td>6.88</td>
<td>242</td>
<td>&quot;</td>
<td>105.6</td>
<td>&quot;</td>
</tr>
<tr>
<td>content in 0,02 %</td>
<td>6.88</td>
<td>241</td>
<td>&quot;</td>
<td>99.6</td>
<td>&quot;</td>
</tr>
<tr>
<td>sample solution 2,0 %</td>
<td>6.71</td>
<td>325</td>
<td>*1</td>
<td>54.6</td>
<td>cleavage product *2</td>
</tr>
</tbody>
</table>

Note: *1: There are found sedimented white foreign materials which are about azuki-bean-size

*2: Rf-value of 0,2 was found besides a Rf-value of 0,5 which is equal to the Rf-value of mabuterol
Example 5

As reported in Example 1, mabuterol.HCl remarkably reduced normal intraocular pressure (IOP) in rabbits, and the effect gradually diminished over time.

In the following experiments, the drug, which was included in β-cyclodextrin (β-CD) to provide a sustained action, was examined in rabbits with normal as well as elevated IOP:

Materials and Methods

Composition of the eye drop

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mabuterol.HCl</td>
<td>0.02</td>
</tr>
<tr>
<td>B-Cyclo-dextrin</td>
<td>0.002</td>
</tr>
<tr>
<td>Na₂HPO₄.12H₂O</td>
<td>0.0002</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0</td>
</tr>
</tbody>
</table>

pH: 7
osmotic pressure ratio: ca. 0.9
β-CD inclusion: ca. 47%

Normal IOP

50 µl of mabuterol β-CD eye drop (0.02 %, 0.002 %, 0.0002 %) were administered into both eyes of 4 male albino rabbits once daily for 17 days. To a control group of 4 animals a phosphate buffer solution (pH 7) containing β-CD was administered in the same way.

On days 1, 10 and 17, IOP was measured as described in the previous examples.
Water load-induced ocular hypertension

20 ml of water (37°C) were orally administered to rabbits. 50 μl of the mabuterol B-CD eye drop was applied to both eyes of the rabbits immediately after water load.

In Experiment I, the animals received the eye drops once daily for 10 days and on days 1, 5 and 10 IOP was measured after water load. In Experiment II, the animals were treated and water loaded daily for 10 days, and on days 1, 5 and 10 IOP was measured.

Results

Effect on normal IOP

On day 1, the 0.02 % and 0.002 % mabuterol solution but not the 0.0002 % mabuterol solution produced a significant fall in IOP. On days 10 and 17, the drug did not reduce IOP at any concentration examined (Figures 4 and 5).

As particularly shown in Fig. 4, it was found that 0.002 % and 0.02 % mabuterol-hydrochloride ophthalmic solutions showed significant decreases of intraocular pressure in comparison to the control group. The decreases of intraocular pressure after 1 hour from the administration were 2.00 ± 0.42 mmHg in the 0.002 % mabuterol-hydrochloride ophthalmic solution, and 2.63 ± 0.75 mmHg in the 0.02 % mabuterol-hydrochloride ophthalmic solution. Though a tendency to decrease intraocular pressure with a 0.0002 % mabuterol-hydrochloride ophthalmic solution was found, this result was not significant.

Fig. 4 is a graph showing a relation between the time after administration and the intraocular pressure change.
Effect on elevated IOP

Experimental animals
Eight male normal adult white rabbits having a body weight of 2.8 to 3.9 kg (Japanese white native species, 7 to 8 months old) were divided into 2 equal groups. All the eyes in each group were used.

Loading with water
The loading with water was carried out by orally administering of 20 ml of warm water (about 37°C) to eight rabbits.

Method of administration
Exactly 50 µl of 0.02 % mabuterol-hydrochloride ophthalmic solution were administered to both eyes of each rabbit, simultaneously to the loading with water. As a control group, a phosphate buffer solution having a pH of 7.0 which contained β-cyclodextrin was administered to both eyes of each rabbit in the same manner as above.

After administering the solutions the intraocular pressures of both eyes of the rabbit were measured twice by using PTG at intervals of 30 minutes over 2 hours. The 0.02 % mabuterol-hydrochloride ophthalmic solution was administered on each day during ten days, the loading with water was carried out at day one, day five and day ten.
a) Results of Experiment I
IOP in controls increased by about 10 mmHg after water load, whereas it was virtually unchanged in those animals receiving the drug indicating that a water load-induced rise in IOP was significantly inhibited by the drug (two way analysis of variance) (Figure 6). Similar results were obtained on days 5 and 10 (Figures 7 and 8).

b) Results of Experiment II
The eye drops significantly inhibited a water load-induced increase in IOP on days 1, 5 and 10 (Figures 9 and 10).

Example 6

Mabuterol-hydrochloride was dissolved in a physiological saline to give an ophthalmic solution containing 0,0002 % or 0,02 % of the mabuterol-hydrochloride (hereinafter referred to as "0,0002 % mabuterol physiological saline" and "0,02 % mabuterol physiological saline" respectively).

These physiological salines were then subjected to the following tests:

Test to show the influence on blood pressure, heart rate and electrocardiogram

Experimental animals
Fifteen male normal adult white rabbits having a body weight of 3,2 to 3,5 kg (Japanese white native species, 7 to 10 months old) were divided into 3 equal groups. In each group, both eyes of each rabbit were used.
Apparatus for measurement

For the measurements of blood pressure a pressure transducer (trade name: MP-4T, made by Nippon Koden Kogyo K.K.) was used, for recording the heart rate and the electrocardiogram a tachometer in connection with a polygraph (trade-name: RT-5, made by Nippon Koden Kogyo K.K.) were used. Measurements were carried out every hour by the same man at the same time.

Method for administration

50 μl of a 0.0002 % or 0.02 % mabuterol-hydrochloride containing physiological saline were carefully measured with a micropipette; the solution was administered to both eyes of each of rabbit test group. To a control group only physiological saline was administered to both eyes of each rabbit in the same manner as above.

Test method

Blood pressure, heart rate and electrocardiogram were measured 5 and 10 minutes after the administration and, then, at intervals of 1 hour, over the length of 6 hours. The administration was carried out once a day during 8 days. The measurement was carried out on the first day and the eighth day.

Measurement results

At the first day, though the blood pressure had a tendency to decrease after administration of a 0.002 % mabuterol-hydrochloride solution, a significant difference could not be observed. However, a significant increase of the heart rate was found. At the eighth day the same result as at the first day was observed. As to electrocardiogram, there was no remarkable change observable either on the first day or on the eighth day. These results show that the ophthalmic solutions containing mabuterol do not remarkably influence blood pressure (shown in Figs. 11 and 12).
Figs. 11 and 12 are graphs showing a relation between time after administration and intraocular pressure and between time after administration and heart rate at the first day and the eighth day respectively. Fig. 13 is a graph showing the results according to Figs. 11 and 12 for each of the ophthalmic solutions used, enabling a better comparison.

In each graph each point shows a mean value of 5 rabbits. In the following Table 5, the blood pressures and the heart rates of subjected animals at the first day and the eighth day are shown in comparison to controls, when a 0,002 % and 0,02 % mabuterol-hydrochloride solution had been administered.

Table 5

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0,0002 % mabuterol-hydrochloride solution</th>
<th>0,002 % mabuterol-hydrochloride solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood pressure (mmHg) at the first day</td>
<td>89,6 ± 5,7</td>
<td>90,0 ± 3,2</td>
<td>99,0 ± 6,0</td>
</tr>
<tr>
<td>blood pressure (mmHg) at the eighth day</td>
<td>87,0 ± 2,5</td>
<td>87,0 ± 4,6</td>
<td>94,0 ± 4,0</td>
</tr>
<tr>
<td>heart rate (beats/min) at the first day</td>
<td>233,2 ± 10,1</td>
<td>217,0 ± 11,4</td>
<td>243,0 ± 6,6</td>
</tr>
<tr>
<td>heart rate (beats/min) at the eighth day</td>
<td>243,0 ± 5,8</td>
<td>230,0 ± 8,8</td>
<td>233,0 ± 5,4</td>
</tr>
</tbody>
</table>
Example 7

Effect on ocular-mucous membrane

Ophthalmic solutions, as described in Example 1, were used in the following test:

Acute repeated administration
Twelve male normal adult white rabbits having a body weight of 2.8 to 3.2 kg (Japanese white native species, 7 to 8 months old) were divided into 4 groups. In each group, 3 eyes were used.

50 μl of a phosphate buffer solution having a pH of 6.7 and containing 0.0002 %, 0.02 % or 2 % mabuterol-hydrochloride obtained according to Example 1 were exactly measured with a micropipette, and this solution was administered to one eye of each rabbit 9 times a day (every 1 hour) for three successive days.

With each concentration of the ophthalmic solution applied, no abnormality could have been found with regard to the external eye, its fundus and the pathological histology of the eyeball.

Administration for two successive weeks
Twelve male normal adult white rabbits having a body weight of 2.8 to 3.2 kg (Japanese white native species, 7 to 8 months old) were divided into 4 groups. In each group, 3 eyes were used.

50 μl of 0.0002 %, 0.002 % and 0.02 % mabuterol-hydrochloride ophthalmic solution obtained according to Example 1 were exactly measured with a micropipette. The solution was administered to one eye of each rabbit 3 times a day (every 3 hours) for fourteen successive days. No external ocular ab-
normality could be found; the fundus and the pathological histology of the eyeball appeared normal.

Effect of the invention:

The ophthalmic solution for intraocular pressure adjustment according to the present invention containing mabuterol as the active ingredient shows a strong effect in decreasing intraocular pressure. Accordingly, it will be very effective as an ophthalmic solution for intraocular pressure adjustment for the treatment of ocular hypertension and glaucoma. Moreover, the ophthalmic solution shows no myosis action as it is the case with pilocarpine ophthalmic solutions, it lacks the problems that would contraindicate it for asthmatic patients or that would give rise to bradycardia, as is the case for ophthalmic solutions containing β-receptor blocking agents.

Therefore, the ophthalmic solution of the present invention will be clinically valuable as an ophthalmic solution for intraocular pressure adjustment for use in treating ocular hypertension and glaucoma.
CLAIMS
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An ophthalmic solution for intraocular pressure adjust-
   which comprises racemic mixtures or optically active isomers of
   a compound of the formula I

\[
\begin{array}{c}
\text{Hal} \\
\text{H}_2\text{N} \\
\text{Hal} \\
\end{array}
\begin{array}{c}
\text{R}_4 \\
\text{R}_3 \\
\text{R}_5 \\
\text{R}_1 \\
\text{R}_2 \\
\end{array}
\begin{array}{c}
\text{CH-C-N} \\
\end{array}
\]

wherein each Hal is chlorine or bromine,

- \( \text{R}_1 \) is hydrogen or hydroxyl,
- \( \text{R}_2 \) and \( \text{R}_3 \) are each hydrogen or alkyl of 1 to 4 carbon atoms, and
- \( \text{R}_4 \) and \( \text{R}_5 \) are each hydrogen, lower alkyl, lower alkenyl, lower alkinyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl, di-lower alkyl-amino-lower alkyl, cycloalkyl, phenyl, benzyl or adamantyl; or, together with each other and the nitrogen atom to which they are attached, pyrrolidino, lower alkyl-pyrrolidino, piperidino, lower alkyl-piperidino, piperazino, N'-lower alkyl-piperazino, morpholino, lower alkyl-morpholino, hexamethylenimino, lower alkyl-hexamethylenimino, camphidino or lower alkyl-camphidino; or a non-toxic, pharmacologically acceptable acid addition salt thereof,

or racemic mixtures or optically active isomers of a com-

 pound of the formula II
wherein

$R_1$ is hydrogen, fluorine, chlorine, bromine, iodine or cyano,

$R_2$ is fluorine, trifluoromethyl, nitro or cyano, and

$R_3$ is alkyl of 3 to 5 carbon atoms, hydroxyalkyl of 3 to 5 carbon atoms, cycloalkyl of 3 to 5 carbon atoms, 1-(3,4-methyleneoxy-phenyl)-2-propyl or 1-(p-hydroxy-phenyl)-2-propyl,

or racemic mixtures or optically active isomers a compound of the formula III

wherein

$R_1$ is hydrogen, halogen or cyano,

$R_2$ is fluorine, cyano, trifluoromethyl, nitro or alkyl of 1 to 4 carbon atoms,
R₃ is alkoxy of 1 to 5 carbon atoms, alkenyloxy of 2 to 5 carbon atoms, aryloxy of 6 to 10 carbon atoms, aralkoxy of 7 to 11 carbon atoms or -NR₅R₆ where R₅ and R₆ are each hydrogen, alkyl of 1 to 5 carbon atoms, alkenyl of 2 to 5 carbon atoms, aryl of 6 to 10 carbon atoms or aralkyl of 7 to 11 carbon atoms, and

R₄ is cycloalkyl of 3 to 5 carbon atoms or alkyl of 3 to 5 carbon atoms,

or non-toxic, pharmacologically acceptable acid addition salts of the compounds of formulae I, II and III in association with ophthalmologically acceptable carriers, diluents and excipients.

2. An ophthalmic solution for intraocular pressure adjustment comprising as an active ingredient 1-(4'-amino-3'-chloro-5'-trifluoromethyl-phenyl)-2-tert.butylaminoethanol or a non-toxic, pharmacologically acceptable acid addition salt thereof.

3. An ophthalmic solution according to claim 1 or 2 in the form of an aqueous solution.

4. An ophthalmic solution according to claim 3, containing physiological saline and phosphate buffers.

5. An ophthalmic solution according to claims 3 and 4, containing a preservative.

6. An ophthalmic solution according to claims 3 to 5 containing means for providing a sustained action.

7. An ophthalmic solution according to claim 6, wherein as means for providing a sustained action β-cyclodextrin is used.
8. Use of compounds of formulae I, II or III, as defined in claim 1, or non-toxic pharmacologically acceptable acid addition salts thereof in the treatment of ocular conditions.

9. Use of 1-(4'-amino-3'-chloro-5'-trifluoromethylphenyl)-2-tert.butylamino-ethanol or a non-toxic pharmacologically acceptable acid addition salt thereof in the treatment of ocular conditions.

10. A method of treating ocular conditions in a subject which comprises administering to the said subject one or more compounds selected from compounds of formulae (I), (II) and (III) as defined in claim 1.

11. The use of a compound of formula (I), (II) or (III) as defined in claim 1 in the preparation of a medicament for the treatment of ocular conditions.
12. An ophthalmic solution according to claim 1, or use thereof substantially as hereinbefore described with reference to the accompanying examples.

13. The steps, features, compositions and compounds disclosed herein or referred to or indicated in the specification and/or claims of this application, individually or collectively, and any and all combinations of any two or more of said steps or features.

DATED this TWENTY FIRST day of APRIL 1989

Basotherm GmbH

by DAVIES & COLLISON
Patent Attorneys for the applicant(s)
FIG. 1.

intraocular pressure change (mmHg)

0  0.002  0.02  0.2  2.0
concentration in ophthalmic solution (%)

FIG. 3.

carotid artery pressure (mmHg)

1 min.
FIG. 2a.

- - control
- - 0.0002 % ophthalmic solution
- - 0.002 % ophthalmic solution

intraocular pressure change (mmHg)

0  1  2  3  4  
time after instillation (hour)
FIG. 2b.

- - - - control
- - - - 2.0% ophthalmic solution
- - - - 0.2% ophthalmic solution
- - - - 0.02% ophthalmic solution

Intraocular pressure change (mmHg)

Time after instillation (hour)
FIG. 4.

Ocular hypotensive effect of β-CD mabuterol (day 1)

- Control
- 0.02% Mabuterol
- 0.002%
- 0.0002%
FIG. 5.

Ocular hypotensive effect of β-CD mabuterol (day 10)

- Control
- 0.02% Mabuterol
- 0.002%
- 0.0002%
Effect on water load-induced ocular hypertension (day 1)
FIG. 7.

Effect of water load-induced ocular hypertension (day 5)

- Control
- 0.02% Mabuterol

Change of IOP (mmHg)

0 30 60 90 120 min.

(n=6)  (n=8)
**FIG. 8.**

- Control
- 0.02% Mabuterol

**Effect on water load-induced ocular hypertension (day 10)**

Change of IOP (mmHg)

(n=6)  
(n=8)
FIG. 9.

Effect on water load-induced hypertension (5 days of instillation and loading)

- Control
- 0.02% Mabuterol

Change of 10P (mm Hg)

Effect on water load-induced hypertension (5 days of instillation and loading)
FIG. 10.

- Control
- 0.02% Mabuterol

Effect on water load-induced hypertension (10 days of instillation and loading)
FIG. 11.

○: control (physiological salt solution)
・: 0.0002 % mabuterol physiological salt solution
△: 0.02 % mabuterol physiological salt solution
FiG. 12.

- ○: control (physiological salt solution)
- •: 0.0002% mabuterol physiological salt solution
- ▲: 0.02% mabuterol physiological salt solution

Time after instillation (hour)

blood pressure change (mmHg)

heart rate change (beats/min)
FIG. 13.

blood pressure change (mmHg)  

heart rate change (beats/min)

○: blood pressure on the first day (mmHg)  
●: blood pressure on the eight day (mmHg)  
□: heart rate on the first day (beats/min)  
■: heart rate on the eight day (beats/min)