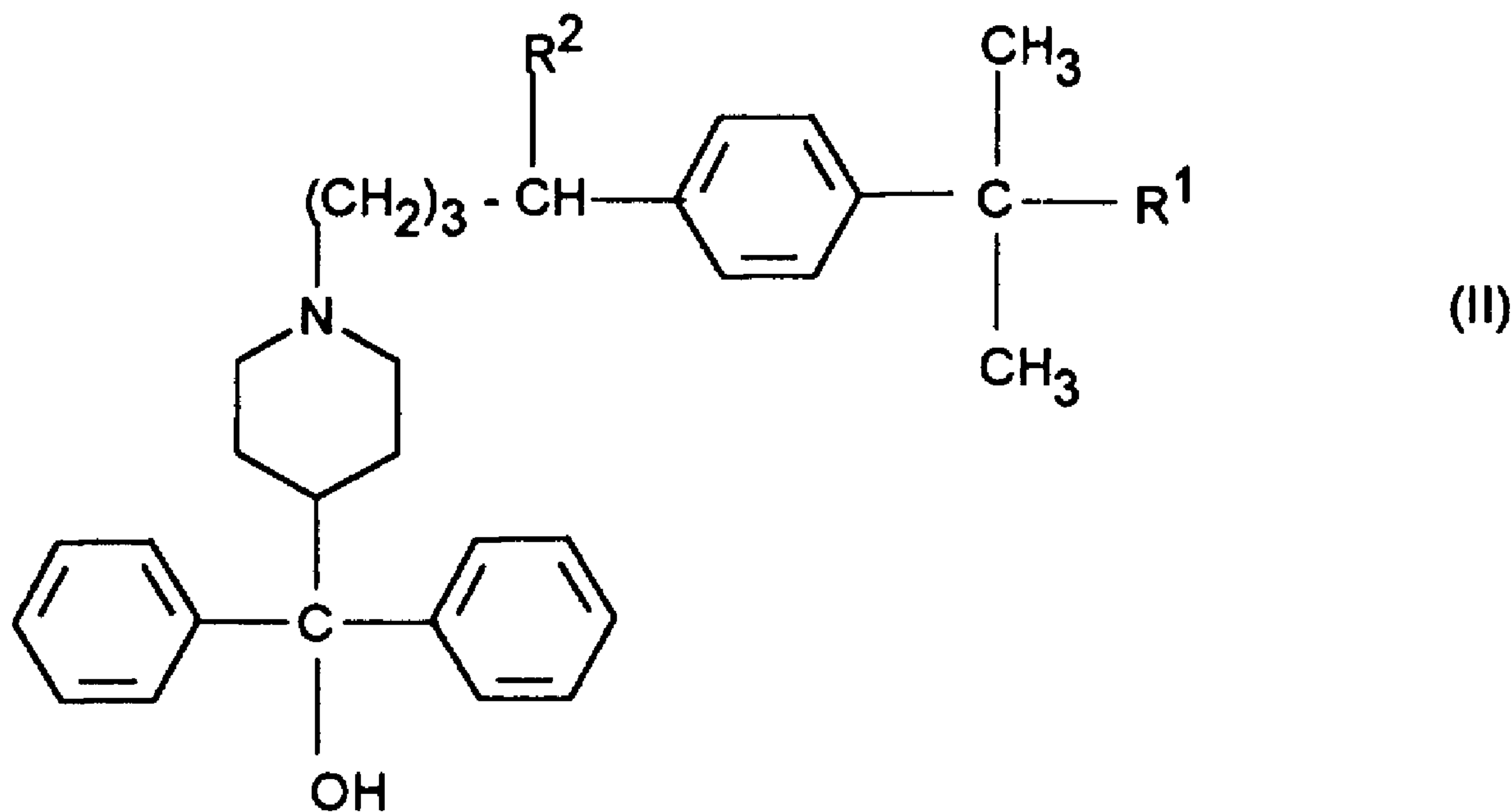




(22) Date de dépôt/Filing Date: 1998/03/09  
(41) Mise à la disp. pub./Open to Public Insp.: 1998/09/11  
(45) Date de délivrance/Issue Date: 2007/10/30  
(30) Priorités/Priorities: 1997/03/11 (DE19709898.3);  
1997/11/21 (DE19751498.7)

(51) Cl.Int./Int.Cl. *C12P 17/12* (2006.01),  
*A61K 31/675* (2006.01), *C07F 9/59* (2006.01)  
(72) Inventeurs/Inventors:  
MEIWES, JOHANNES, DE;  
WORM, MANFRED, DE  
(73) Propriétaire/Owner:  
AVENTIS PHARMA DEUTSCHLAND GMBH, DE  
(74) Agent: BERESKIN & PARR

(54) Titre : METHODE POUR PREPARER L'ACIDE 4-(4-(4-(HYDROXYDIPHENYL)-1-PIPERIDINYL)-1-HYDROXY-  
BUTYL)- $\alpha,\alpha$ -DIMETHYLPHENYLACETIQUE ET SES DERIVES PHOSPHORYLES  
(54) Title: PROCESS FOR THE PREPARATION OF 4-(4-(4-(HYDROXYDIPHENYL)-1-PIPERIDINYL)-1-  
HYDROXYBUTYL)- $\alpha,\alpha$ -DIMETHYLPHENYLACETIC ACID AND PHOSPHORYLATED DERIVATIVES



(57) Abrégé/Abstract:

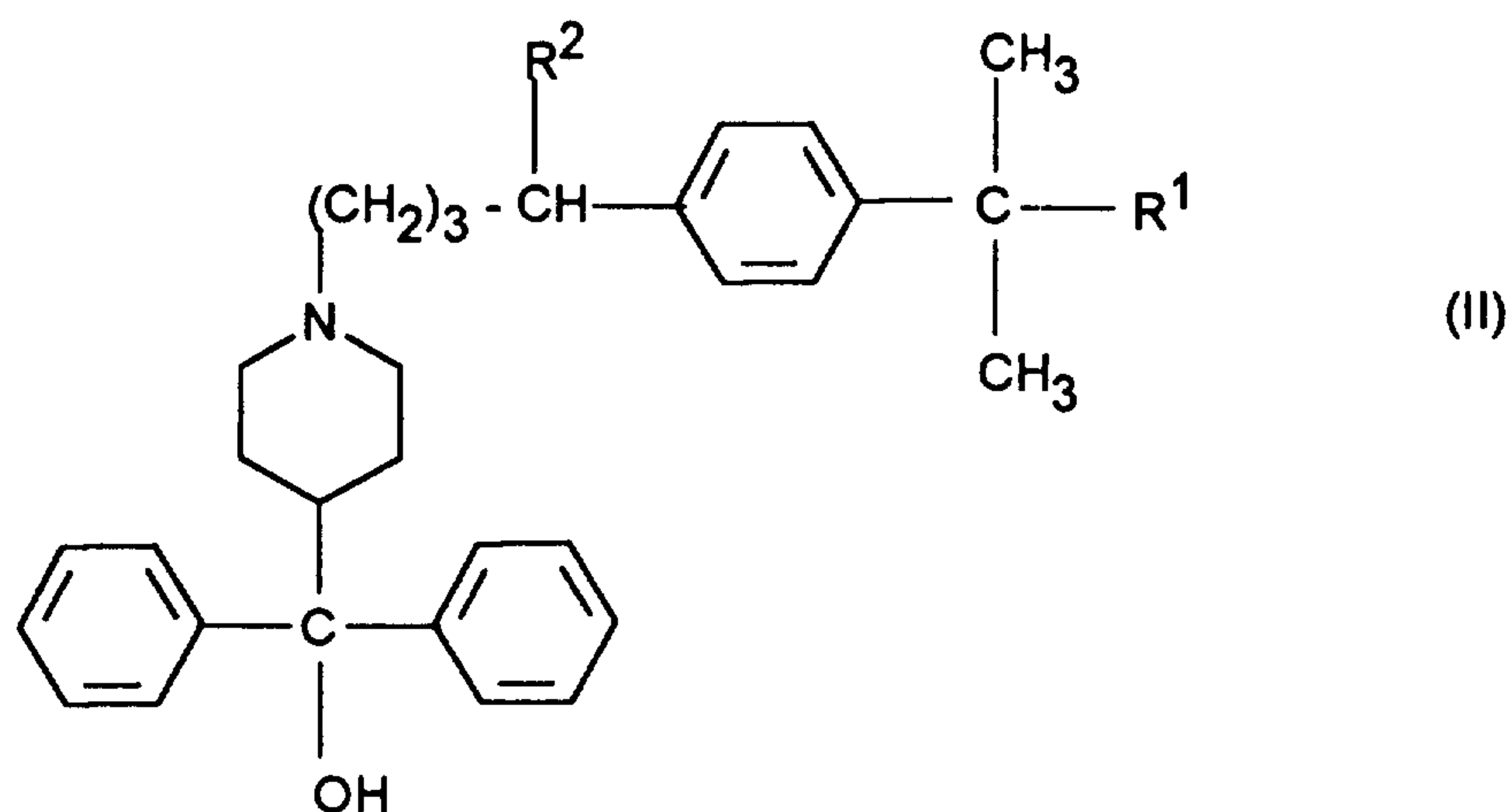
The compound 4-(4-(4-(hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylphenylacetic acid and its phosphorylated derivatives can be prepared from  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol with the aid of fungi of the genus *Cunninghamella* or *Absidia*. Compounds of the formula II (see formula II) in which R<sup>1</sup> is -CH<sub>2</sub>-O-P(O)(OH)<sub>2</sub> and R<sup>2</sup> is -OH, R<sup>1</sup> is -CH<sub>3</sub> and R<sup>2</sup> is -O-P(O)(OH)<sub>2</sub> or R<sup>1</sup> is -COOH and R<sup>2</sup> is -O-P(O)(OH)<sub>2</sub> are suitable as pharmaceuticals with antihistamine action.

## Abstract

Process for the preparation of 4-(4-(4-(hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylphenylacetic acid and phosphorylated derivatives

The compound 4-(4-(4-(hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylphenylacetic acid and its phosphorylated derivatives can be prepared from  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol with the aid of fungi of the genus *Cunninghamella* or *Absidia*.

Compounds of the formula II



in which  $R^1$  is  $-\text{CH}_2-\text{O}-\text{P}(\text{O})(\text{OH})_2$  and  $R^2$  is  $-\text{OH}$ ,  $R^1$  is  $-\text{CH}_3$  and  $R^2$  is  $-\text{O}-\text{P}(\text{O})(\text{OH})_2$  or  $R^1$  is  $-\text{COOH}$  and  $R^2$  is  $-\text{O}-\text{P}(\text{O})(\text{OH})_2$  are suitable as pharmaceuticals with antihistamine action.

Hoechst Marion Roussel Deutschland GmbH HMR 97/L 242K

Dr. TH/St

## Description

- 5 Process for the preparation of 4-(4-(4-(hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylphenylacetic acid and phosphorylated derivatives

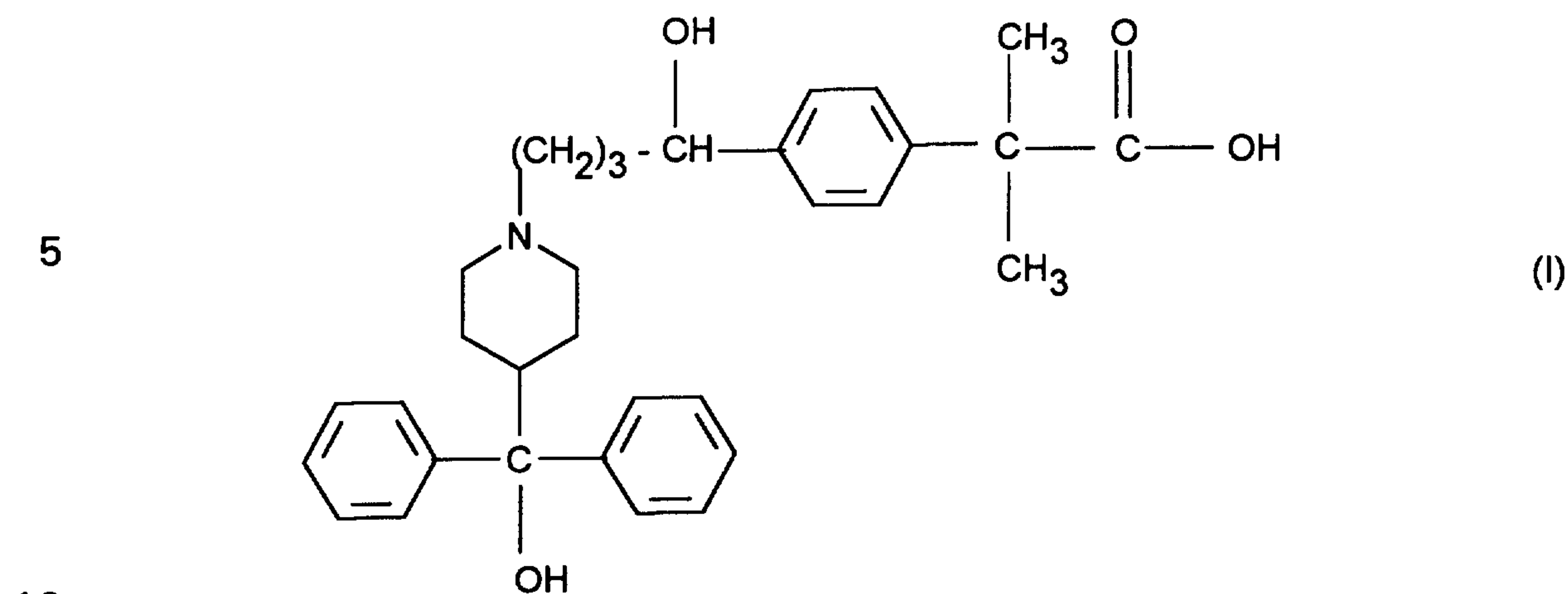
The invention relates to a microbial process for the preparation of 4-(4-(4-(hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylphenylacetic acid  
10 (compound 1) and phosphorylated derivatives and phosphorylated derivatives of  $\alpha$ -(p-tertiary-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol (compound 2), and their use as pharmaceuticals. The invention furthermore relates to a microbial process for the phosphorylation of the compound 1.

- 15 It is known that compound 2 is converted into compound 1 in the human body (DE 23 03 306, US 4,254,129, US 4,285,957, DE 30 07 498). It is furthermore known that ebastine is oxidized to carebastine with the aid of microorganisms, for example of the genus *Cunninghamella* (DE 40 34 218; Schwarz et al. Appl. Microbiol. Biotechnol. (1996) 44: pages 731-735).

20 It has now been found that fungi of the genera *Cunninghamella* and *Absidia* convert the compound 2 selectively into the compounds of the formula I or II. The selective oxidation only at the p-tertiary-butylphenyl radical of the compound 2 to give the corresponding carboxyl group is surprising, since the two hydroxyl groups in the  
25 compound 2 which could also be oxidized are not oxidized.

The invention therefore relates to a process for obtaining the compound of the formula I

2



which comprises incubating  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol with a fungus of the genus *Cunninghamella* or *Absidia*.

15 Preferably, a fungus from the group consisting of *Cunninghamella blakesleeana*, *Cunninghamella elegans* and *Cunninghamella echinulata*, in particular ATCC 8688a, DSM 1905, DSM 1908 and ATCC 9244, is employed. Mutants and selectants of the fungi of the genus *Cunninghamella* are furthermore suitable for use in the process according to the invention as long as they convert the compound 2 into a compound of the formula I and/or II. Fungi of the genus *Cunninghamella* can

20 furthermore be employed for the phosphorylation of the compound 1.

The nutrient solution contains carbon sources such as sucrose, corn starch, dextrose or molasses and nitrogen sources such as soybean flour, groundnut flour, malt extract or ammonium acetate.

25 The nutrient medium also contains inorganic salts such as sodium hydrogenphosphate, sodium chloride, calcium chloride, calcium sulphate, calcium carbonate, magnesium sulphate or potassium hydrogen-phosphate. Furthermore, fat such as methyl oleate or soybean oil can be added to the nutrient medium. In

30 addition, trace elements such as iron, manganese, copper, zinc, cobalt or other metal salts are also added.

The fungi are cultured at temperatures from 20°C to 35°C, preferably at 28°C and at

pHs from 5 to 9, preferably at pH 8. Culturing is carried out aerobically, first in shaker flasks and then in a fermenter with stirring and aeration with air or pure oxygen. Culturing of the microorganisms in the fermenters is carried out over a time interval of 48 to 240 hours, preferably from 70 to 110 hours.

5

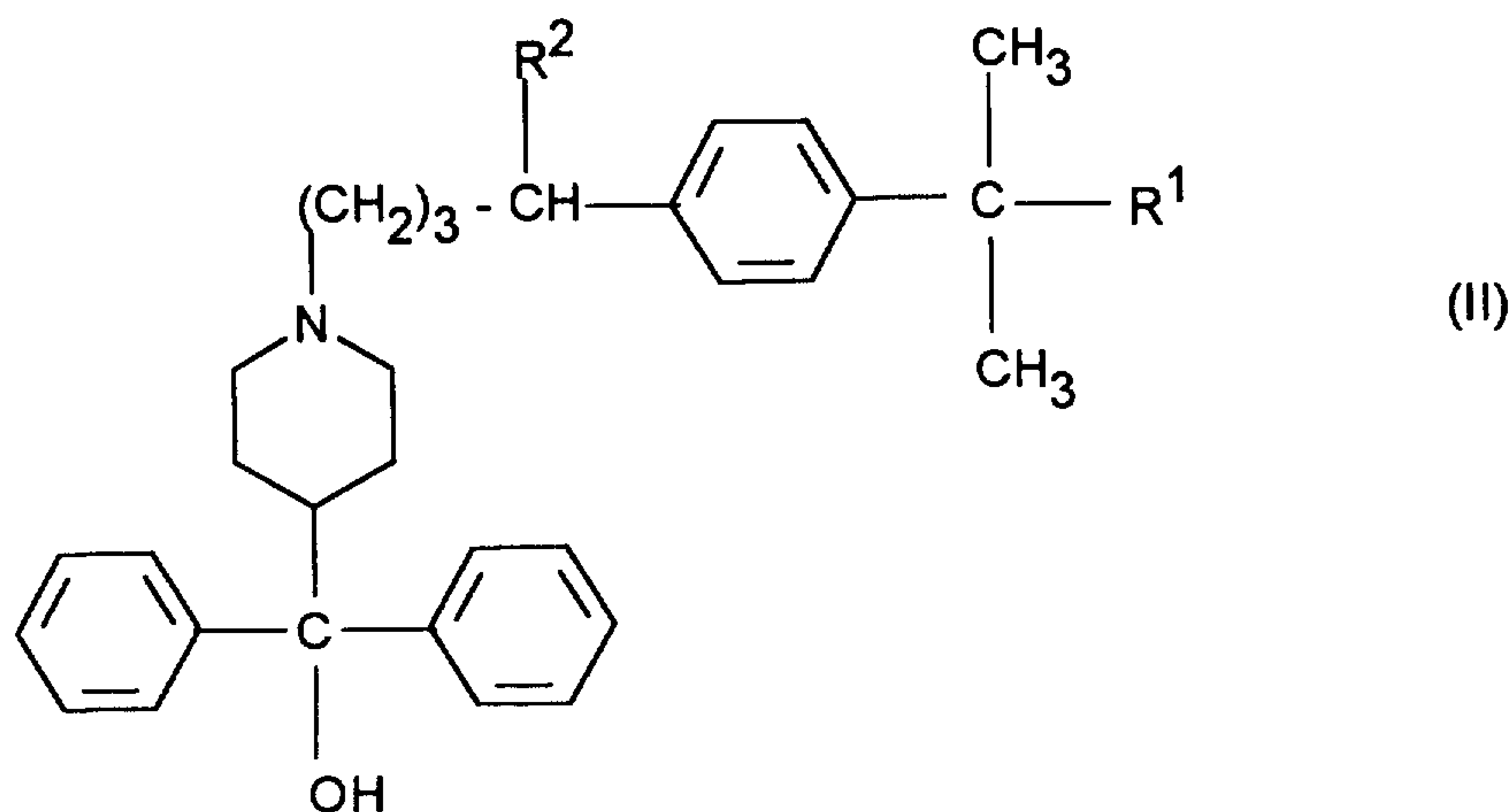
The addition of the compound 2 is carried out directly at the start of culturing but can also be carried out later, preferably after approximately 48 hours. The addition of the compound 2 is carried out as a solid substance, as a suspension or in solution.

10 Suitable solvents are ethanol, dimethylformamide (DMF), but also dimethyl sulfoxide (DMSO), dimethylacetamide (DMA), dimethoxyethane (DME), tetrahydrofuran (THF) and also dibutyl-, diisopropyl- or diethylformamide, 1-methyl-, 1-ethyl- or 1-cyclohexylpyrrolidone, 4-formylmorpholine, 1-formylpiperidine, 1-formylpyrrolidine,  
 15 oxide, sulfolane or N-methylcaprolactam or mixtures of the solvents mentioned.

Solubilizers such as ®Tween 80 or sodium dodecylsulfate can also be added.

20 The compound 1 is isolated directly from the nutrient solution or after separation of the cells, for example by centrifugation or filtration. The compound 1 can be isolated by extraction with solvents or by adsorption on hydrophobic resins such as XAD 16, HP 20 or ion exchangers.

25 The invention furthermore relates to a process for the preparation of the compound of the formula II



30

in which

$R^1$  is  $-OH$  or  $-CH_2-O-P(O)(OH)_2$  and  $R^2$  is  $-OH$ ,

$R^1$  is  $-CH_3$  and  $R^2$  is  $-O-P(O)(OH)_2$  or

$R^2$  is  $-O-P(O)(OH)_2$  and  $R^1$  is  $-C(O)OH$ ,

- 5 which comprises incubating  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol or, if appropriate, 4-(4-(4-(hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylacetic acid with a fungus of the genus *Cunninghamella* or *Absidia*.
- 10 The reaction is carried out essentially as in the process for the preparation of the compound of the formula I. For the preparation of the compound of the formula II in which  $R^1$  is  $-COOH$  and  $R^2$  is  $-O-P(O)(OH)_2$ , compound 1 is preferably employed as a substrate for the reaction.
- 15 The invention furthermore relates to novel compounds of the formula II, in which  $R^1$  is  $-CH_2-O-P(O)(OH)_2$  and  $R^2$  is  $-OH$  (compound 3),  $R^1$  is  $-CH_3$  and  $R^2$  is  $-O-P(O)(OH)_2$  (compound 4) or  $R^2$  is  $-O-P(O)(OH)_2$  and  $R^1$  is  $-COOH$ .
- 20 The invention furthermore relates to a process for the preparation of the compound of the formula II in which  $R^1$  is  $-OH$  and  $R^2$  is  $-OH$  (triol), which comprises incubating  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol with a fungus of the genus *Cunninghamella* or *Absidia*.
- 25 The invention also relates to pharmaceuticals comprising an efficacious amount of at least one compound of the formula II and/or of a physiologically tolerable salt of the compound of the formula II, together with a pharmaceutically suitable and physiologically tolerable excipient.
- 30 The invention also includes pharmaceutically tolerable salts of the compound of the formula II. Pharmaceutically tolerable acid addition salts of compounds according to the invention are those with suitable inorganic or organic acids. Suitable inorganic acids are, for example, hydrochloric acid, hydrobromic acid, sulfuric acid and

phosphoric acid. Suitable organic acids include carboxylic acids such as acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, malonic acid, succinic acid, fumaric acid, malic acid, tartaric acid, citric acid, cyclamen acid, ascorbic acid, hydroxymaleic acid, dihydroxymaleic acid, benzoic acid, phenylacetic acid, 4-aminobenzoic acid, 4-hydroxybenzoic acid, anthranilic acid, cinnamic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid and mandelic acid, sulfonic acids such as methanesulfonic acid, ethanesulfonic acid and  $\beta$ -hydroxyethanesulfonic acid. Nontoxic salts of the compounds of the above formulae with inorganic or organic bases also come within the scope of the invention. These include, for example, the salts with alkali metals such as sodium, potassium and lithium, with alkaline earth metals such as calcium and magnesium, light metals of group IIIA such as aluminum, with organic amines such as primary, secondary and tertiary amines, for example cyclohexylamine, ethylamine, pyridine, methylaminoethanol and piperazine. The salts are formed in a conventional manner, for example by reacting a compound of the formula II with the appropriate acid or base.

On account of the pharmacological properties, the compounds of the formula II according to the invention are suitable as antihistamines, antiallergens and bronchodilators.

The compounds of the formula II according to the invention are suitable for the treatment of allergic rhinitis, asthma or other allergic disorders.

The compounds of the formula II according to the invention can be administered orally, parenterally, for example subcutaneously, intravenously, intramuscularly or intraperitoneally, by intranasal introduction or application to mucous membranes, for example of the nose, of the throat or of the bronchial tract, for example in the form of an aerosol spray which contains small particles of a compound according to the invention in mist or powder form.

The amounts of the compounds to be administered depend on the patient and the manner of administration. The amount to be administered can vary within a wide

range, such that dose units having an efficacious amount of approximately 0.01 to 20 mg/kg of body weight/day for achieving the desired effect result. For example, the desired antihistaminic, antiallergenic or bronchodilating action can be achieved by absorption of a dose unit such as, for example, a tablet having 5 to 300 mg of the compound according to the invention, preferably 10 to 200 mg, which is taken 1 to 4 times daily.

The invention also relates to a process for the production of a pharmaceutical, which comprises bringing at least one compound of the formula II into a suitable administration form with a pharmaceutically suitable and physiologically tolerable carrier and, if appropriate, further suitable active compounds, additives or auxiliaries.

Suitable solid or pharmaceutical preparation forms are, for example, granules, powders, coated tablets, tablets, (micro)capsules, suppositories, syrups, juices, suspensions, emulsions, drops or injectable solutions and also preparations with protracted release of active compound, in whose preparation are used customary auxiliaries, such as excipients, disintegrants, binders, coating agents, swelling agents, glidants or lubricants, flavorings, sweeteners and solubilizers. Frequently used auxiliaries which may be mentioned are magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, lactoprotein, gelatin, starch, cellulose and its derivatives, animal and vegetable oils such as cod liver oil, sunflower oil, groundnut or sesame oil, polyethylene glycol and solvents such as, for example, sterile water and mono- or polyhydric alcohols such as glycerol.

The compounds of the formula II according to the invention can also be administered as injectable solutions or suspensions in a physiologically tolerable diluent with a pharmaceutical carrier which is a sterile liquid such as water or an oil, it being possible to add surface-active and other pharmaceutically permissible auxiliaries. Examples of suitable oils are those of petroleum, animal, vegetable or synthetic origin such as groundnut oil, soybean or mineral oil. In general, water, saline solution, aqueous dextrose solution or similar sugar solutions and glycols such as propylene glycol or polyethylene glycol are preferred as liquid carriers, in

particular for injectable solutions.

For use as aerosols, the compounds according to the invention are put under pressure in solution or suspension into an aerosol container together with suitable  
5 propellants, for example hydrocarbon propellants such as propane, butane or isobutane, or carbon dioxide or nitrogen or other ecologically permissible propellants and customary auxiliaries. The compounds can also be administered in unpressurized form, for example using atomizers.

10 For administration, possible animals are warm-blooded animals, birds, mammals, for example humans, cats, dogs, horses, sheep, cattle, cows, pigs, lambs, rats, mice and guinea-pigs.

#### 15 Example 1

##### Maintenance of the strain

100 µl of a spore suspension of the Cunninghamella strains Cunninghamella  
blakesleeana FH 001, Cunninghamella blakesleeana FH 002, Cunninghamella  
20 blakesleeana FH 003, Cunninghamella echinulata DSM 1905, Cunninghamella echinulata variant elegans ATCC 8688a, Cunninghamella elegans DSM 1908 and Cunninghamella echinulata variant echinulata ATCC 9244 are plated out on an agar plate (20 g/l of malt extract, 2 g/l of peptone and 20 g/l of agar pH 6.5) and incubated at 28°C for 72 h. The plate can then be stored at 4°C for at least eight  
25 weeks.

Alternatively, the strains can be subjected to submerge culture in the same medium without addition of agar. After culturing for 72 h at 28°C, the culture is treated with 20% glycerol and the suspension is stored at approximately -120°C over liquid nitrogen.

30

## Example 2

## High-pressure liquid chromatography (HPLC) analysis

5	Sample preparation :	Dilute culture broth 1:1 with methanol and stir suspension for 30 minutes. Separation of the clear phase by centrifugation.	
	Eluent A :	900 ml of	H <sub>2</sub> O
		100 ml of	acetonitrile
		1 ml of	trifluoroacetic acid (TFA)
10	Eluent B :	900 ml of	acetonitrile
		100 ml of	H <sub>2</sub> O
		1 ml of	TFA
	Detection :	215 nm	
	Flow rate :	1 ml/min	
15	Gradient :	0 min	50% B
		10 min	80% B
		15 min	80% B
		16 min	50% B
		21 min	50% B
20	Column temperature :	55°C	
	Column :	Merck RP 18 5 µm Licrospher™ 100	
		Length 25 cm Diameter 4 mm	
	Retention times :	Compound 1 5.4 minutes (min)	
		Compound 2 9 min	
25		Triol	5.7 min
		Compound 3 3.5 min	
		Compound 4 6.9 min	

## Example 3

30

## Screening with Cunninghamella strains

100 ml of screening medium (5 g/l of soybean peptone, 5 g/l of yeast extract, 5 g/l of NaCl, 5 g/l of K<sub>2</sub>HPO<sub>4</sub>, 3 g/l of agar and 20 g/l of glucose / separately autoclaved,

pH 5.0 in an Erlenmeyer flask of 1000 ml volume) were inoculated with a piece of agar of one of the strains mentioned in Example 1. Culturing was first carried out for 48 h at 28°C and 180 revolutions per minute (rpm) on a rotating shaker. After this time, the addition of 20 mg of the compound 2 dissolved in 100 µl of DMF or ethanol was carried out. After 72 hours (h), or alternatively an earlier time, an aliquot of the culture was taken and treated with 50% methanol. The suspension obtained was suspended with an Ultra-Turrax™ for 30 seconds and the suspension was then centrifuged at 10,000 g. The clear supernatant obtained was then analyzed by means of HPLC (see Example 2).

10 It was possible to detect the compound 1 in a concentration of up to 5 mg/l in the culture broth of all strains after an incubation time of 72 h.

#### Example 4

15 Production of the compound 1 on the flask scale using various Cunninghamella strains

100 ml of preculture medium (5 g/l of soybean peptone, 5 g/l of yeast extract, 5 g/l of NaCl, 5 g/l of K<sub>2</sub>HPO<sub>4</sub>, 3 g/l of agar and 20 g/l of glucose / separately autoclaved, pH 5.0 in an Erlenmeyer flask of 1000 ml volume) were inoculated with a piece of agar on which one of the strains mentioned in Example 1 had been grown in each case. Culturing was carried out for 48 h at 28°C and 180 rpm on a rotating shaker. 100 ml of production medium (5 g/l of soybean peptone, 10 g/l of corn steep liquor, 20 g/l of glucose, 5 g/l of NaCl, 1 g/l of K<sub>2</sub>HPO<sub>4</sub>, 3 g/l of agar, 25.1 g/l of 2-([hydroxy-1,1-bis-(hydroxymethyl)ethyl]amino)ethanesulfonic acid (TES) (sodium salt), 100 µl of Desmophen, pH 8.0 in an Erlenmeyer flask of 500 ml volume) were inoculated with 10 ml of the preculture described above. Culturing was first carried out for 48 h at 28°C and 180 rpm on a rotating shaker.

25 After this time, 20 mg of compound 1 dissolved in 100 µl of DMF were added. After 30 72 h an aliquot of the culture was taken and treated with 50% methanol. The suspension obtained was suspended for 30 seconds using an Ultra-Turrax and the suspension was then centrifuged at 10,000 g. The clear supernatant obtained was then analyzed by means of HPLC (Example 2).

The analysis gives the yields compiled in Table 1; the following compounds were found:

compound 1 is the compound of the formula I;

compound 2 is the compound of the formula II in which R<sup>1</sup> is -CH<sub>3</sub> and R<sup>2</sup> is -OH;

5 compound 3 is the compound of the formula II in which R<sup>1</sup> is -CH<sub>2</sub>-O-P(O)(OH)<sub>2</sub> and R<sup>2</sup> is -OH;

compound 4 is the compound of the formula II in which R<sup>1</sup> is -CH<sub>3</sub> and R<sup>2</sup> is -O-P(O)(OH)<sub>2</sub> and

triol is the compound of the formula II in which R<sup>1</sup> is -OH and R<sup>2</sup> is -OH.

10

Table 1

Strain	Triol [mg/l]	Compound 1 [mg/l]	Compound 3 [mg/l]	Compound 4 [mg/l]
C. blak. FH 001	4.9	< 1.0	11.9	60.4
C. blak. FH 002	26.0	50.7	42.5	3.0
15 C. blak. FH 003	2.1	< 1.0	1.6	18.4
C. echinu. ATCC 8688a	4.7	43.2	27.3	2.2
C. echinu. DSM 1905	13.8	3.3	40.4	23.1
20 C. el. DSM 1908	17.3	1.1	15.2	37.0
C. ech. ATCC 9244	18.5	5.1	20.7	7.4

## 25 Example 5

Improved preparation of the compound 1 on the flask scale with *Cunninghamella echinulata* variant *elegans* ATCC 8688a

100 ml of preculture medium (5 g/l of soybean peptone, 5 g/l of yeast extract, 5 g/l of NaCl, 5 g/l of K<sub>2</sub>HPO<sub>4</sub>, 3 g/l of agar and 20 g/l of glucose (separately autoclaved), pH 5.0 in an Erlenmeyer flask of 1000 ml volume) were inoculated with a piece of agar on which one of the strains mentioned in Example 1 had been grown in each case. Culturing was carried out for 48 h at 28°C and 180 rpm on a rotating shaker.

100 ml of production medium (5 g/l of soybean peptone, 5 g/l of casein peptone, 20 g/l of soluble starch, 5 g/l of NaCl, 1 g/l of K<sub>2</sub>HPO<sub>4</sub>, 1 g/l of NH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>, 3 g/l of

35

agar, 25.1 g/l of TES (sodium salt), 100 µl of Desmophen, pH 8.0 (in an Erlenmeyer flask of 500 ml volume) were inoculated with 10 ml of the preculture described above. Culturing was first carried out for 24 h at 28°C and 180 rpm on a rotating shaker.

5

After this time, 20 mg of compound 2 dissolved in 800 µl of ethanol were added. After 72 h an aliquot of the culture was taken and treated with 50% methanol. The suspension obtained was suspended for 30 seconds using an Ultra-Turrax and the suspension was then centrifuged at 10,000 g. The clear supernatant obtained was then analyzed by means of HPLC (Example 2). Analysis gave a yield of 145 mg/l of compound 1, 6.5 mg/l of triol (2), 22 mg/l of compound 3 and 5 mg/l of compound 4.

10

#### Example 6

15 Production of compounds of the formula II in a 10 l fermenter

100 ml of preculture medium (5 g/l of soybean peptone, 5 g/l of yeast extract, 5 g/l of NaCl, 5 g/l of K<sub>2</sub>HPO<sub>4</sub>, 3 g/l of agar and 20 g/l of glucose / separately autoclaved, pH 5.0 in an Erlenmeyer flask of 1000 ml volume) were inoculated with a piece of agar of the strain *Cunninghamella echinulata* var. *elegans* ATCC 8688a. Culturing was carried out for 48 h at 28°C and 180 rpm on a rotating shaker. 10 l of production medium (5 g/l of soybean peptone, 5 g/l of yeast extract, 20 g/l of glucose (separately autoclaved), 5 g/l of NaCl, 1 g/l of K<sub>2</sub>HPO<sub>4</sub>, 100 µl of Desmophen, pH 5.0) were inoculated with 100 ml of the preculture described above. Compound 2 was then added such that a final concentration of 200 mg/l of production medium was achieved in the fermenter. Fermentation was carried out at 28°C, 700 rpm and 0.5 vvm for 96 hours.

20

25

According to HPLC analysis, the culture broth contained approximately 10 mg/l of compound 1, 10 mg/l of triol (2), 100 mg/l of compound 3 and 100 mg/l of compound 4.

30

## Example 7

## Isolation of the compounds of the formula II

The culture broth obtained according to Example 6 (8800 ml) was separated into  
 5 supernatant (8000 ml) and biomass (800 g) by means of a bucket centrifuge (5000  
 g/10 minutes) and both phases were worked up separately.

160 g of macroporous acrylamide/polystyrene adsorber resin such as XAD 7 were  
 added to the supernatant. Adsorption was carried out with gentle stirring at pH 2.5  
 and room temperature for 2 hours. The adsorber resin was filtered off and eluted  
 10 with 4 times 160 ml of methanol. The eluates were combined and concentrated to  
 dryness under reduced pressure (approximately 50 g of oily solid after  
 lyophilization).

Fine purification was carried out by means of preparative HPLC according to the  
 following procedure:

15

Eluent A :                800 ml of        H<sub>2</sub>O  
                               200 ml of        acetonitrile  
                               1 ml of            TFA

20

Eluent B :                1000 ml of      acetonitrile  
                               0.9 ml of        TFA

Detection :              215 nm

Flow rate:                50 ml/min

Gradient :                0 min            0% B

                              60 min           0% B

25

                              133 min         43% B

                              134 min         100% B

                              157 min         100% B

Column temperature: Room temperature

Column :                 Kromasil™ C18, 7 μm

30

Length 350 mm, diameter 50 mm

Sample                    5 g of crude product, dissolved in 350 ml of methanol/water  
 (1:1)

After combination of the appropriate fractions, concentration under reduced pressure and lyophilization of the aqueous residue, 20 mg of compound 1 and 160 mg of compound 3 were obtained from two runs.

5 The biomass (800 g) was extracted with n-propanol, 30% strength and 100% strength (total 1500 ml) and the solid is separated off by means of filtration through filter layers (K300). The filtrates were concentrated under reduced pressure and 26.2 g of oily solid were obtained.

10 Fine purification was carried out by means of preparative HPLC according to the following procedure:

Eluent A : 800 ml of H<sub>2</sub>O  
200 ml of acetonitrile  
1 ml of TFA

15

Eluent B : 1000 ml of acetonitrile  
0.9 ml of TFA

Detection : 215 nm

Flow rate : 50 ml/min

20

Gradient : 0 min 0% B

34 min 0% B

121 min 51% B

122 min 100% B

25

148 min 100% B

Column temperature: Room temperature

Column: Kromasil, C18, 7 µm

Length 350 mm, diameter 50 mm

Sample 5 g of crude product, dissolved in 400 ml of

30

methanol/water (1:1)

After combination of the appropriate fractions, concentration under reduced pressure and lyophilization of the aqueous residue, 4 mg of triol (2), 20 mg of

compound 4 and 80 mg of compound 1 were obtained from two runs.

### Example 8

#### 5 Characterization of the compounds of the formula II

In addition to characterization by means of UV/VIS spectroscopy, the components were characterized by  $^1\text{H-NMR}$  and ESI-MS or FAB-MS.

MS data:

Triol (2) ESI-MS:  $m/e = 488.4 [M+H]^+$

10 Compound 1 ESI-MS:  $m/e = 502.3 [M+H]^+$

Compound 3 ESI-MS:  $m/e = 568.3 [M+H]^+$

HR-FAB-MS:  $m/e = 568.2827 [M+H]^+$

Compound 4 ESI-MS:  $m/e = 552.3 [M+H]^+$

15  $^1\text{H-NMR}$  data are shown in Table 2:

$^1\text{H}$  chemical shifts in DMSO at 300°K.

Table 2:

20

	Compound 1	Compound 3	Triol 2	Compound 4
1-CH <sub>3</sub>	1.45	1.25	1.20	1.25
1-CH <sub>2</sub>	-	3.83	3.39	-
3	7.30	7.34	7.30	7.32
4	7.28	7.26	7.23	7.27
6	4.52	4.52	4.51	5.11
7	1.66/1.59	1.68/1.59	1.68/1.58	1.71
8	1.59	1.58	1.58	1.73
9	2.98	2.98	2.97	2.97
10	3.43/2.89	3.43/2.89	3.41/2.88	3.31/2.83
11	1.66/1.46	1.66/1.45	1.66/1.46	1.74/1.37
12	2.83	2.83	2.82	2.81
15	7.49	7.49	7.49	7.50
16	7.28	7.29	7.30	7.28
17	7.16	7.16	7.16	7.15

35

## Example 9

## Action on the bronchial diameter of the anesthetized guinea-pig

- 5 The investigations were carried out according to the method of Konzett-Rössler (Arch. exp. Path. U. Pharmacol. 195 (71-74) 1940), on male albino guinea-pigs having a body weight of 312-415 g. The test preparations were administered in a dose of 10 mg/kg in a suspension with the administration volume of 2 ml/kg of body weight. After two preliminary values with histamine 20 µg/kg intravenously (i.v.), the
- 10 substance was administered a single time by means of a duodenal tube and the asthma testing was repeated 15, 30, 60, 90, 120, 150, 180, 210 and 240 min after administration of preparations.
- The alterations of the bronchial diameter in comparison to the control measurements were given in percentages. 6 animals were employed per dose. Statistics were
- 15 obtained by means of the t-test.

16

The results are shown in Table 3.

Table 3:

Preparation	Inhibition of the histamine-induced asthma attack in %											
	15 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min			
5 Terfenadine (TELDANE®)*	11.7 ± 9.4	26.8 ± 14.3	38.2 ± 13.9	61.8 ± 11.5	83.5 ± 12.5	88.0 ± 4.8	87.6 ± 6.6	81.2 ± 9.3	75.4 ± 11.2			
10 Compound 3	30.7 ± 21.7	66.2 ± 17.2	85.7 ± 8.9	84.3 ± 15.7	88.7 ± 6.3	91.8 ± 3.8	91.3 ± 3.8	94.0 ± 2.7	92.6 ± 4.9			
Compound 4	68.3 ± 16.7	91.8 ± 3.3	94.2 ± 2.0	93.5 ± 1.9	94.5 ± 1.5	93.8 ± 1.2	91.7 ± 1.7	93.7 ± 1.2	92.0 ± 3.6			

Dose = 10 mg/kg intraduodenally

N = 6/dose

\* = suspension; converted to active compound

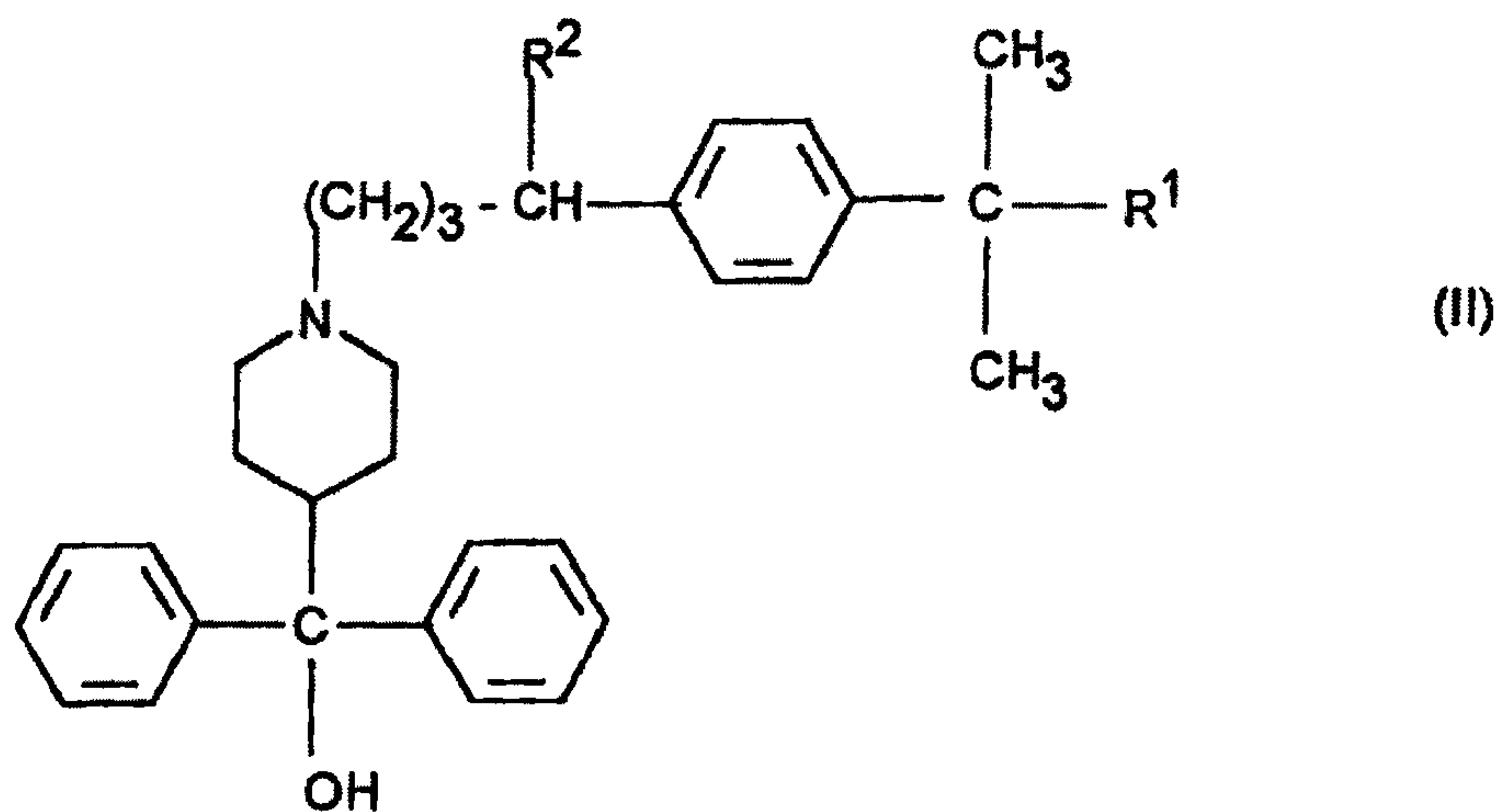
15

Compound 3 achieved its maximal action most rapidly (60 min post-administration (p.a.)) and the action lasted almost undecreased for up to 240 min p.a., compound 4 showed a slower onset of action (approximately 150 min p.a.).

5 Terfenadine showed a maximum action after 150 min p.a. and the onset of action after 60 min. p.a.. compounds 3 and 4 are more rapidly and highly active than terfenadine.

## Patent Claims:

1. A compound of the formula II



5

in which

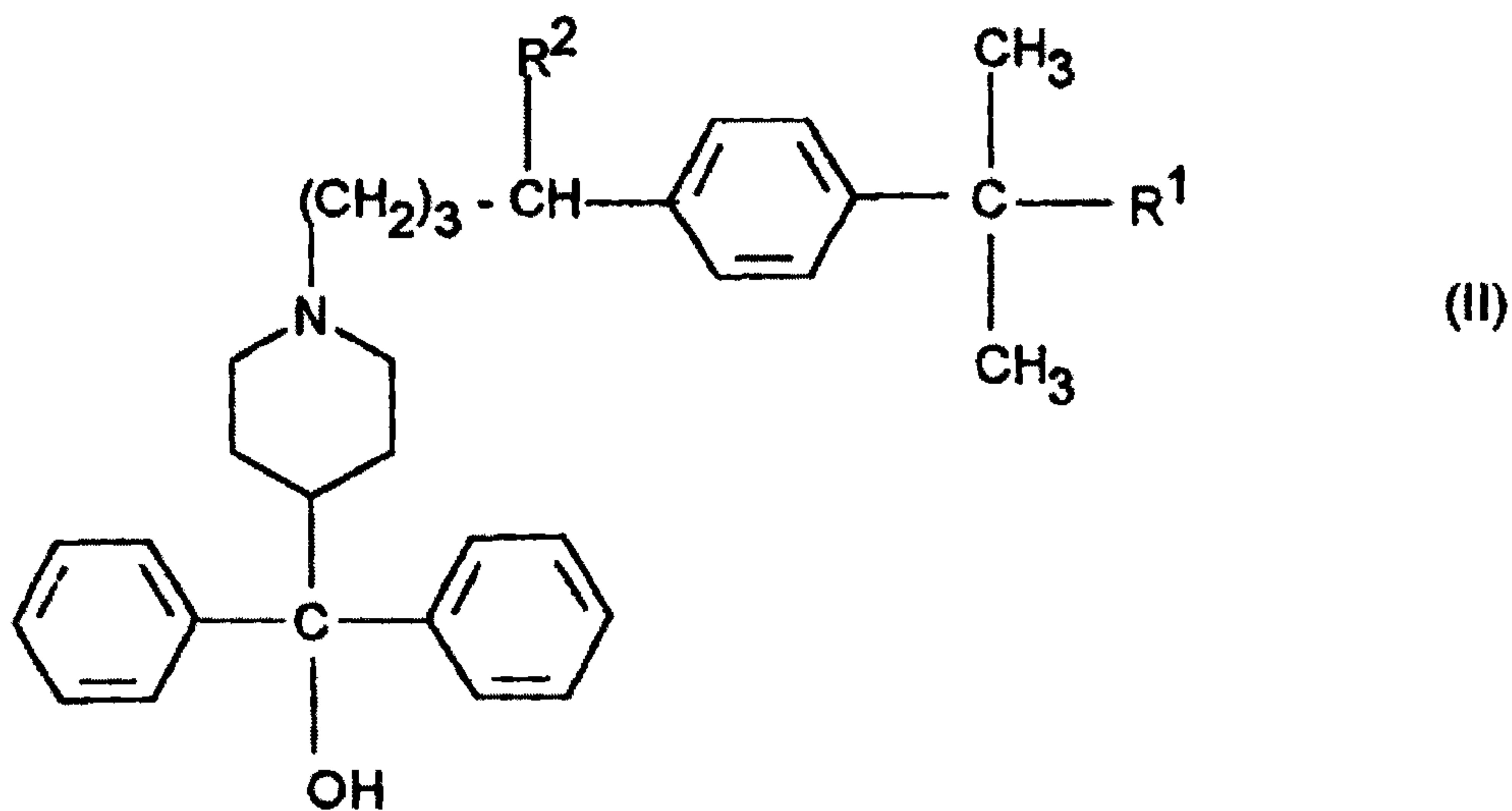
R<sup>1</sup> is -CH<sub>2</sub>-O-P(O)(OH)<sub>2</sub> and R<sup>2</sup> is -OH,

R<sup>1</sup> is -CH<sub>3</sub> and R<sup>2</sup> is -O-P(O)(OH)<sub>2</sub> or

R<sup>2</sup> is -O-P(O)(OH)<sub>2</sub> and R<sup>1</sup> is -COOH.

10

2. A process for the preparation of the compound of the formula II



15

in which

$R^1$  is  $-OH$  or  $-CH_2-O-P(O)(OH)_2$  and  $R^2$  is  $-OH$ ,

$R^1$  is  $-CH_3$  and  $R^2$  is  $-O-P(O)(OH)_2$  or

$R^2$  is  $-O-P(O)(OH)_2$  and  $R^1$  is  $-COOH$ ,

5

which comprises incubating  $\alpha$ -(p-tertiary-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol or, optionally, 4-(4-(4-hydroxydiphenyl)-1-piperidiny)-1-hydroxybutyl)- $\alpha,\alpha$ -dimethylacetic acid with a fungus of the genus *Cunninghamella* or *Absidia*.

10

3. A pharmaceutical composition comprising an effective amount of at least one compound of the formula II as claimed in claim 1 together with a pharmaceutically suitable and physiologically tolerable excipient.

15

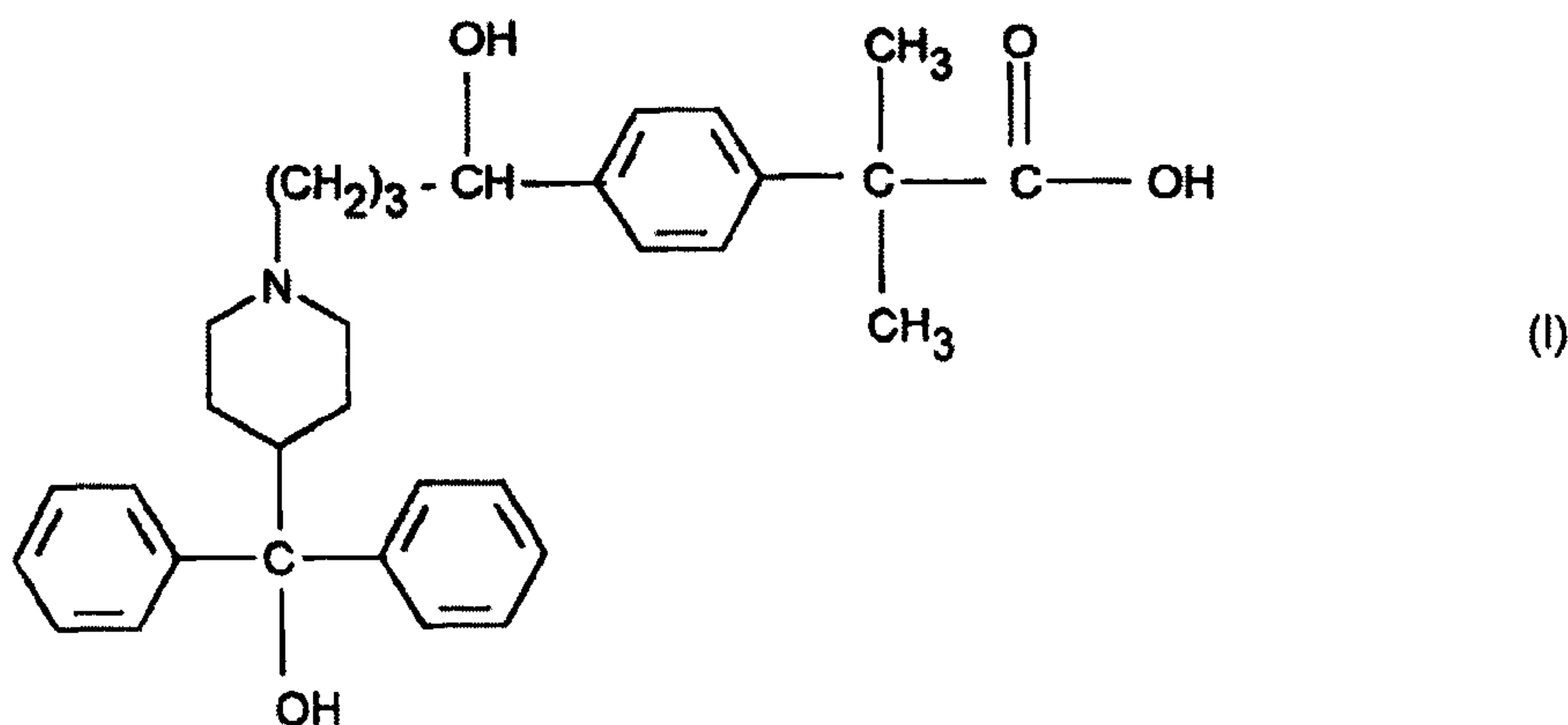
4. The use of at least one compound of the formula II as claimed in claim 1 for the production of pharmaceuticals for the treatment of allergic disorders, allergic rhinitis or asthma.

20

5. A process for the production of a pharmaceutical composition as claimed in claim 3, which comprises bringing at least one compound as claimed in claim 1 and a pharmaceutically suitable and physiologically tolerable excipient into a suitable administration form.

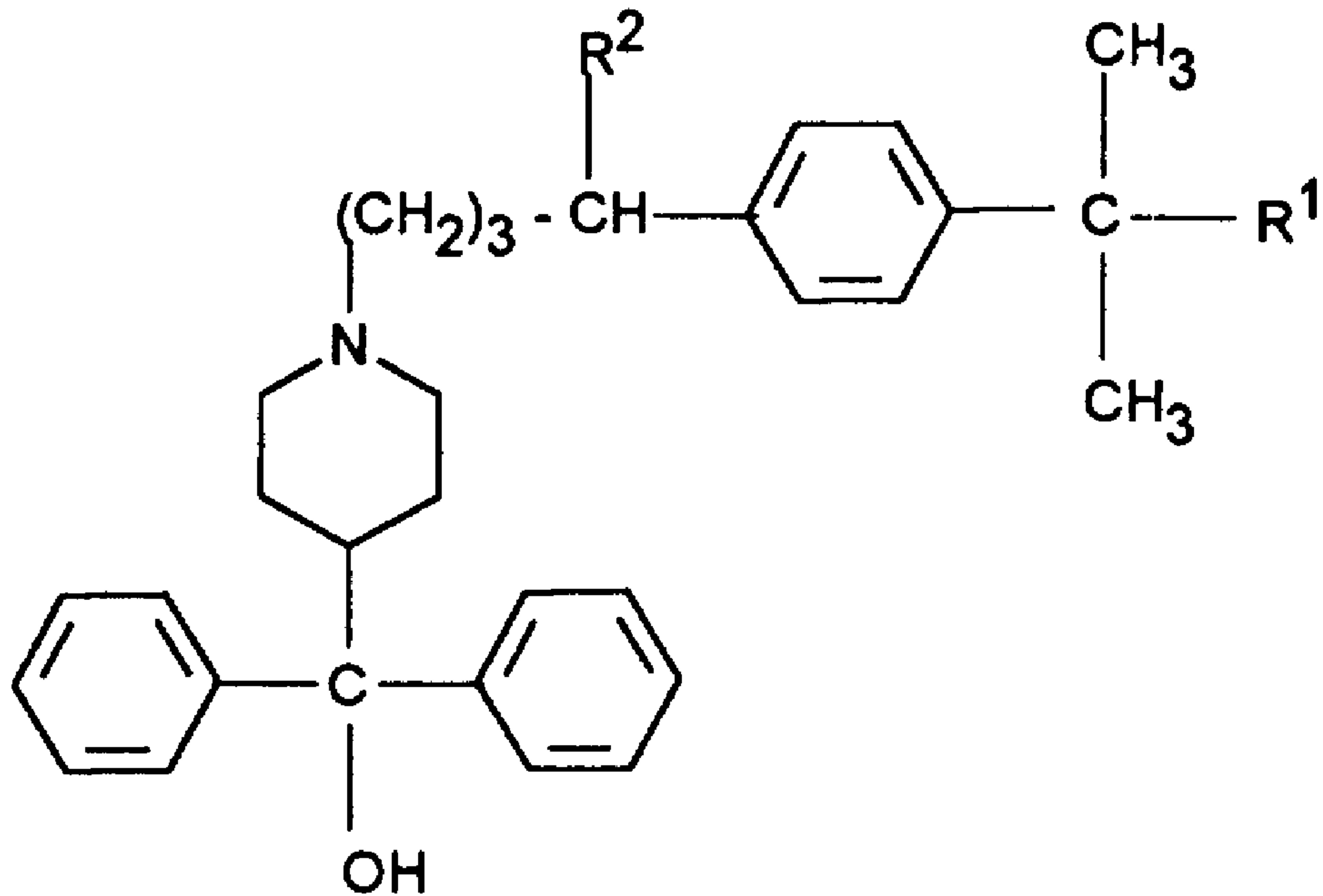
6. A process for obtaining the compound of the formula I

25



which comprises incubating  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol with a fungus of the genus *Cunninghamella* or *Absidia*.

- 5 7. The process as claimed in claim 6, wherein a fungus selected from the group consisting of *Cunninghamella balakesleena*, *Cunninghamella elegans* and *Cunninghamella echinulata* is employed.
- 10 8. The process as claimed in claim 7, wherein the fungus selected from the group consisting of *Cunninghamella echinulata* variant *elegans* ATCC 8688a, *Cunninghamella echinulata* DSM 1905, *Cunninghamella elegans* DSM 1908 and *Cunninghamella echinulata* ATCC 9244.
- 15 9. The process as claimed in any one of claims 2, 6, 7 and 8 wherein  $\alpha$ -(p-tert-butylphenyl)-4-( $\alpha$ -hydroxy- $\alpha$ -phenylbenzyl)-1-piperidinybutanol is added at the start of the culturing of the fungus of the genus *Cunninghamella* or 24 hours after the start.



(II)