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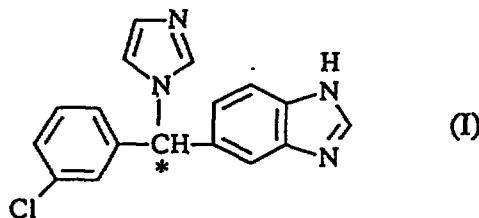
ENANTIOMERICALLY PURE (+)-LIARAZOLE

10 This invention relates to novel enantiomerically pure compounds of formula (I) useful in treating disorders which are characterized by an increased proliferation and/or abnormal differentiation of normal, preneoplastic or neoplastic epithelial cells. These compounds are particularly useful in the field of dermatology. Also disclosed are compositions containing said novel compounds as well as methods of using the mentioned compounds
15 to treat the mentioned disorders. The dextrorotatory compounds of formula (I) are useful for the manufacture of a medicine for treating keratinization disorders. Further the present invention provides methods of preparing the present novel compounds.

The novel compounds subject to the present invention are the dextrorotatory isomer of
20 the compound liarozole and the pharmaceutically acceptable acid addition salts thereof.

Liarozole is a racemic mixture, i.e. a mixture of its optical isomers, and is specifically mentioned as compound 28 in EP-0,371,559. Said patent application mentions the use of compounds like liarozole in the treatment of epithelial disorders. EP-0,260,744
25 describes the use of compounds like liarozole for inhibiting or lowering androgen formation. Whereas EP-0,371,559 and EP-0,260,744 recognize that compounds like liarozole have stereochemically isomeric forms, no example of an enantiomerically pure form is given of liarozole.

30 Chemically liarozole is $(\pm)$ -5-[3-chlorophenyl]-1H-imidazol-1-ylmethyl]-1H-benzimidazole, and is represented by formula (I). As can be seen from the chemical structure, liarozole has one stereogenic center (indicated with an asterisk in formula (I)).



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The subject of this invention is the enantiomerically pure dextrorotatory isomer or (+)-isomer of liarozole. Said isomer will hereinafter be referred to as (+)-liarozole.

Many organic compounds exist in optically active forms, i.e. they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes (+) and (-) or d and l are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is laevorotatory and with (+) or d meaning that the compound is dextrorotatory. For a given chemical structure the optically active isomers having an opposite sign of optical rotation are called enantiomers. Said enantiomers are identical except that they are mirror images of one another. A 1:1-mixture of such enantiomers is called a racemic mixture.

Stereochemical purity is of importance in the field of pharmaceuticals since the respective enantiomers may have a different potency or may have a different activity. The enantiomer of a beneficial isomer may even be deleterious rather than simply inert. Several examples of such differences are known in the art.

The term "enantiomerically pure" as used herein means that the product contains at least 90% by weight of one enantiomer and 10% by weight or less of the other enantiomer. In the most preferred embodiment the term "enantiomerically pure" means that the composition contains at least 99% by weight of one enantiomer and 1% or less of the other enantiomer.

It should be noted that optical rotation of chemical substances is dependent upon experimental parameters. The values shown in the experimental part hereinafter are specific rotations and the experimental conditions such as temperature, the wavelength of the plane polarized light used, the solvent as well as the concentration of the sample are indicated in the conventional way. The optical rotation may vary (it may even change sign!) when for instance an acid addition salt is formed. When reference is made to the dextrorotatory isomer of liarozole or (+)-liarozole then the sign of the optical rotation of the base form is intended under the given experimental conditions shown hereinafter.

It should also be noted that when a chemical reaction does not involve the stereocenter then the absolute configuration of said stereocenter remains the same, although the optical rotation of the compound which results from said chemical reaction may be different or even have an opposite sign. Hence, in order to avoid confusion, the intermediate with the same absolute configuration of the stereocenter as the desired enantiomer of the final product will be designated with the prefix (B) before the reference number.

The pharmaceutically acceptable acid addition salts as mentioned hereinabove are meant to comprise the therapeutically active non-toxic acid addition salt forms which the compounds of formula (I) are able to form. The latter can conveniently be obtained by treating the base form with such appropriate acids as inorganic acids, for example, hydrohalic acids, e.g. hydrochloric, hydrobromic and the like; sulfuric acid; nitric acid; phosphoric acid and the like; or organic acids, for example, acetic, propanoic, hydroxyacetic, 2-hydroxypropanoic, 2-oxopropanoic, ethanedioic, propanedioic, butanedioic, (Z)-2-butenedioic, (E)-2-butenedioic, 2-hydroxybutanedioic, 2,3-dihydroxybutanedioic, 2-hydroxy-1,2,3-propanetricarboxylic, methanesulfonic, ethanesulfonic, benzenesulfonic, 4-methylbenzenesulfonic, cyclohexanesulfamic, 2-hydroxybenzoic, 4-amino-2-hydroxybenzoic and the like acids. Conversely the salt form can be converted by treatment with alkali into the free base form. The term addition salt also comprises the hydrates and solvent addition forms which the compounds of formula (I) are able to form. Examples of such forms are e.g. hydrates, alcoholates and the like.

Preferred pharmaceutically acceptable acids are hydrochloric acid and (E)-2-butenedioic acid.

General preparation of structures including liarozole have been extensively described in EP-0,371,559 and EP-0,260,744.

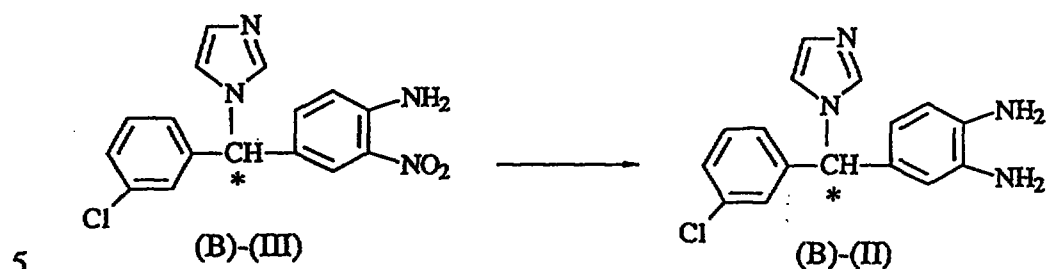
Enantiomerically pure (+)-liarozole may be prepared by reacting an enantiomerically pure intermediate diamine of formula (B)-(II) with formic acid or a functional derivative thereof.



Said functional derivative of formic acid is meant to comprise the halide, anhydride, amide and ester, including the ortho and imino ester form thereof. Also methanimidamide or an acid addition salt thereof can be used as cyclizing agent.

The general reaction conditions, work-up procedures and conventional isolation techniques for carrying out the above and following reactions are described in the prior art. When more specific conditions are required they are mentioned hereinunder.

The enantiomerically pure intermediate diamine of formula (B)-(II) may be prepared by reducing an intermediate of formula (B)-(III) by a standard nitro-to-amine reduction reaction.



10 The desired enantiomer of the intermediate of formula (B)-(III) can be prepared by fractional crystallization of a racemic mixture of the intermediate of formula (III) with an enantiomerically pure chiral acid. Preferred chiral acid for the above fractional crystallization is 7,7-dimethyl-2-oxobicyclo[2.2.1]heptane-1-methanesulfonic acid (i.e. 10-camphorsulfonic acid).

15 Appropriate solvents for carrying out said fractional crystallization are water, ketones, e.g. 2-propanone, 2-butanone; alcohols, e.g. methanol, ethanol, 2-propanol. Mixtures of ketones and water are very suitable for the above fractional crystallization. Preferably a mixture of 2-propanone and water is used.

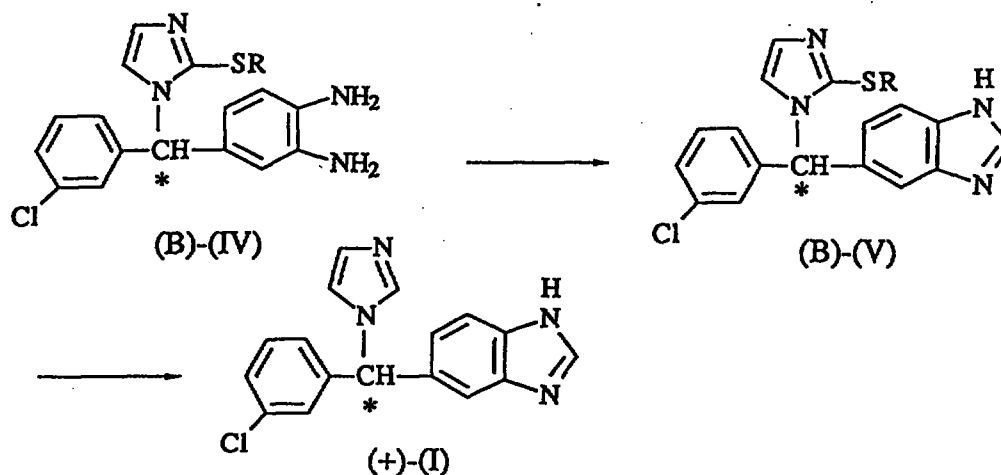
The ratio of water/2-propanone by volume may vary from 1/10 to 1/2. Preferred range of said ratio is 1/5 to 1/3.

20 The fractional crystallizations are suitably carried out below room temperature, preferably below 5°C.

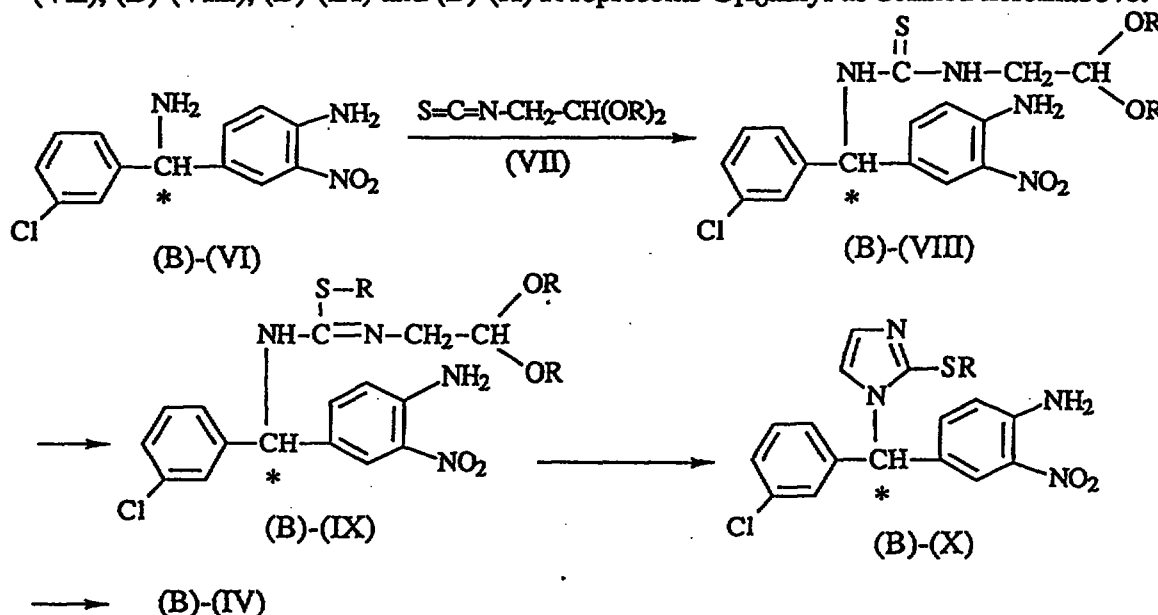
25 It was also found that the subsequent reaction step can be carried out without any appreciable racemization.

Alternatively the (+)-isomer of the compound of formula (I) may be prepared by cyclizing an intermediate of formula (B)-(IV) following procedures as described above for the cyclization of intermediates of formula (B)-(II) and desulfurating the thus obtained intermediate of formula (B)-(V). In formulas (B)-(IV) and (B)-(V) R represents C₁₋₆alkyl, wherein C₁₋₆alkyl means a straight or branch chained saturated hydrocarbon radicals having 1 to 6 carbon atoms such as, for example, methyl, ethyl, propyl, butyl, pentyl, hexyl. Preferably R is methyl.

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- 5 The intermediates of formula (B)-(IV) may be prepared by reacting an intermediate of formula (B)-(VI) with a reagent of formula (VII), alkylating the thus formed thiourea derivative of formula (B)-(VIII) subsequently cyclizing the intermediate of formula (B)-(IX), and reducing the nitro group of the intermediate (B)-(X). In the formulas (VII), (B)-(VIII), (B)-(IX) and (B)-(X) R represents C₁₋₆alkyl as defined hereinabove.



- 10 The enantiomerically pure intermediate of formula (B)-(VI) can be prepared by art-known resolution techniques, e.g. by chromatography using chiral stationary phases or by forming diastereomeric compounds such as forming an amide with an enantiomerically pure chiral acid, e.g. α -hydroxybenzeneacetic acid (mandelic acid), or by forming diastereomeric salt forms using enantiomerically pure chiral acid.

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Liarozole has retinoid mimetic effects *in vivo* and *in vitro*. This means that the compound is thought to inhibit retinoic acid (RA) catabolism, so that increased retinoic acid (RA) levels lead to pronounced RA effects at the tissue or cell level. Liarozole has

also been shown to be a potent inhibitor of androgen biosynthesis. Preclinical and clinical studies are ongoing showing the utility of liarozole in the field of oncology and dermatology.

- 5 Unexpectedly it has been found that (+)-liarozole shows increased retinoic mimetic activity when compared with racemic liarozole or with the enantiomerically pure leavorotatory isomer of liarozole, hereinafter referred to as (-)-liarozole. More in particular, (+)-liarozole is a stronger inhibitor of the retinoic acid metabolism in human skin epidermis and human tongue squamous carcinoma cells (SCC25). Moreover, the
10 increased effectiveness of (+)-liarozole as a retinoic mimetic, especially in the field of dermatology, can be evidenced by the test "Induction of Pinnal Epidermal Hyperplasia in Hairless Mice". The effect of retinoic acid at the level of normal human keratinocytes is also more potentiated by (+)-liarozole. Furthermore, it has been found from toxicity tests that, unexpectedly, the use of (+)-liarozole is more suitable, when compared to
15 (-)-liarozole, for the manufacture of a medicament for treating keratinization disorders. The increased retinoic mimetic activity of (+)-liarozole is described in more detail in the experimental part hereinafter. From the above, it can be conceived that by administering an effective amount of (+)-liarozole it is possible to accomplish a more "targeted" dermatological therapy. A more "targeted" dermatological therapy means that by using
20 the (+)-isomer of liarozole, the compound is used which has a higher retinoic mimetic activity.

The use of (+)-liarozole and its pharmaceutically acceptable acid addition salts in the method of the present invention is based on their useful property to delay the catabolism
25 of retinoids, such as, all-*trans*-retinoic acid, 13-*cis*-retinoic acid and their derivatives. The latter results in more sustained / higher tissue concentrations of retinoids and improved control of differentiation and growth of various cell types. This action of (+)-liarozole is also called retinoic mimetic activity because administering (+)-liarozole causes the same effect as if retinoid would be administered. As such, (+)-liarozole can
30 be used to control the rate of growth and differentiation of normal, preneoplastic and neoplastic epithelial cells.

(+)-Liarozole and its pharmaceutically acceptable acid addition salts is therefore useful in a method of treating disorders which are characterized by an increased proliferation
35 and/or abnormal differentiation of epithelial cells. (+)-Liarozole shows activity on cells of which the growth and differentiation is not substantially mediated by or insensitive to the actions of androgens or estrogens, in particular on cells of which the growth and differentiation is sensitive to the actions of retinoids. Special uses include the ability to

cure and/or reduce a variety of disorders of keratinization such as, for example, rosacea, acne, psoriasis, ichthyosis, warts, callosities, acanthosis nigricans, lichen planus, corneal epithelial abrasion, geographic tongue, Fox-Fordyce disease, precancerous skin conditions, such as, actinic keratoses, and keloids, epidermolytic hyperkeratosis, Darier's disease, pityriasis rubra pilaris, congenital ichthyosiform erythroderma, hyperkeratosis palmaris et plantaris, melasma, hyperpigmentation. (+)-Liarozole and its pharmaceutically acceptable acid addition salts is useful for the manufacture of a medicine for treating keratinization disorders.

- 10 In general it is contemplated that an effective amount to treat disorders which are characterized by an excessive proliferation and/or abnormal differentiation of tissues, would be from 0.001 mg/kg to 20 mg/kg body weight and more preferably from 0.01 mg/kg to 10 mg/kg body weight.
- 15 The compounds of formula (I) used in the method of the invention are most preferably applied in the form of appropriate compositions. As appropriate compositions there may be cited all compositions usually employed for systemically or topically administering drugs. To prepare the pharmaceutical compositions of this invention, an effective amount of the particular compound, optionally in acid-addition salt form, as the active
- 20 ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirable in unitary dosage form suitable, particularly, for administration orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the compositions in oral dosage form,
- 25 any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets and capsules
- 30 represents the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of
- 35 saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations. In the compositions suitable for percutaneous

administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious effect on the skin. As appropriate compositions for topical application there may be cited all compositions usually employed for topically administering drugs, e.g., creams, gellies, dressings, shampoos, tinctures, pastes, ointments, salves, powders, liquid or semi-liquid formulation and the like. Application of said compositions may be by aerosol e.g. with a propellant such as nitrogen carbon dioxide, a freon, or without a propellant such as a pump spray, drops, lotions, or a semisolid such as a thickened composition which can be applied by a swab. In particular compositions, semisolid compositions such as salves, creams, pastes, gellies, ointments and the like will conveniently be used.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used in the specification and claims herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powders packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

Other compositions are preparations of the cosmetic type, such as toilet waters, packs, lotions, skin milks or milky lotions. Said preparations contain, besides the active ingredient, components usually employed in such preparations. Examples of such components are oils, fats, waxes, surfactants, humectants, thickening agents, antioxidants, viscosity stabilizers, chelating agents, buffers, preservatives, perfumes, dyestuffs, lower alkanols, and the like. If desired, further ingredients may be incorporated in the compositions, e.g. antiinflammatory agents, antibacterials, antifungals, disinfectants, vitamins, sunscreens, antibiotics, or other anti-acne agents.

In a further aspect of the invention there are provided particular pharmaceutical or cosmetical compositions which comprise an inert carrier, an effective amount of (+)-liarozole or an acid addition salt form thereof and an effective amount of a retinoic acid, a derivative thereof, in particular retinol, or a stereochemically isomeric form thereof.

It can be demonstrated that the retinoic acids and (+)-liarozole act in a synergistic manner. Indeed, the combined effect of both substances is greater than the sum of their

respective effects when administered separately. The above described retinoic acid containing compositions are particularly useful for treating acne or for retarding the effects of aging of the skin and generally improve the quality of the skin, particularly human facial skin. A pharmaceutical or cosmetical composition containing retinoic acid or a derivative thereof as the active ingredient in intimate admixture with a dermatologically acceptable carrier can be prepared according to conventional compounding techniques, such as those known for topical application of retinoic acid and its derivatives optionally in admixture with cyclodextrines or derivatives thereof known in the art. Preferred composition for topical application are in form of a cream, ointment or lotion comprising from 0.001 to 0.5% (particularly from 0.01 to 0.1%) all-*trans*-retinoic acid, 13-*cis*-retinoic acid or a derivative thereof, in particular retinol, and from 0.1 to 5% of a (+)-liarozole or a dermatologically acceptable acid addition salt thereof, in a semi-solid or liquid diluent or carrier.

These preferred compositions should preferably be non-irritating and as far as possible they should be odorless and non-toxic. For convenience in applying to the skin, the composition usually contain, besides water or an organic solvent, several of certain organic emollients, emulsifiers for the aqueous and/or non aqueous phases of the compositions, wetting agents preservatives and agents that facilitate the penetration and remainence of the active agents in the skin.

In use, the retinoic acid containing compositions of the invention are applied topically to the area to be treated or protected, at regular intervals, as needed, generally about 7 to about 21 times per week. The duration of the treatment will depend upon the nature and severity of the condition to be treated as well as the frequency of application of the composition.

Experimental part

A. Preparation of the intermediates

Example 1

a) A heterogeneous mixture of ($\pm$)-4-[(3-chlorophenyl)-1H-imidazol-1-ylmethyl]-2-nitrobenzenamine (the preparation of which is described in EP-371,559) (500 g) in 2-propanone (2000 ml) and water (100 ml) was stirred at 22°C. (-)-(1R)-7,7-dimethyl-2-oxo-bicyclo[2.2.1]heptane-1-methanesulfonic acid (353.2 g) was added and the mixture became homogeneous after 10 minutes. The mixture was first stirred for 18 hours at 20°C and then for 3 hours at 0-5°C. The precipitate was filtered off, washed with 2-propanone/water 95/5 (150 ml) and dried, yielding 308.9 g (36.2%) of product. A sample (306.7 g) was partitioned between dichloromethane (500 ml) and water (750 ml). Ammonium hydroxide (100 ml) was added. This mixture was stirred for 15

minutes. The aqueous layer was separated and extracted twice with dichloromethane (250 ml each time). The separated organic layer was washed with water (250 ml), dried, filtered and the solvent was evaporated, yielding 179.7 g of (-)-(B)-4-[(3-chlorophenyl)-1H-imidazol-1-ylmethyl]-2-nitrobenzenamine; mp. 89.8°C; $[\alpha]_D^{20} = -19.80^\circ$ (c = 0.5% in

5 methanol) (interm. 1).

b) A mixture of intermediate (1) (179.7 g) in methanol (656 ml) and a solution of ammonia in methanol (32.7 ml) was hydrogenated at 20-25 °C with platinum on activated carbon (13.1 g) as a catalyst in the presence of thiophene (0.27 g). After uptake of hydrogen (3 eq.) the catalyst was filtered off and washed with 2-propanol (30
10 ml). A solution of hydrochloric acid in 2-propanol (522 ml) was added to the filtrate at <30°C. The mixture was stirred for 3 hours at 20 °C, then for 3 hours at 0-5 °C. The resulting precipitate was slowly filtered off, washed with methanol (100 ml) and dried (50 °C), yielding 185.60 g (83.2%) (+)-(B)-4-[(3-chlorophenyl)-1H-imidazol-1-yl-methyl]-1,2-benzenediamine trihydrochloride; mp. 172.5°C; $[\alpha]_D^{20} = +23.73^\circ$

15 (c = 1% in methanol) (interm. 2).

Example 2

a) A mixture of (4-amino-3-nitrophenyl) (3-chlorophenyl)methanone (50 g), formamide (375 ml) and formic acid (63 ml) was stirred and refluxed for 17 hours. After cooling,
20 the mixture was poured on ice. The precipitate was filtered off and dried, yielding 55 g (99.4%) of (±)-N-[(4-amino-3-nitrophenyl) (3-chlorophenyl)methyl]formamide (interm. 3).

b) A mixture of intermediate (3) (50.7 g), hydrochloric acid 6N (350 ml) and 2-propanol (70 ml) was stirred and refluxed for 17 hours. The yellow precipitate was
25 filtered off and dried in vacuo, yielding 51 g (97.8%) of (±)-4-amino-α-(3-chloro-phenyl)-3-nitrobenzenemethanamine monohydrochloride; mp. 263°C (interm. 4).

c) To a solution of intermediate (4) (43 g) in tetrahydrofuran (400 ml) at room temperature was added succesively N,N-diethylethanamine (13.8 g) and (R)-(-)-α-hydroxybenzeneacetic acid (20.8 g). Then a solution of 1-hydroxybenzotriazole
30 monohydrate (22.2 g) in tetrahydrofuran (200 ml) was added. After complete addition a solution of N,N'-dicyclohexylcarbodiimide (33.9 g) in dichloromethane (300 ml) was introduced to the mixture. After stirring for 2 hours at room temperature N,N'-dicyclohexylurea was filtered off. The filtrate was washed with a solution of potassium carbonate (10%) and the organic layer was dried to give a mixture of diastereomers (60g)
35 (fraction 1). The same experiment with intermediate (4) (16 g) as starting material resulted in a yield of 26 g of a mixture of diastereomers (fraction 2). Fraction 1 and 2

were combined and purified by HPLC (eluent : CH₂Cl₂/ethyl acetate 90:10), yielding 30g (32.3%) of (±)-(R,B)-N-[(4-amino-3-nitrophenyl)(3-chlorophenyl)methyl]-α-hydroxybenzeneacetamide (interm. 5).

5 d) A mixture of intermediate (5) (30 g), hydrochloric acid 12N (300 ml) and 1-propanol (100 ml) was stirred and refluxed for 17 hours and poured on ice. The mixture was extracted with ethyl acetate. The aqueous phase was basified with ammonium hydroxide and extracted with dichloromethane. The dichloromethane extracts were dried, filtered and evaporated, yielding 7.3 g (36.0%) of (+)-(B)-4-amino-α-(3-chlorophenyl)-3-nitrobenzenemethanamine (interm. 6).

10 e) A mixture of intermediate (6) (7.3 g), 2-isothiocyanato-1,1-dimethoxyethane (4.8 g) and methanol (75 ml) was stirred and refluxed for 2 hours. The mixture was evaporated to an oily residue, yielding 11 g (100%) of (+)-(B)-N-[(4-amino-3-nitrophenyl)(3-chlorophenyl)methyl]-N'-(2,2-dimethoxyethyl)thiourea (interm. 7).

15 f) A mixture of intermediate (7) (11 g), iodomethane (2 ml) and potassium carbonate (4.97 g) was stirred at room temperature for 48 hours. The solvent was evaporated and the residue was taken off with dichloromethane and washed with water. The organic layer was dried, filtered and evaporated, yielding 11.4 g of (+)-(S)-methyl (B)-N-[(4-amino-3-nitrophenyl)(3-chlorophenyl)methyl]-N'-(2,2-dimethoxyethyl)carbamimidothioate as an oily residue (interm. 8).

20 g) To intermediate (8) (11.4 g) at 0°C was added sulfuric acid (100ml) (precooled to 5°C). The mixture was stirred at 5°C until complete dissolution and then was warmed to room temperature. After stirring for 2 hours, the solution was poured on ice and basified with ammonium hydroxide. The aqueous solution was extracted with ethyl acetate. The organic layer was dried, filtered and evaporated. The residue was purified by column
25 chromatography (eluent : CH₂Cl₂/CH₃OH 98:2). The eluent of the desired fraction was evaporated, yielding 3.7 g (38.0%) of (+)-(B)-4-[(3-chlorophenyl)[2-(methylthio)-1H-imidazol-1-yl]methyl]-2-nitrobenzenamine (interm. 9).

h) A mixture of intermediate (9) (6.2 g), Raney nickel (6 g) and methanol (100 ml) was hydrogenated for 2 hours at 2 bar and at room temperature. After the calculated amount
30 of hydrogen was taken up, the catalyst was filtered off. The filtrate, (+)-(B)-4-[(3-chlorophenyl)[2-(methylthio)-1H-imidazol-1-yl]methyl]-1,2-benzenediamine (interm. 10), was used for the next step.

i) A mixture of intermediate (10) (5.7 g), methanimidamide monoacetate (5.2 g) and methanol (100 ml) was stirred and refluxed for 3 hours. The reaction mixture was
35 evaporated and the residue was taken off in dichloromethane and washed with sodium hydrogen carbonate (10%). The organic layer was dried, filtered and evaporated. The oily residue was purified by column chromatography (eluent : CH₂Cl₂/CH₃OH 95:5).

The eluent of the desired fraction was evaporated, yielding 4.9 g (83.7%) of (+)-(B)-5-[(3-chlorophenyl)[2-(methylthio)-1H-imidazol-1-yl]methyl]-1H-benzimidazole (interm. 11).

5 B. Preparation of the final compounds

Example 3

A mixture of intermediate (2) (185 g) in water (512 ml) was stirred at 20 °C. Hydrochloric acid (289 ml) was added. Formic acid (85%) (61.17 ml) was added and this mixture was heated to 55°C. The reaction mixture was stirred for 3 hours at 55 °C
10 and then cooled to 20°C. Dichloromethane (1223 ml) was added. Ammonium hydroxide (730 ml) was added dropwise at < 25°C. The separated organic layer was washed with water (500 ml), dried, filtered and the solvent was evaporated, yielding 152.88 g (108.5%) of product. A sample was dried (18 hours at 55 °C), yielding 3.18 g of (+)-(B)-5-[(3-chlorophenyl)-1H-imidazol-1-ylmethyl]-1H-benzimidazole; mp.
15 113.7°C; $[\alpha]_D^{20} = +43.46^\circ$ (c = 1% in methanol) (comp. 1).

Example 4

A mixture of intermediate (11) (4.9 g), Raney nickel (2 g) and ethanol (100ml) was stirred and refluxed for 5 days, while every day an additional amount of Raney nickel (2
20 g) was added. The catalyst was filtered off and rinsed with dichloromethane. The filtrate was evaporated and the residue was purified twice by column chromatography (silica gel; CH₂Cl₂/CH₃OH 95:5 ; CH₂Cl₂/CH₃OH/NH₄OH 80:20:3). The eluent of the desired fraction was evaporated and the residue was converted into the hydrochloride salt in 2-propanol and ethanol. The salt was recrystallized from 2-butanone, yielding 1.8 g
25 (37.2%) of (+)-(B)-5-[(3-chlorophenyl)(1H-imidazol-1-yl)methyl]-1H-benzimidazole monohydrochloride; mp. 212.1°C; $[\alpha]_D^{20} = +42.43^\circ$ (c = 1% in ethanol) (comp. 2)

Example 5

Compound (1) (149.7 g) was dissolved in 2-butanone (2424 ml). A mixture of
30 hydrochloric acid in 2-propanol (82.6 ml) in 2-butanone (727 ml) was added over a 2 hour period at 20 °C. The reaction mixture was stirred for 16 hours at 20 °C. The precipitate was filtered off, washed with 2-butanone (242 ml) and dried (vacuum; 80°C); yielding 147.5 g (99.3%) of (+)-(B)-5-[(3-chlorophenyl)-1H-imidazol-1-ylmethyl]-1H-benzimidazole monohydrochloride; mp. 214.5°C; $[\alpha]_D^{20} = +36.20^\circ$ (c = 1% in methanol)
35 (comp. 2).

Example 6

A mixture of compound (1) (0.72 g) in ethanol (5.1 ml; denaturated) was stirred at 20 °C until it became homogeneous. (E)-2-butenedioic acid (0.54 g) was added. The mixture was stirred for 18 hours at 20 °C and then cooled 0-5 °C and precipitation resulted. More
5 denaturated ethanol (2 ml) was added and the mixture was stirred for 2 hours at 20 °C. The precipitate was filtered off, washed with ethanol (3 ml; denaturated) and dried (vacuum; 50 °C), yielding 0.26 g (23.4%) (B)-5-[(3-chlorophenyl)-1H-imidazol-1-yl-methyl]-1H-benzimidazole (E)-2-butenedioate (2:3).ethanolate (2:1); mp. 111.2°C (comp. 3).

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C. Pharmacological Examples

Example 7 : Retinoic acid metabolism in human tongue squamous carcinoma cells.

Human tongue squamous carcinoma cells (SCC25) were seeded in 6-well plates and grown for 4 days at 37 °C. The medium used consisted of a 1:1 mixture of Hans' F12
15 and Dulbecco's modified Eagle's medium supplemented with hydrocortisone and fetal calf serum. After 4 days of growth, the medium was replaced by a keratinocyte serum free medium and the confluent cells were further incubated for 3 days. The medium was refreshed 16 hours before the onset of the experiment. To measure the effect of the test compound on retinoic acid metabolism, test compound and/or 2 µl DMSO were added to
20 the media before the reaction was initiated by the addition of 1 µCi [11,12-³H]-retinoic acid. After 3 hours of incubation at 37 °C, the medium plus the cells were extracted and [11,12-³H]-retinoic acid was analyzed by HPLC as described by Van Wauwe *et al.*, J. Pharmacol. Exp. Ther. (1992), 261 : 773-779. The results of this test showed that compound No. 2, i.e. the HCl salt of (+)-liarozole, inhibited the hydroxylation of
25 retinoic acid in human tongue squamous carcinoma cells with an IC₅₀ value of 1.0 µM, the HCl salt of racemic liarozole had an IC₅₀ value of 2.9 µM and the HCl salt of (-)-liarozole was almost inactive.

Example 8 : Induction of Pinnal Epidermal Hyperplasia in Hairless Mice

30 One of the cutaneous effects of retinoids is their potent ability to induce epidermal hyperplasia *in vivo* (Conner, Models in dermatology 1987, Vol. 3 Karger, Basel, 1987, p. 23-28). Therefore, liarozole and both its stereochemically isomers were compared with all-*trans*-retinoic acid for their ability to induce epidermal hyperplasia in hairless mice.

35 Female hairless mice, weighing 25-30 g, were treated orally and once daily for 14 consecutive days with either placebo (PEG 200), all-*trans*-retinoic acid or the test compound. On day 15, the animals were killed and ear tissue was collected from which

2 μ m thick sections were prepared for morphological analysis. The thickness of the total viable epidermis was measured and the epidermis of placebo treated mice consisted of a thin epithelium. In contrast, the epidermis of animals treated with RA or a test compound was hyperplastic. The results are presented in Table 1.

5

Table 1

Compound	dose (mg/kg)	% increase versus placebo
all- <i>trans</i> retinoic acid	5	156
HCl salt of racemic liarozole	10	48
HCl salt of (+)-liarozole (Comp. No. 2)	10	49
HCl salt of (-)-liarozole	10	21
HCl salt of racemic liarozole	20	123
HCl salt of (+)-liarozole (Comp. No. 2)	20	93
HCl salt of (-)-liarozole	20	10

Example 9 : Toxicological test.

- 10 Dogs were administered daily and for one month an oral dose of the test compounds, compound No. 2 and the HCl salt of (-)-liarozole. At a dose of 10 mg/kg/day, the concentrations in most dog tissues for compound No. 2 were at least a tenfold lower than for the HCl salt of (-)-liarozole.

15 D. Composition Examples

The following formulations exemplify typical pharmaceutical compositions suitable for systemic or topical administration to animal and human subjects in accordance with the present invention.

- 20 "Active ingredient" (A.I.) as used throughout these examples relates to a compound of formula (I) or a pharmaceutically acceptable acid addition salt thereof.

Example 10 : Oral drops

- 25 500 g of the A.I. was dissolved in 0.5 l of 2-hydroxypropanoic acid and 1.5 l of the polyethylene glycol at 60-80°C. After cooling to 30-40°C there were added 35 l of polyethylene glycol and the mixture was stirred well. Then there was added a solution of 1750 g of sodium saccharin in 2.5 l of purified water and while stirring there were added 2.5 l of cocoa flavor and polyethylene glycol q.s. to a volume of 50 l, providing an oral drop solution comprising 10 mg/ml of A.I. The resulting solution was filled into suitable containers.

Example 11 : Oral solution

9 g of methyl 4-hydroxybenzoate and 1 g of propyl 4-hydroxybenzoate were dissolved in 4 l of boiling purified water. In 3 l of this solution were dissolved first 10 g of
5 2,3-dihydroxybutanedioic acid and thereafter 20 g of the A.I. The latter solution was combined with the remaining part of the former solution and 12 l 1,2,3-propanetriol and 3 l of sorbitol 70% solution were added thereto. 40 g of sodium saccharin were dissolved in 0.5 l of water and 2 ml of raspberry and 2 ml of gooseberry essence were added. The latter solution was combined with the former, water was added q.s. to a
10 volume of 20 l providing an oral solution comprising 5 mg of the A.I. per teaspoonful (5 ml). The resulting solution was filled in suitable containers.

Example 12 : Capsules

20 g of the A.I., 6 g sodium lauryl sulfate, 56 g starch, 56 g lactose, 0.8 g colloidal
15 silicon dioxide, and 1.2 g magnesium stearate were vigorously stirred together. The resulting mixture was subsequently filled into 1000 suitable hardened gelatin capsules, each comprising 20 mg of the A.I.

Example 13 : Film-coated tablets

20 Preparation of tablet core

A mixture of 100 g of the A.I., 570 g lactose and 200 g starch was mixed well and thereafter humidified with a solution of 5 g sodium dodecyl sulfate and 10 g polyvinylpyrrolidone (Kollidon-K 90 ®) in about 200 ml of water. The wet powder mixture was sieved, dried and sieved again. Then there was added 100 g microcrystalline
25 cellulose (Avicel ®) and 15 g hydrogenated vegetable oil (Sterotex ®). The whole was mixed well and compressed into tablets, giving 10.000 tablets, each comprising 10 mg of the active ingredient.

Coating

To a solution of 10 g methyl cellulose (Methocel 60 HG ®) in 75 ml of denaturated
30 ethanol there was added a solution of 5 g of ethyl cellulose (Ethocel 22 cps ®) in 150 ml of dichloromethane. Then there were added 75 ml of dichloromethane and 2.5 ml 1,2,3-propanetriol. 10 g of polyethylene glycol was molten and dissolved in 75 ml of dichloromethane. The latter solution was added to the former and then there were added 2.5 g of magnesium octadecanoate, 5 g of polyvinylpyrrolidone and 30 ml of
35 concentrated color suspension (Opaspray K-1-2109 ®) and the whole was homogenated. The tablet cores were coated with the thus obtained mixture in a coating apparatus.

Example 14 : Injectable solution

1.8 g methyl 4-hydroxybenzoate and 0.2 g propyl 4-hydroxybenzoate were dissolved in about 0.5 l of boiling water for injection. After cooling to about 50°C there were added while stirring 4 g lactic acid, 0.05 g propylene glycol and 4 g of the A.I. The solution
5 was cooled to room temperature and supplemented with water for injection q.s. ad 1 l volume, giving a solution of 4 mg/ml of A.I. The solution was sterilized by filtration (U.S.P. XVII p. 811) and filled in sterile containers.

Example 15 : Suppositories

10 3 g A.I. was dissolved in a solution of 3 g 2,3-dihydroxy-butanedioic acid in 25 ml polyethylene glycol 400. 12 G surfactant (SPAN ®) and triglycerides (Witepsol 555 ®) q.s. ad 300 g were molten together. The latter mixture was mixed well with the former solution. The thus obtained mixture was poured into moulds at a temperature of 37-38°C to form 100 suppositories each containing 30 mg of the active ingredient.

Example 16 : 2% cream

75 mg stearyl alcohol, 2 mg cetyl alcohol, 20 mg sorbitan monostearate and 10 mg isopropyl myristate are introduced into a doublewall jacketed vessel and heated until the mixture has completely molten. This mixture is added to a separately prepared mixture of
20 purified water, 200 mg propylene glycol and 15 mg polysorbate 60 having a temperature of 70 to 75°C while using a homogenizer for liquids. The resulting emulsion is allowed to cool to below 25°C while continuously mixing. A solution of 20 mg A.I., 1 mg polysorbate 80 and purified water and a solution of 2 mg sodium sulfite anhydrous in purified water are next added to the emulsion while continuously mixing. The cream, 1 g
25 of the A.I. is homogenized and filled into suitable tubes.

Example 17 : 2% topical gel

To a solution of 200 mg hydroxypropyl β -cyclodextrine in purified water is added 20 mg of A.I. while stirring. Hydrochloric acid is added until complete dissolution and then
30 sodium hydroxide is added until pH 6.0. This solution is added to a dispersion of 10 mg carrageenan PJ in 50 mg propylene glycol while mixing. While mixing slowly, the mixture is heated to 50°C and allowed to cool to about 35°C whereupon 50 mg ethyl alcohol 95% (v/v) is added. The rest of the purified water q.s. ad 1 g is added and the mixture is mixed to homogenous.

Example 18 : 2% topical cream

To a solution of 200 mg hydroxypropyl β -cyclodextrine in purified water is added 20 mg

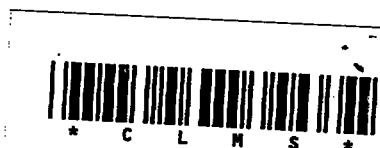
- of A.I. while stirring. Hydrochloric acid is added until complete dissolution and next sodium hydroxide is added until pH 6.0. While stirring, 50 mg glycerol and 35 mg polysorbate 60 are added and the mixture is heated to 70°C. The resulting mixture is added to a mixture of 100 mg mineral oil, 20 mg stearyl alcohol, 20 mg cetyl alcohol, 20 mg glycerol monostearate and 15 mg sorbate 60 having a temperature of 70°C while mixing slowly. After cooling down to below 25°C, the rest of the purified water q.s. ad 1 g is added and the mixture is mixed to homogenous.

Example 19 : 2% liposome formulation

- 10 A mixture of 2 g A.I. microfine, 20 g phosphatidyl choline, 5 g cholesterol and 10 g ethyl alcohol is stirred and heated at 55-60°C until complete dissolution and is added to a solution of 0.2 g methyl paraben, 0.02 g propyl paraben, 0.15 g disodium edetate and 0.3 g sodium chloride in purified water while homogenizing. 0.15 g Hydroxypropyl-methylcellulose in purified water ad 100 g is added and the mixing is continued until swelling is complete.

Example 20 : 2% liposome formulation

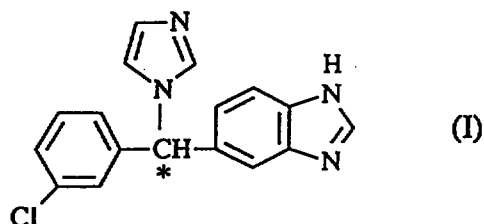
- 20 A mixture of 10 g phosphatidyl choline and 1 g cholesterol in 7.5 g ethyl alcohol is stirred and heated at 40°C until complete dissolution. 2 g A.I. microfine is dissolved in purified water by mixing while heating at 40°C. The alcoholic solution is added slowly to the aqueous solution while homogenizing during 10 minutes. 1.5 g Hydroxypropyl-methylcellulose in purified water is added while mixing until swelling is complete. The resulting solution is adjusted to pH 5.0 with sodium hydroxide 1 N and diluted with the rest of the purified water ad 100 g.



Claims

1. The dextrorotatory compound of formula (I)

5



or a pharmaceutically acceptable acid addition salt thereof.

2. A compound according to claim 1, wherein the compound is (+)-5-[3-chlorophenyl]-1H-imidazol-1-ylmethyl]-1H-benzimidazole hydrochloride (1:1).

3. A compound according to claim 1, wherein the compound is (+)-5-[3-chlorophenyl]-1H-imidazol-1-ylmethyl]-1H-benzimidazole (E)-2-butenedioate (2:3).

15

4. A composition for treating keratinization disorders comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound as claimed in any one of claims 1 to 3.

5. A composition according to claim 4 wherein said composition is in a form suitable for topical administration.

6. A composition according to claim 5 wherein said composition also comprises an effective amount of a retinoic acid, a derivative thereof or a stereochemically isomeric form thereof.

25

7. Use of a compound as claimed in any one of claims 1 to 3 as a medicine.

8. Use of a compound as claimed in any one of claims 1 to 3 for the manufacture of a medicament for treating keratinization disorders.

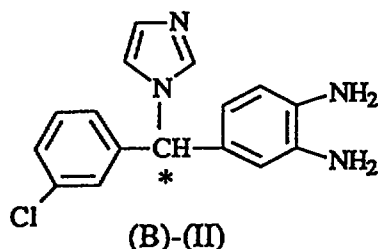
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9. Use of a compound as claimed in any one of claims 1 to 3 for the manufacture of a medicament for treating disorders in the field of dermatology.



10. Use of a compound as claimed in any one of claims 1 to 3 for the manufacture of a medicament for treating acne, ichthyosis or psoriasis.

11. An enantiomerically pure intermediate of formula (B)-(II)

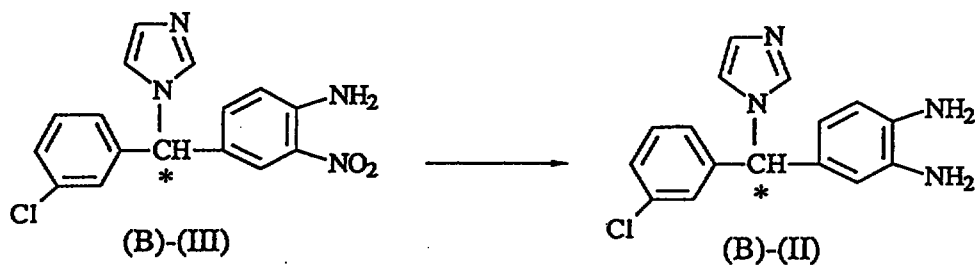


or an acid addition salt thereof.

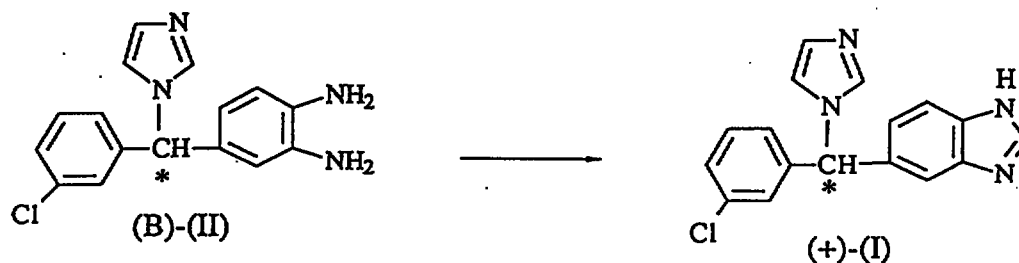
10 12. A process for preparing a compound as claimed in claim 1, characterized by

a) resolving an intermediate of formula (III) with an enantiomerically pure chiral acid, such as 7,7-dimethyl-2-oxobicyclo[2.2.1]heptane-1-methanesulphonic acid, in an appropriate solvent;

15 b) reducing the thus obtained enantiomerically pure intermediate of formula (B)-(III);



20 c) cyclizing the enantiomerically pure intermediate of formula (B)-(II), with methanimidamide, formic acid or functional derivatives thereof, yielding an enantiomerically pure compound of formula (I);





and if desired , converting the compound of formula (I) into a pharmaceutically acceptable acid addition salt form thereof by treatment with an appropriate acid or, conversely, converting the acid-addition salt into the free base form with alkali.