FORM 1

636609

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

F.Hoffmann-La Roche AG, of 124 Grenzacherstrasse, CH-4002, Basel, SWITZERLAND, hereby apply for the grant of a standard patent for an invention entitled:

Dehydrocholecalciferol Derivatives

which is described in the accompanying complete specification.

Details of basic application(s):-

Basic Applic. No:	Country:	Application Date:
353,716	US	18 May 1989

The address for service is:-

Spruson & Ferguson
Patent Attorneys
Level 33 St Martins Tower
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Sydney New South Wales Australia

DATED this SEVENTEENTH day of MAY 1990

F.Hoffmann-La Roche AG

By: J. Spruson.

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
OUR REF: 128409
S&F CODE: 55541

501490 17/05/90

5845/2

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the convention application made for a patent for an invention entitled

Dehydrocholecalciferol Derivatives

I, Fridolin Klausner, of 187 Baselmattweg, 4123 Allschwil, Switzerland, do solemnly and sincerely declare as follows:

1. I am authorised by F.Hoffmann-La Roche AG, the applicant for the patent, to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was made in United States of America on 18 May 1989 by Barbara J. Baggiolini (executrix to the deceased inventor Enrico G. Baggiolini), Bernard Michael Hennesy, Shian-Jan Shiuey, Gary Arthur Truitt and Milan Radoje Uskokovic.

3. Enrico Giuseppe Baggiolini, deceased, formerly of 30 Evergreen Drive, North Caldwell, New Jersey 07006, United States of America, Bernard Michael Hennessy, Shian-Jan Shiuey, Gary Arthur Truitt and Milan Radoje Uskokovic, of 111 Passaic Avenue, Nutley, New Jersey 07110; 331 Bloomfield Avenue, Nutley, New Jersey 07110; 109 Garner Avenue, Bloomfield, New Jersey 07003; and 253 Highland Avenue, Upper Montclair, New Jersey 07043, all in United states of America, respectively, are the actual inventors of the invention and the facts upon which the applicant is entitled to make the application are as follows:-

   The said applicant is the assignee of Bernard Michael Hennessy, Shian-Jan Shiuey, Gary Arthur Truitt, Milan Radoje Uskokovic and Barbara J. Baggiolini the said legal representative of the deceased other inventor Enrico Giuseppe Baggiolini.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Basle, Switzerland this 10th day of July, 1990

Fridolin Klausner

TO: THE COMMISSIONER OF PATENTS
AUSTRALIA
S&F Ref: 128409
cjd:5903D

AU9055165

(12) PATENT ABRIDGMENT (11) Document No. AU-B-55165/90
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 636609

(54) Title
DEHYDROCHOLECALCIFEROL DERIVATIVES

International Patent Classification(s)
(51)[5] **C07C 401/00** **A61K 031/59**

(21) Application No. : **55165/90** (22) Application Date : **17.05.90**

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(43) Publication Date : **22.11.90**

(44) Publication Date of Accepted Application : **06.05.93**

(71) Applicant(s)
F.HOFFMANN-LA ROCHE AG

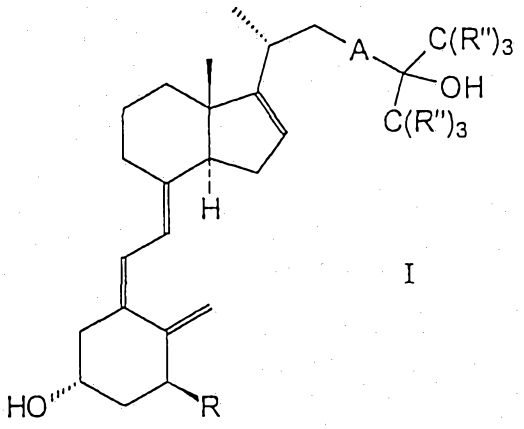
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(56) Prior Art Documents
AU 622139 28644/89 A61K 031/59
AU 28643/89 C07C 401/00 A61K 031/59

(57) Claim

1. A method of treating sebaceous gland diseases in a patient requiring or desiring such treatment, comprising administering to said patient an effective amount of a compound of formula

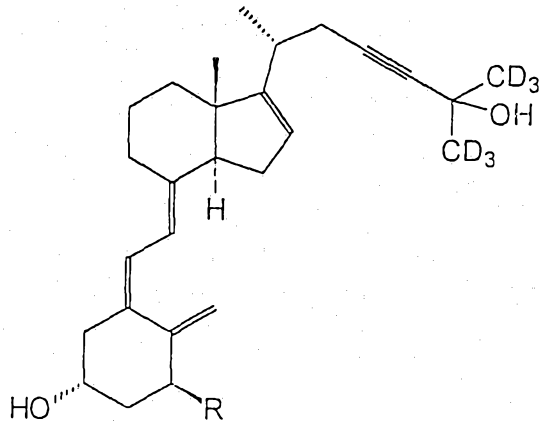


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wherein R is H or OH, A is -C≡C-, -CH=CH- with E-configuration or $-CH_2CH_2-$ and R" is H or, where A is -C≡C-, R" may also be deuterium or of a pharmaceutical composition containing said compound together with a pharmaceutically effective carrier, diluent, excipient and/or adjuvant.

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6. A compound of formula



wherein R is H or OH.

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S & F Ref: 128409

FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE:

Class Int Class

Complete Specification Lodged:
Accepted:
Published:

Priority:

Related Art:

Name and Address of Applicant: F.Hoffmann-La Roche AG
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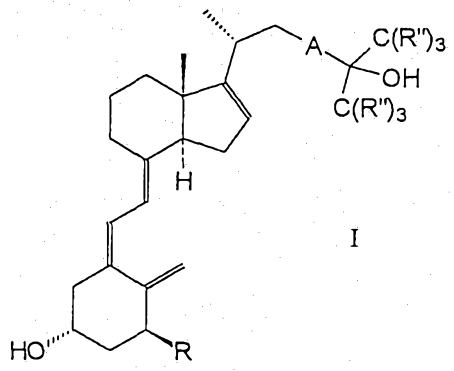
Complete Specification for the invention entitled:

Denydrocholecalciferol Derivatives

The following statement is a full description of this invention, including the best method of performing it known to me/us

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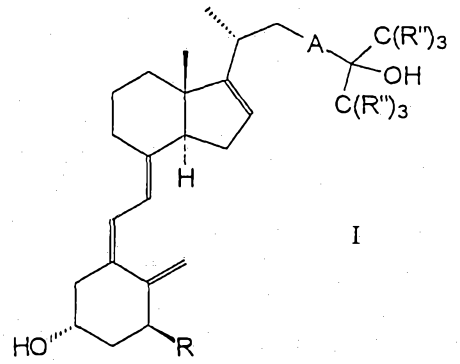
The invention is based on the finding of novel physiological properties of dehydrocholecalciferol derivatives. Specifically, it has been found that the compounds of formula I



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wherein R is H or OH, A is -C≡C-, -CH=CH- with E-configuration or $-CH_2CH_2-$ and R" is H or, where A is -C≡C-, R" may also be deuterium, are useful for the treatment of sebaceous gland diseases, such as acne and seborrheic dermatitis.

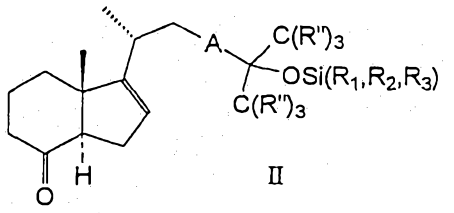
According to a first embodiment of the present invention there is provided a method of treating sebaceous gland diseases in a patient requiring or desiring such treatment, comprising administering to said patient an effective amount of a compound of formula



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wherein R is H or OH, A is -C≡C-, -CH=CH- with E-configuration or $-CH_2CH_2-$ and R" is H or, where A is -C≡C-, R" may also be deuterium.

Among the compounds of formula I, those wherein R" is deuterium are novel and as such are part of the invention. The deuterated compounds can be prepared in a manner similar to the non-deuterated compounds, e.g. as described in the European patent application No. 325 279, via intermediates of formula



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wherein R" is as above, R^1 and R^3 are lower alkyl and R^2 is lower alkyl, aryl or aryl-lower alkyl.

The invention relates to the above mentioned deuterated compounds, further to the use of the compounds of formula I for the treatment of sebaceous gland diseases such as seborrheic dermatitits and, specifically, acne; to pharmaceutical compositions in dosage unit form suitable for oral or topical administration for the above treatment, containing one or more compounds of formula I, as well as to commercial packages containing as active pharmaceutical ingredient one or more compounds of formula I, together with instructions for the use thereof as indicated above. The invention also relates to a method for treating the above-mentioned disease states by administration of a compound of formula I or a mixture of two or more compounds of formula I.

Compounds of formula I are compounds A to H as defined below:

A: 1α,25-dihydroxy-16-dehydrocholecalciferol;
B: 25-hydroxy-16-dehydrocholecalciferol;
C: 1α,25-dihydroxy-16,23E-bisdehydrocholecalciferol;
D: 25-hydroxy-16,23E-bisdehydrocholecalciferol;
E: 1α,25-dihydroxy-16-dehydro-23-didehydrocholecalciferol;
F: 25-hydroxy-16-dehydro-23-didehydrocholecalciferol;
G: 26,26,26,27,27,27-hexadeutero-1α,25-dihydroxy-16-dehydro-23-didehydrocholecalciferol and
H: 26,26,26,27,27,27-hexadeutero-25-hydroxy-16-dehydro-23-didehydrocholecalciferol.

The useful activity of compound of formula I as agents for the treatment of acne can be demonstrated by the following test procedures:

Sebaceous cells are isolated from adult human sebaceous glands and cultured on a layer of mouse 3T3 fibroblasts in a

manner analogous to that described in Cell 6, 1975, 331-334 and In Vitro 22:3, 1986, Part II, p. 22a, abstract #46. It involves the separation of the epidermal layer from the dermis by an electrokeratome. The dermal tissue is then treated by enzymatic and mechanical methods, to generate a single cell suspension of sebaceous cells.

The cells are cultured in either Iscove's medium containing 2% human serum, 8% fetal calf serum and 4 µg/ml dexamethasone, or Iscove's medium containing 10% fetal calf serum and 4 µg/ml dexamethasone.

Cells are plated in medium without a compound of the invention and then given the compound in fresh medium 24-48 hours after the initial plating. The cultures are given fresh medium, containing a compound of the invention, every 48 hours. On the day of harvesting, the cultures are rinsed with 0.03% EDTA in PBS, to remove only the 3T3 fibroblasts. The remaining sebocyte colonies are incubated in 0.05% trypsin/0.03% EDTA to create a single cell suspension of sebocytes. The cells are diluted and counted in a hemocytometer.

Stock solutions of the test compounds are made up as 10^{-2} M solutions in ethanol and then diluted to concentrations of 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M. Also tested for the inhibition of proliferation of sebaceous cells in vitro was 1α,25-dihydroxycholecalciferol (compound X in the tables below).

The results are given in Table 1 as the amount of compound necessary to inhibit the proliferation of the sebaceous cells by 50% as compared to a control. The control was a culture treated with diluent only.

Table 1

Compund	X	A	D	E	F
ED_{50} (µM)	0.005	0.001	>1	0.001	0.1

The results demonstrate that the compounds of the invention inhibit human sebocyte proliferation in vitro and, therefore, are useful as agents for the treatment of acne.

Several compounds were evaluated for topical anti-acne activity in the hamster ear sebaceous gland model. 50 µl of a solution of the compounds in acetone, were applied daily to the dorsal side of the right ear of the hamster. Control hamsters received 50 µl of acetone. The animals were sacrificed after 4 weeks. The ears were removed and processed for histological evaluation. The areas of the sebaceous glands were determined from the cross sections by image analysis.

In Table 2, the data are expressed as percent change from control animals of the hamster ear sebaceous gland size cross section.

Table 2

Compound	Dose (µg/hamster)	% Change
D	0.10	-16
	1.00	-16
	10.00	-43
F	0.01	- 8
	0.10	-23
	1.00	-40
	10.00	-64

In order to evaluate the calcification of soft tissues by the compounds of the invention, rats were labelled with a

subcutaneous injection of 40 µCi ^{45}Ca. The compounds were then administered either subcutaneously or topically for four consecutive days. The rats were sacrificed one day after the last injection. The hearts and kidneys were removed and digested with nitric acid. An aliquot of the digest was counted in a scintillation counter. The calcification ratio

(cpm of X - control cpm)/(cpm of compound - control cpm)

is given below:

Table 3

Compound	Subcutaneous	Topical
X	1	1
D	>1400	>34
E	47	< 1
F	>1400	>34

The effect of compounds of the invention on the sebaceous glands of the hamster ear after oral administration of the compounds was evaluated. 200 µl of a compound of the invention was dissolved in propylene glycol, administered daily to male Golden Syrian hamsters. The animals were sacrificed after 4 weeks and the ears were processed for histological evaluation. The area of the sebaceous glands was measured on histologically prepared cross sections of the ear by image analysis. The data obtained are given below as % change in hamster ear sebaceous gland size cross section:

Table 4

Compound	Dose (µg/kg)	% Change
D	2.50	-15*
	5.00	-22**
	10.00	-27***
	20.00	-36***
F	0.05	-16*
	0.50	-23**
	5.00	-42***
	50.00	-55***

* p <0.05; ** p <0.01; *** p <0.001

The above data demonstrate that compounds of the invention are useful as agents in the treatment of sebaceous gland diseases, such as acne or seborrheic dermatitits. Moreover, they bring about less soft tissue calcification than does 1α,25-dihydroxycholecalciferol. Soft tissue calcification is an undesirable side effect in a compound to be used for treating sebaceous gland diseases.

The compounds of formula I can be administered topically or orally for the treatment of sebaceous gland diseases such as acne or seborrheic dermatitits. Thus, they can be administered orally for the treatment of acne in humans at a dosage of about 0.7 to 700, preferably 7.0 to 70 µg per day.

Oral dosage forms comprising compounds of formula I may be incorporated e.g. in capsules or tablets with pharmaceutically acceptable carriers. Examples of such carrier materials which may be incorporated into capsules are binders, such as gum tragacanth or gelatin; excipients, such as dicalcium phosphate; disintegrating agents, such as corn starch; lubricants, such as magnesium stearate; sweetening

agents, such as sucrose; flavoring agents, such as peppermint. Tablets may be coated with shellac, sugar or both. A syrup or elixir may contain a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring agent.

Topical dosage forms comprising compounds of formula I include ointments and creams encompassing formulations having oleaginous, adsorbable, water soluble and emulsion-type bases, such as lanolin and polyethylene glycols. Topical dosage forms also comprise gels, lotions, powders and aerosols.

Lotions, i.e. liquid preparations varying from simple solutions to aqueous of hydroalcoholic preparations containing finely divided substances, can contain suspending or dispersing agents, such as cellulose derivatives, e.g. ethyl or methyl cellulose; gelatin or gums, which incorporate the active ingredient in a vehicle made up of water, alcohol or glycerin. Gels are semi-solid preparations made by gelling a solution or suspension of the active ingredient in a carrier vehicle. The vehicles, which can be hydrous or anhydrous, are gelled using a gelling agent, e.g. carboxy polymethylene, and neutralized to a proper gel consistency with the use of alkalies, e.g. sodium hydroxide, or amines, such as polyethylenecocoamine.

The following Examples illustrate the composition of soft gelatine capsules for oral administration and of a topical cream:

Example A

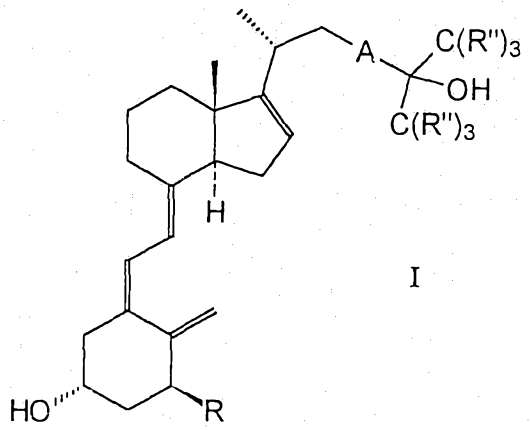
	mg/capsule
Compound E	0.0001-0.010
Butylated hydroxytoluene	0.016
Butylated hydroxyanisole	0.016
Fractionated coconut oil	160.0

Example B

	mg/g of cream
Compound E	0.001-1.0
Cetyl alcohol	1.5
Stearyl alcohol	2.5
Sorbitan monostearate	2.0
Glyceryl monostearate and polyoxyethylene glycol stearate	4.0
Polysorbate 60	1.0
Mineral oil	4.0
Propylene glycol	5.0
Propylparaben	0.05
Butylated hydroxyanisole	0.05
Sorbitol solution	2.0
Edetate disodium	0.01
Methylparaben	0.18
Distilled water	q.s. to 100 g

The claims defining the invention are as follows:

1. A method of treating sebaceous gland diseases in a patient requiring or desiring such treatment, comprising administering to said patient an effective amount of a compound of formula



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wherein R is H or OH, A is -C≡C-, -CH=CH- with E-configuration or $-CH_2CH_2-$ and R" is H or, where A is -C≡C-, R" may also be deuterium or of a pharmaceutical composition containing said compound together with a pharmaceutically effective carrier, diluent, excipient and/or adjuvant.

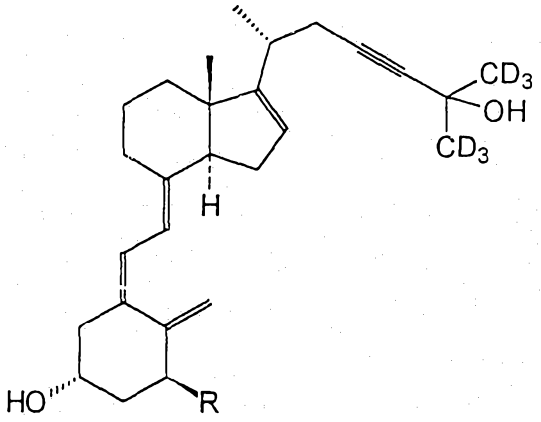
2. A method according to claim 1, wherein in the compound of formula I according to claim 1, R is H or OH, A is -C≡C- and R" is deuterium.

3. A method according to claim 1 or claim 2, wherein the compound or composition is administered in a daily dosage individually determined by the attending physician.

4. A method according to any one of claims 1 to 3, wherein said disease is seborrheic dermatitis.

5. A method according to any one of claims 1 to 3, wherein said disease is acne.

6. A compound of formula



wherein R is H or OH.

7. A dehydrocholecalciferol derivative substantially as hereinbefore described

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with reference to Compounds G and H of the Examples.

8. A pharmaceutical composition comprising an effective amount of one or more compounds according to claim 6 or claim 7 and a pharmaceutically acceptable excipient, diluent, carrier and/or adjuvant.

Dated 2 February, 1993

F.Hoffmann-La Roche AG

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

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