

PATENT SPECIFICATION

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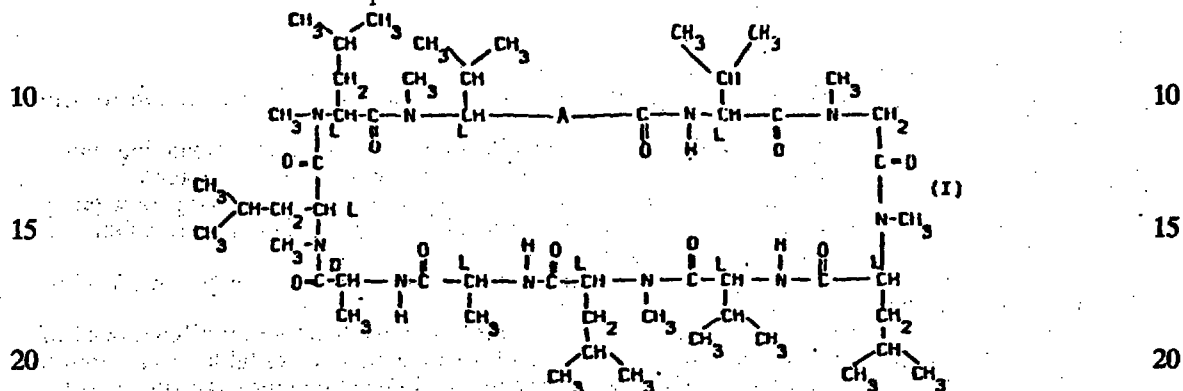
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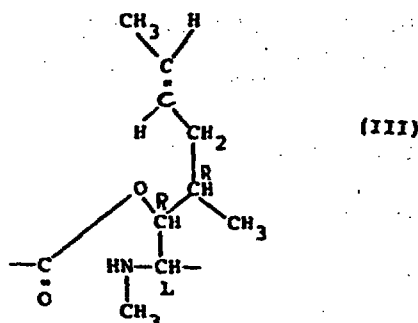
(54) CYCLOSPORIN DERIVATIVES

(71) We, SANDOZ LTD., of 35 Lichtstrasse, 4002 Basle, Switzerland, a Swiss Body Corporate, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

5 The present invention relates to cyclosporin derivatives. More particularly the present invention relates to compounds of formula I

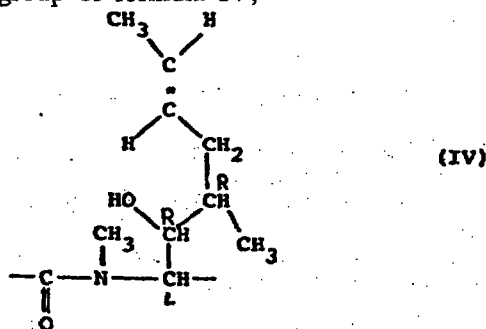


or of formula III



which compounds are hereinafter referred to as dihydrocyclosporin D and isocyclosporin D, respectively.

The present invention also provides a process for the production of:-
a) dihydrocyclosporin D which comprises hydrogenating a compound of formula I above wherein A is a group of formula IV,



which compound is hereinafter referred to as cyclosporin D, and

b) isocyclosporin D which comprises isomerising cyclosporin D by subjection to acid conditions.

(The symbols "D" and "L" in the formulae I to IV above and in the accompanying claims indicate D and L optically active centres in accordance with standard usage).

The required starting material for the above process, cyclosporin D, may be produced by cultivating a cyclosporin D producing strain of the species *Tolypocladium inflatum* Gams in a nutrient medium and isolating cyclosporin D.

The cultivation process may be effected in conventional manner for analogous strains, e.g. as described in Example 1 hereinafter.

A preferred cyclosporin D producing strain is the freely available strain NRRL 8044 of the species *Tolypocladium inflatum* Gams, a culture of which is available from United States Department of Agriculture (Northern Research and Development Division), Peoria, Ill., USA. This strain was formerly described as a strain of the species *Trichoderma polysporum* (Link ex Pers.) and is described in the literature, e.g. in U.K. Patent Specification No. 1,491,509.

Alternatively cyclosporin D producing strains obtained by selection or mutation of NRRL 8044, or treatment of this strain by ultraviolet light or X-rays, or treatment of this strain with laboratory chemicals, may be used.

Cyclosporin D may be isolated in conventional manner, e.g. as described in Example 1, from other natural products that may be produced in greater amounts, e.g. the somewhat more polar cyclosporin A (also known as S 7481/F-1), the more polar cyclosporin B (also known as S 7481/F-2) and the yet more polar cyclosporin C.

The compound cyclosporin D is production and compositions containing the same and the subject of our co-pending application No. 8006316 (Serial No. 1591934).

The hydrogenation process (a) may be effected in conventional manner, e.g. by catalytic hydrogenation. Preferred solvents include methanol, ethanol, isopropanol or ethyl acetate. The process is preferably effected in a neutral medium at a temperature of from 20 to 30°C and at atmospheric or slightly elevated pressure. A preferred catalyst is palladium on charcoal.

The acid treatment of process (b) may be effected in conventional manner, e.g. with trifluoroacetic acid or preferably methanesulphonic acid or p-toluenesulphonic acid. The mole ratio of acid to cyclosporin D is preferably from 1:1 to 4:1. Preferred solvents include methanol, chloroform, and dioxane. Preferred temperatures are from 20 to 65°C, more preferably from 40 to 55°C.

In the following examples all temperatures are in degrees Centigrade. All ratios are by volume unless otherwise stated.

EXAMPLE 1

5 Preparation of starting material - cyclosporin D

500 litres of a nutrient solution containing per litre, 40 g of glucose, 2 g of sodium caseinate, 2.5 g of ammonium phosphate, 5 g of $MgSO_4 \cdot 7H_2O$, 2 g of KH_2PO_4 , 3 g of $NaNO_3$, 0.5 g of KCl, 0.01 g of $FeSO_4$ and demineralized water to 1 litre, are inoculated with 50 litres of a pre-culture of the strain NRRL 8044 and are incubated in a steel fermenter under stirring (170 rpm) and aeration (1 litre air/minute/litre nutrient solution) for 13 days at 27° (see U.K. Patent Specification No. 1,491,509).

The culture liquor is stirred with the same amount of n-butyl acetate, concentrated by evaporation in a vacuum after separation of the organic phase and the crude extract is de-fatted by a 3-stage partition between methanol/water (9:1) and petroleum ether. The methanolic phase is separated, concentrated by evaporation in a vacuum and the crude product is precipitated by the addition of water. The material obtained upon filtration is chromatographed on silica gel with n-hexane/acetone (2:1) as eluant. The initially eluted fractions contain predominantly cyclosporin A and cyclosporin D and the later eluted fractions contain predominantly cyclosporin C. For further purification the cyclosporin A-and D-containing fractions are crystallized from a 2- to 2.5-fold amount of acetone at -15°. The crystallate is further chromatographed twice on silica gel, the fractions initially eluted with water-saturated ethyl acetate, containing cyclosporin D in greatly enriched form. These are dissolved in twice the amount of acetone and are allowed to crystallize at -15°. The resulting crude crystalline product of cyclosporin D is dissolved for further purification in a 10-fold amount of acetone, 2% by weight of active charcoal are added and heating is effected for 5 minutes to 60°C. The clear and almost colourless filtrate obtained upon filtration over talc is concentrated by evaporation to a third of its volume and is allowed to cool down at room temperature, whereupon cyclosporin D crystallizes spontaneously. The crystallization is completed by allowing to stand at -17°. The crystals obtained by filtration are washed with a small amount of ice-cold acetone and are subsequently dried in a high vacuum at 80° for 2 hours.

CHARACTERIZATION OF cyclosporin D:

Colourless, prismatic crystals. M.P. 148-151°

35 $[\alpha]_D^{20} = -245^\circ$ (c = 0.52 in chloroform)

$[\alpha]_D^{20} = -211^\circ$ (c = 0.51 in methanol)

EXAMPLE 2

40 Preparation of end-product: dihydrocyclosporin D

400 mg of palladium on charcoal [10% (w/w) palladium] are pre-hydrogenated in 15 ml of ethanol during the course of 20 minutes. To this suspension of the palladium catalyst, there is added the solution of 3.66 g of cyclosporin D in 30 ml of ethanol. The mixture is hydrogenated at 24° and at a pressure of 736 mm of Hg until the hydrogen take-up is complete. Subsequently the catalyst is filtered off and the filtrate is evaporated to dryness in a vacuum at 20 to 40°C. The dihydrocyclosporin D, which is pure by thin layer chromatography, precipitates as colourless amorphous powder, which is dried in a high vacuum for 4 hours at 70°.

CHARACTERIZATION OF dihydrocyclosporin D: M.P. 153-156°

50 $[\alpha]_D^{20} = -237^\circ$ (c = 0.56 in chloroform)

$[\alpha]_D^{20} = -196^\circ$ (c = 0.58 in methanol)

EXAMPLE 3

55 Preparation of end-product: isocyclosporin D

A solution of 3.6 g of methanesulphonic acid in 60 ml of dioxane is added to the solution of 18.25 g of antibiotic cyclosporin D in 120 ml of absolute dioxane and the mixture is kept at 50° in the absence of moisture. The reaction course is followed by thin layer chromatography [polygram SIL G-foils; chloroform/methanol/glacial acetic acid (90:6:4); iodine vapour for detection]. After 17 hours, the mixture is cooled to room temperature. After 3.38 g of anhydrous sodium acetate is added the precipitated salt is filtered with suction after stirring for 15 minutes and the filtrate is concentrated by evaporation in a vacuum at 45°. The 21 g of residue are chromatographed on 1.5 kg of silica gel, using chloroform/methanol (98:2) for elution. The fractions consisting of practically pure

isocyclosporin D are combined, concentrated by evaporation in a vacuum at 50° and the residue is crystallized twice or thrice from diethyl ether, whereupon the isocyclosporin D precipitates out.

CHARACTERIZATION OF isocyclosporin D: M.P. 142-144°

$[\alpha]_D^{20} = -205.5^\circ$ (c = 0.51 in chloroform)

$[\alpha]_D^{20} = -144.4^\circ$ (c = 0.64 in methanol)

The compounds dihydrocyclosporin D and isocyclosporin D exhibit pharmacological activity. In particular, they exhibit anti-inflammatory activity and anti-arthritis activity as indicated in standard tests with animals, for example by an inhibition of swellings in the Freund's adjuvant arthritis test in rats on p.o. administration of 3 to 100 mg/kg of the compounds.

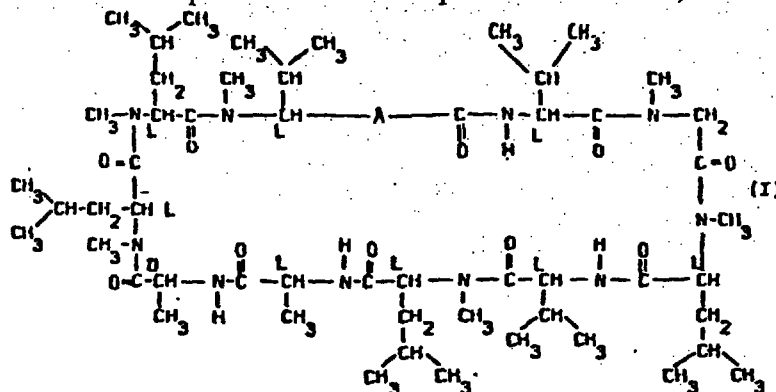
The compounds are therefore indicated for the treatment and prophylaxis of chronic inflammations, e.g. arthritis and rheumatic disorders. For this use an indicated daily dose is from 50 to 900 mg, conveniently administered in divided doses 2 to 4 times a day in unit dosage form containing from 15 to 450 mg, or in sustained release form.

The present invention also provides a pharmaceutical composition comprising dihydrocyclosporin D or isocyclosporin D in association with a pharmaceutical carrier or diluent. Such compositions may be in the form of, for example, a solution or a tablet.

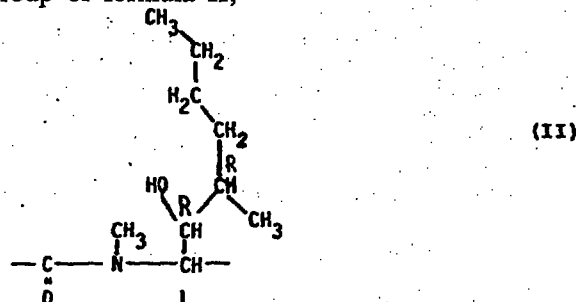
The compound of Example 3 exhibits especially interesting activity.

WHAT WE CLAIM IS:

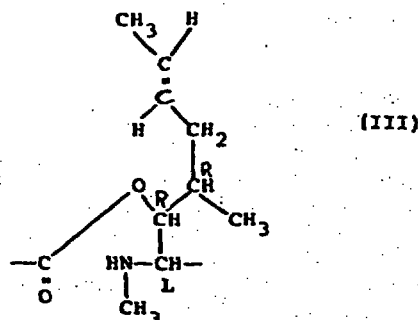
1. A process for the production of a compound of formula I,



wherein A is a group of formula II,



or of formula III,



which compounds are hereinafter referred to as hydrocyclosporin D or isocyclosporin D

isocyclosporin D are combined, concentrated by evaporation in a vacuum at 50° and the residue is crystallized twice or thrice from diethyl ether, whereupon the isocyclosporin D precipitates out.

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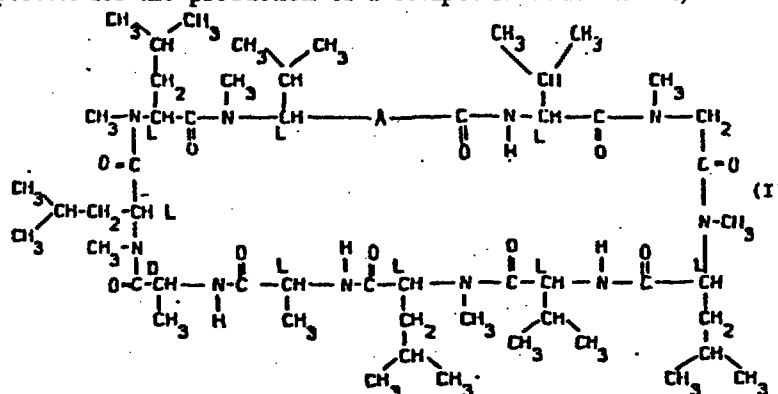
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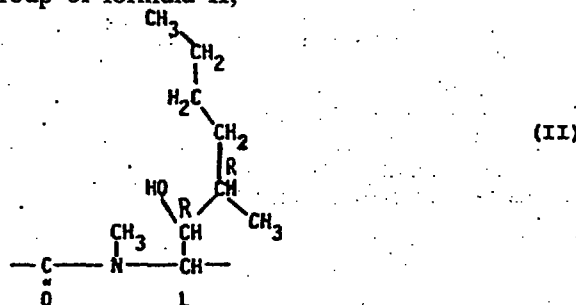
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WHAT WE CLAIM IS:

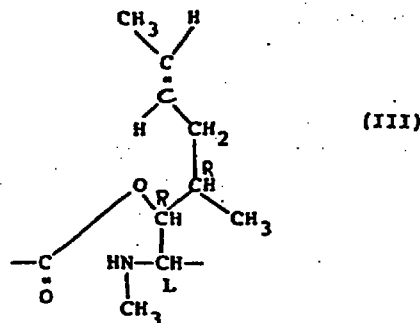
1. A process for the production of a compound of formula I,



wherein A is a group of formula II,



or of formula III,



which compounds are hereinafter referred to as hydrocyclosporin D or isocyclosporin D



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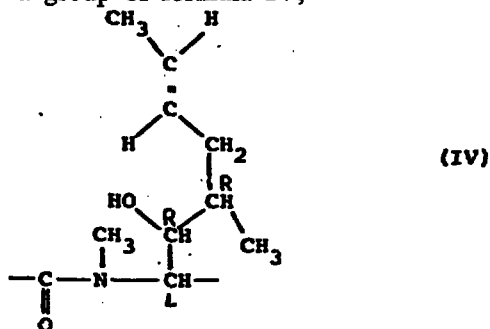
respectively, which process comprises

a) for the production of dihydrocyclosporin D, hydrogenating a compound of formula I, above, wherein A is a group of formula IV,

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which compound is hereinafter referred to as cyclosporin D, or

b) for the production of isocyclosporin D, isomerising cyclosporin D by subjection to acid conditions.

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2. A process as claimed in Claim 1, substantially as hereinbefore described with reference to Example 2 or 3.

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3. Dihydrocyclosporin D or isocyclosporin D according to Claim 1 whenever prepared by a process as claimed in Claim 1 or Claim 2.

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4. Dihydrocyclosporin D of formula I as illustrated in Claim 1, wherein A is a group of formula II as illustrated in Claim 1.

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5. Isocyclosporin D of formula I as illustrated in Claim 1, wherein A is a group of formula III as illustrated in Claim 1.

6. A pharmaceutical composition comprising dihydrocyclosporin D as claimed in Claim 4 in association with a pharmaceutical carrier or diluent.

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7. A pharmaceutical composition comprising isocyclosporin D as claimed in Claim 5 in association with a pharmaceutical carrier or diluent.

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