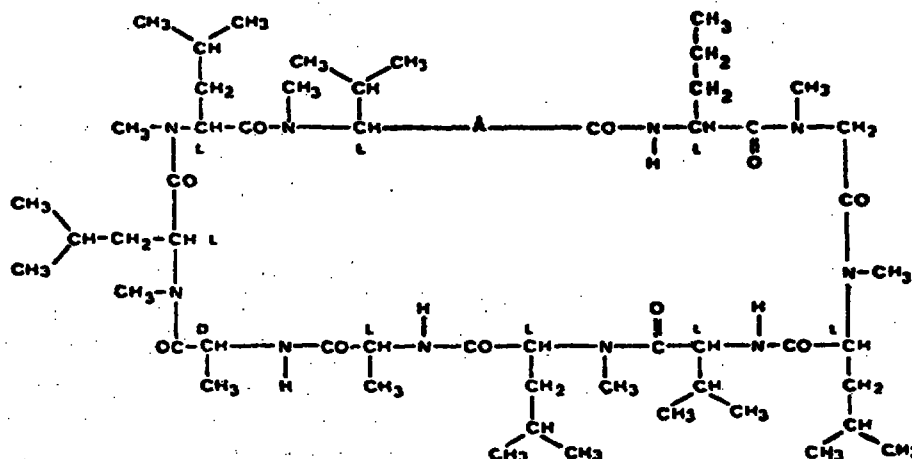


SPECIFICATION

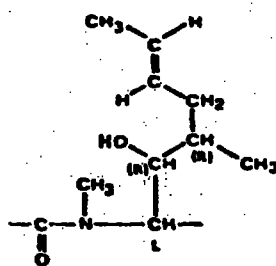
Cyclosporin Derivatives, their Production and Pharmaceutical Compositions Containing them

This invention relates to cyclosporin derivatives, which are monocyclic undecapeptide derivatives, their production and pharmaceutical compositions containing them.

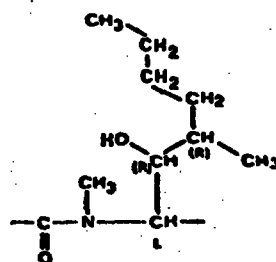
5 The present invention provides cyclosporin G, dihydrocyclosporin G or isocyclosporin G of formula I



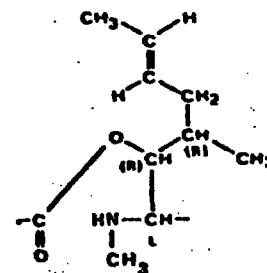
wherein A is



or



or



10 respectively.

The present invention provides a process for the production of cyclosporin G, dihydrocyclosporin G or isocyclosporin G which comprises,

- a) for the production of cyclosporin G, cultivating a cyclosporin G-producing strain of the fungal species *Tolypocladium* in the presence of a nutrient medium and isolating cyclosporin G,
- 15 b) for the production of dihydrocyclosporin G, reducing cyclosporin G, or
- c) for the production of iso-cyclosporin G, rearranging cyclosporin G.

The cultivation according to process a) may be effected in known manner for the cultivation of analogous strains, e.g. as described in Example 1.

20 A preferred cyclosporin G producing strain is a strain of *Tolypocladium inflatum* Gams. A preferred strain is freely available and has been described in U.K. Patent Specification No. 1,451,509. This has been deposited with the United States Department of Agriculture (Northern Research and Development Division), Peoria, Ill, U.S.A. under the code NRRL 8044 as well as with the Fermentation Research Institute of Japan, Inage, Chiba City, Japan, under the code FERM P No. 2796. This strain was formerly classed as *Trichoderma polysporum* (Link ex Pers) and has now been reclassified as a

25 *Tolypocladium inflatum* Gams strain. Other cyclosporin G-producing strains of *Tolypocladium inflatum* Gams may be produced e.g. by selection, mutation of the above mentioned preferred strain under the influence of ultra-violet or x-ray radiation, or reaction of this strain with appropriate chemicals.

30 Cyclosporin G may be isolated in known manner for isolating fermentation products, e.g. as described in Example 1, from the culture broth. The purification conditions should be chosen to separate cyclosporin G from other natural products that may be present, e.g. the less polar cyclosporin D, and the more polar cyclosporins A and B (also known as S 7481/F-1 and S 7481/F-2 respectively) as well as the yet more polar cyclosporin C.

35 Process b) may be effected using conventional reduction processes, e.g. catalytic hydrogenation. Suitable solvents include ethyl acetate, methanol, ethanol or isopropanol. Conveniently neutral conditions are used. Suitable reaction temperatures are from 20 to 30°C at atmospheric pressure or at a slightly elevated pressure. Suitable catalysts include platinum or preferably palladium, e.g. palladium-on-charcoal.

Process c) may be effected in conventional manner for the rearrangement of analogous cyclosporins, e.g. under acid conditions. Conveniently a strong organic acid is used, e.g. trifluoroacetic acid, methanesulphonic acid or p-toluenesulphonic acid.

The amount of strong acid present is conveniently from 1 to 4 moles per mole of cyclosporin G. As solvent may be used, for example, methanol, chloroform or dioxane. Reaction temperatures are conveniently from 20 to 65°C and preferably from 45 to 55°C.

In the following Examples, all temperatures are in degrees Centigrade and are uncorrected. All ratios are in parts by volume unless stated otherwise. Silicagel is e.g. silicagel 60 (Merck; particle size 0.063—0.2 mm: the word "Merck" is a Registered Trade Mark).

10 Example 1: Cyclosporin G

500 litres of a nutrient solution (containing per litre 40 g glucose, 2 g sodium caesinate, 2.5 g ammonium phosphate, 5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 g KH_2PO_4 , 3 g NaNO_3 , 0.5 g KCl, 0.01 g FeSO_4 and demineralized water to 1 litre) are inoculated with 50 litres of a preculture of the strain NRRL 8044 and incubated in a steel fermenter under stirring (170 r.p.m.) and under aeration (1 litre air/minute/litre nutrient solution) for 13 days at 27° (see also the above-mentioned U.K. Patent Specification).

The culture broth is stirred with the same volume of n-butyl acetate. The organic layer is separated, concentrated in a vacuum and partitioned between methanol/water (9:1) and petroleum ether. The methanolic phase is shaken with petroleum ether twice more to remove further fatty material. The methanolic phase is concentrated in a vacuum and water is added to precipitate the product which is filtered off. The product is chromatographed on a 5 to 7 fold amount of Sephadex LH 20 with methanol as eluant (The word "Sephadex" is a Registered Trade Mark). The leading fractions are then chromatographed on silicagel using n-hexane/acetone (2:1) as eluant, affording first fractions containing mainly cyclosporins A and D (as well as some cyclosporin G) and then fractions containing cyclosporin C.

The first fractions are each treated with a 2 to 2.5 fold volume of acetone and at -15° crystals are produced. The crystals are chromatographed twice on silicagel using hexane/acetone (2:1) as eluant, yielding first fractions containing cyclosporin D (as well as some cyclosporin G). These fractions are dissolved in a 2 fold volume of acetone and allowed to crystallize at -15°. The crystals are crude cyclosporin D. The mother liquor contains, besides cyclosporin D, cyclosporin G and is concentrated to dryness. The residue is chromatographed on silicagel using water-saturated ethyl acetate as eluant. After cyclosporin D is eluted, further fractions contain cyclosporin G, which are chromatographed on silicagel first using chloroform/methanol (98:2) as eluant and then, for separation, hexane/acetone (2:1). The latter fractions contain cyclosporin G in purified form which is crystallised twice from diethyl ether/petroleum ether (1:1) at room temperature. The final product is homogeneous by thin layer chromatography, >95% pure and comprises colourless polyhedrons of m.pt. 193—194° (after drying of the crystals at 80° in a high vacuum for 2 hours).

$[\alpha]_D^{20} = -245^\circ$ (c=1 in CHCl_3)

$= -191^\circ$ (c=1.04 in CH_3OH)

UV spectrum in CH_3OH : end absorption

IR spectrum in CH_2Cl_2 : see Figure 1

$^1\text{H-NMR}$ spectrum in CDCl_3 90 MHz (Tetramethylsilane as internal standard): see Figure 2

Formula: $\text{C}_{63}\text{H}_{113}\text{N}_{11}\text{O}_{12}$

Example 2: Dihydrocyclosporin G

700 mg palladium/charcoal (10% w/w Palladium) are hydrogenated in 20 ml ethanol over 30 minutes. A solution of 4.55 g cyclosporin G in 60 ml ethanol is added. The mixture is hydrogenated at 21° and at a pressure of 742 mm Hg until hydrogen uptake is complete. The catalyst is filtered off and the filtrate evaporated in a vacuum at 20 to 40° to dryness. The residue, dihydrocyclosporin G, is pure by t.l.c and is obtained in the form of a white amorphous powder. M.pt. 150—153° (after drying in a high vacuum at 80° for 4 hours).

$[\alpha]_D^{20} = -232^\circ$ (c=0.64 in CHCl_3)

UV spectrum in CH_3OH : end absorption

IR spectrum in CH_2Cl_2 : see Figure 3

$^1\text{H-NMR}$ spectrum in CDCl_3 90 MHz (Tetramethylsilane as internal standard): see Figure 4

Formula: $\text{C}_{63}\text{H}_{115}\text{N}_{11}\text{O}_{12}$

55 Example 3: Iso-Cyclosporin G

A solution of 1.2 g methanesulphonic acid in 20 ml anhydrous dioxane is added to a solution of 6.08 g cyclosporin G in 40 ml anhydrous dioxane, and maintained at 50° under anhydrous conditions. The progress of the rearrangement is followed by thin layer chromatography on silicagel [Polygram SIL G foil $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{acetic acid}$ 90:6:4; detection by iodine]. After 16 hours the mixture is cooled to room temperature. 1.13 g anhydrous sodium acetate are added to neutralise the acid. After 45 minutes, the mixture is filtered and the filtrate evaporated in a vacuum at 45°. The 8.1 g residue is chromatographed on 500 g silicagel using as eluant $\text{CHCl}_3/\text{CH}_3\text{OH}$ (98:2). The fractions containing practically pure isocyclosporin G are combined, evaporated to dryness at 50° in a vacuum and the

residue crystallised from diethyl ether at +7° to give the title compound m.pt. 143—146°.

$[\alpha]_D^{20} = -196^\circ$ (c=0.72 in CHCl_3)

$= -128^\circ$ (c=0.73 in CH_3OH)

UV spectrum in CH_3OH : end absorption

5 IR spectrum in CH_2Cl_2 : see Figure 5

$^1\text{H-NMR}$ spectrum in CDCl_3 90 MHz (Tetramethylsilane as internal standard): see Figure 6.

The compounds of formula I exhibit pharmacological activity. In particular the compounds exhibit anti-inflammatory activity and anti-arthritic activity as indicated by an inhibition of swellings in the

10 Friends adjuvant arthritis test in rats on p. o. administration of 30 to 100 mg/kg of the compounds. inflammations, e.g. arthritis and rheumatic disorders.

Furthermore, the compounds exhibit immuno-suppressive activity, e.g. by their effect on humoral and cellular immunity, as indicated in standard tests, e.g.

15 a) in the lymphocyte stimulation test according to Janossy *in vitro* at concentrations of 0.01 to 10.0 $\mu\text{g/ml}$ a strong inhibition of H^3 -thymidine incorporation, of the proliferation rate and of the blastogenesis of mice spleen lymphocytes stimulated with Concanavalin A was ascertained.

b) Oxazolone test in mice: A decrease in the ear swelling is observed upon administration of 5x70 mg/kg p.o. of the compounds.

20 c) A local hemolysis in gel: Jerne test [J. F. Borel, Agents and Actions (1974) 4 p.277]. The inhibition of hemolytic plaque-forming cells, immunoglobulin antibodies and immunoglobulin G_{2a} antibodies is observed at a dose of 3x50 mg/kg.

The compounds are therefore indicated for use in the treatment of autoimmune diseases.

For these uses 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 12 to 450 mg, or in sustained release

25 form. The present invention also provides a pharmaceutical composition comprising a compound of formula I in association with a pharmaceutical carrier or diluent.

Such compositions may be in the form of, for example, a solution or a tablet.

The preferred activity is the immunosuppressant activity.

30 The compound of Example 2 exhibits especially interesting activity.

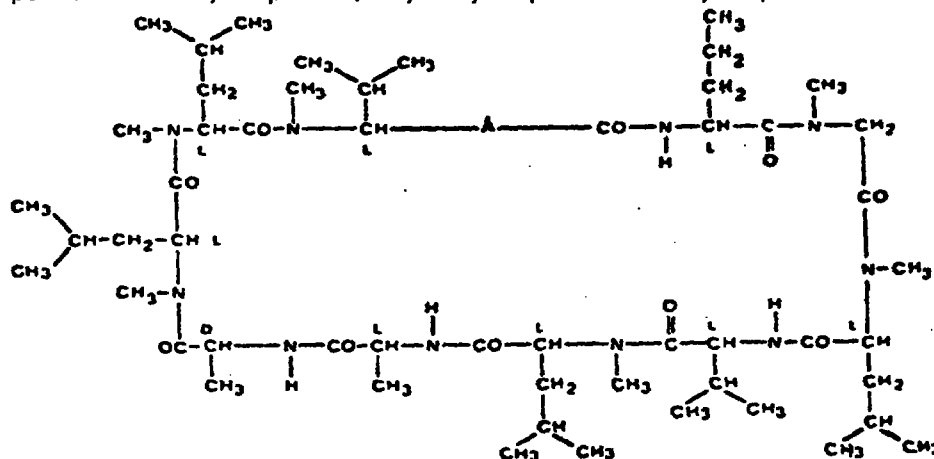
Specific embodiments of the present invention are:

1) Cyclosporin G substantially free from cyclosporin D; and

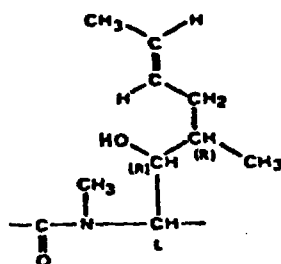
2) Cyclosporin G in greater than 95% purity.

Claims

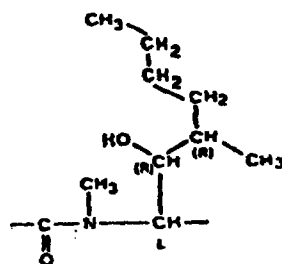
35 1. A compound which is cyclosporin G, dihydrocyclosporin G or isocyclosporin G of formula I



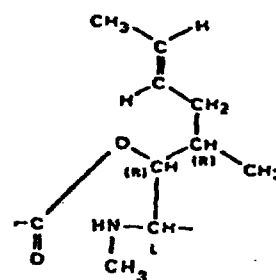
wherein A is



OR



OR



respectively.



residue crystallised from diethyl ether at +7° to give the title compound m.pt. 143—146°.

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$= -128^\circ$ (c=0.73 in CH_3OH)

UV spectrum in CH_3OH : end absorption

5 IR spectrum in CH_2Cl_2 : see Figure 5

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$^1\text{H-NMR}$ spectrum in CDCl_3 90 MHz (Tetramethylsilane as internal standard): see Figure 6.

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10 The compounds are therefore indicated for use in the treatment and prophylaxis of chronic inflammations, e.g. arthritis and rheumatic disorders.

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Furthermore, the compounds exhibit immuno-suppressive activity, e.g. by their effect on humoral and cellular immunity, as indicated in standard tests, e.g.

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25 form. The present invention also provides a pharmaceutical composition comprising a compound of formula I in association with a pharmaceutical carrier or diluent.

25

Such compositions may be in the form of, for example, a solution or a tablet.

The preferred activity is the immunosuppressant activity.

30 The compound of Example 2 exhibits especially interesting activity.

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Specific embodiments of the present invention are:

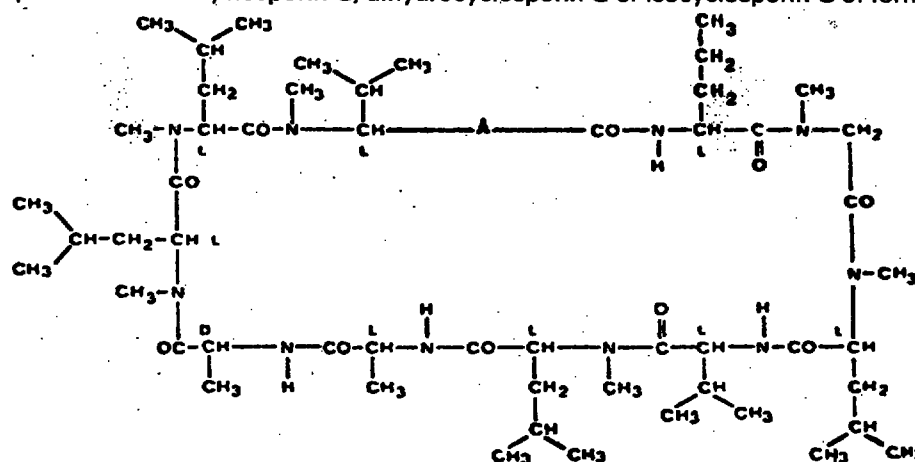
1) Cyclosporin G substantially free from cyclosporin D; and

2) Cyclosporin G in greater than 95% purity.

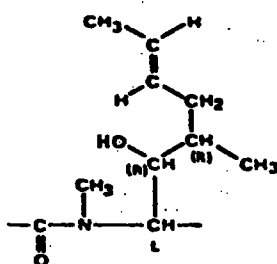
Claims

35 1. A compound which is cyclosporin G, dihydrocyclosporin G or isocyclosporin G of formula I

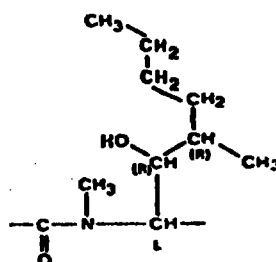
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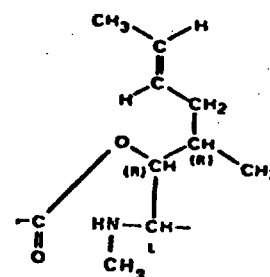
wherein A is



OR



OR



respectively.

20



- 4.
2. The compound of claim 1 which is cyclosporin G substantially free from cyclosporin D.
3. The compound of claim 1 which is cyclosporin G in greater than 95% purity.
4. The compound of claim 1 which is isocyclosporin G.
5. A process for the production of a compound of claim 1 which comprises,
- 5 a) for the production of cyclosporin G, cultivating a cyclosporin G-producing strain of the fungal species *Tolypocladium* in the presence of a nutrient medium and isolating cyclosporin G, 5
- b) for the production of dihydrocyclosporin G, reducing cyclosporin G, or
- c) for the production of iso-cyclosporin G, rearranging cyclosporin G.
6. A process for the production of a compound of claim 1 substantially as hereinbefore described
- 10 with reference to any one of the Examples. 10
7. A pharmaceutical composition which comprises a compound of any one of claims 1 to 4 and 6 in association with a pharmaceutical carrier or diluent.