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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| (51) International Patent Classification 5 : | | (11) International Publication Number: | WO 94/05685 |
|---|---|---|---|
| C07H 19/01, A61K 31/71 | A1 | (43) International Publication Date: | 17 March 1994 (17.03.94) |

(21) International Application Number: PCT/GB93/01850

(22) International Filing Date: 1 September 1993 (01.09.93)

(30) Priority data:
9218797.0 4 September 1992 (04.09.92) GB

(71) Applicant *(for all designated States except US):* FISONS PLC [GB/GB]; Fison House, Princes Street, Ipswich, Suffolk IP1 1QH (GB).

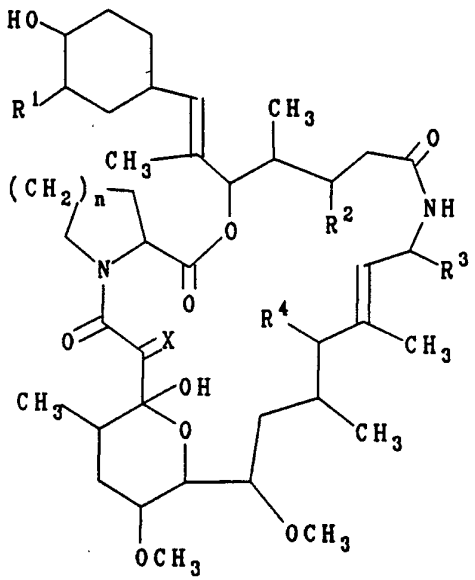
(72) Inventor; and
(75) Inventor/Applicant *(for US only) :* FURBER, Mark [GB/GB]; 22 Windmill Way, Kegworth, Derbyshire DE74 2FA (GB).

(74) Agent: WRIGHT, Robert, Gordon, McRae; Fisons plc, 12 Derby Road, Loughborough, Leicestershire LE11 0BB (GB).

(81) Designated States: AU, CA, FI, JP, KR, NO, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: MACROCYCLIC COMPOUNDS USEFUL IN THERAPY



(I)

(57) Abstract

Compounds of formula (I), wherein $R^1$ represents OH or $OCH_3$; $R^2$ represents H or OH; $R^3$ represents methyl, ethyl, propyl, allyl or 2-oxopropyl; $R^4$ represents H or OH; X represents O, (H,OH) or (H,H); n represents 1 or 2; and pharmaceutically acceptable derivatives thereof; are disclosed. The compounds are useful *inter alia* as immunosuppressants, and in the treatement of reversible obstructive airways diseases and certain skin diseases.

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# Macrocyclic compounds useful in therapy

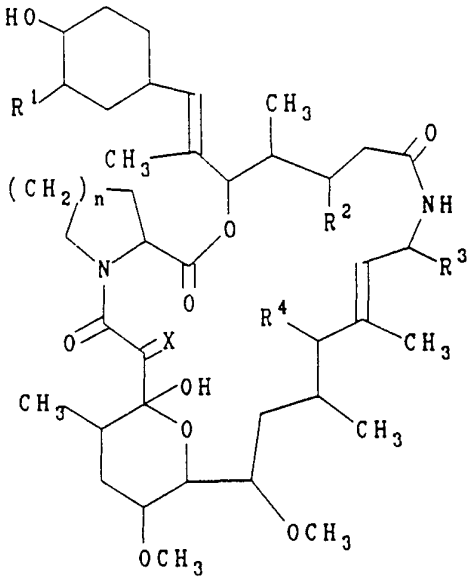
This invention relates to novel macrocyclic compounds, their use as medicaments, and pharmaceutical formulations including them.

European Patent Application 184162 (to Fujisawa Pharmaceuticals Co Ltd) discloses a number of macrocyclic compounds isolated from microorganisms belonging to the genus *Streptomyces*. The compounds are numbered FR-900506, FR-900520, FR-900523 and FR-900525, and the preparation of some of their derivatives is also described. The compounds are indicated as immunosuppressive agents.

European Patent Application 323042 and International Patent Application WO 91/02736 (both to Fisons plc) disclose many macrolides which may be derived from those disclosed in European Patent Application 184162. The compounds are primarily indicated as immunosuppressive agents. European Patent Applications 349049, 349061 and 388153 (to Merck & Co Inc) disclose the 3,4-dihydroxycyclohexyl analogues of FR-900506, FR-900520 and FR-900523 respectively and indicate them primarily as immunosuppressive agents. European Patent Application 405994 (to Merck & Co Inc) discloses a method of converting compounds such as FR-900506 which contain a piperidine ring into their pyrrolidine ring-containing analogues.

At the 6th National American Chemical Society Meeting (Medicinal Chemistry Symposium) held in Buffalo NY, USA, in June 1992, 18-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-24,26-dimethoxy-13,20,22,28-tetramethyl-11,29-dioxa-4,16-diazatricyclo[23.3.1.$0^{4,9}$]nonaconta-19-ene-2,3,10,17-tetraone was disclosed. A group of macrocyclic compounds possessing advantageous properties over this compound has now been found.

Thus, according to the invention, there is provided a compound of formula I,



I

wherein

$R^1$ represents OH or $OCH_3$;

$R^2$ represents H or OH;

$R^3$ represents methyl, ethyl, propyl, allyl or 2-oxopropyl;

$R^4$ represents H or OH;

X represents O, (H,OH) or (H,H);

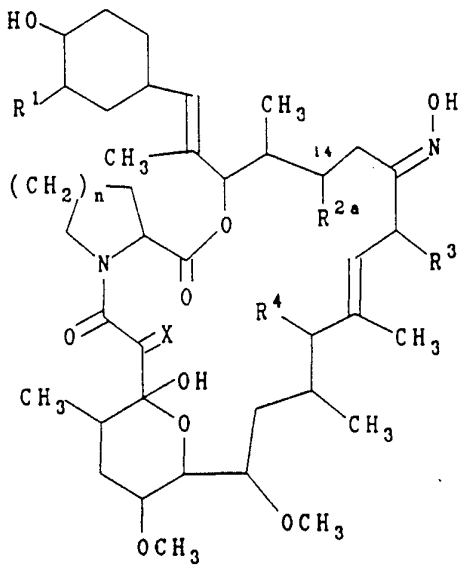
n represents 1 or 2;

and pharmaceutically acceptable derivatives thereof (hereinafter referred to *en bloc* as "the compounds of the invention").

Pharmaceutically acceptable derivatives of compounds of formula I include prodrugs, i.e. compounds which may be metabolized *in vivo* to the compound of formula I. These include compounds which differ from the compound of formula I in that some or all of the OH groups are derivatized to esters or carbamates. Such esters may be prepared by conventional methods, for example reaction with a carboxylic acid and a coupling agent, or an acyl chloride, and the carboxylic acid moiety preferably contains from 1-6 carbon atoms. Such carbamates may also be prepared by conventional methods, for example reaction with an isocyanate, and the carbamic acid moiety preferably contains from 1-6 carbon atoms. Specific esters and carbamates are $CH_3CO_2$-, $C_6H_5CO_2$-, $H_2NCO_2$- and $CH_3NHCO_2$-.

Preferably, $R^4$ represents H.

Compounds of formula I may be prepared by a Beckmann rearrangement of an E-oxime of formula II,



II

wherein $R^1$, $R^3$, $R^4$, X and n are as defined above, and $R^{2a}$ represents H or protected hydroxy, followed where necessary by deprotection of $R^{2a}$. The reaction may be carried out using a mild dehydrating agent (for example 1,3-dimethyl-2-fluoropyridinium 4-toluenesulphonate) in the presence of a base (for example triethylamine), in a solvent which does not adversely affect the reaction (for example dimethoxyethane), at a reduced temperature (for example 0°C).

Protected hydroxy groups which $R^{2a}$ may represent include silyl ethers (for example 'butyldimethylsilyloxy). Such silyl protecting groups may be removed using conventional methods (for example by the action of hydrofluoric acid). Other protected hydroxy groups are described in "Protective Groups in Organic Chemistry", ed: J W F McOmie, Plenum Press (1973), and "Protecting Groups in Organic Synthesis", 2nd edition, T W Greene and P G M Wuts, Wiley & Son Inc (1991)]. In addition, EP 184162 describes the use of protecting groups in macrocyclic compounds.

Compounds of formula II are either known from, or may be made by the methods of, the patent applications mentioned above.

The compounds of the invention are useful because they possesses pharmacological activity in animals, eg as indicated in Tests A-F below.

Thus, the compounds of the invention are indicated for use in the treatment or prevention of rejection of transplanted organs or tissues, such as kidney, heart, lung, bone marrow, skin, cornea, liver, medulla ossium, pancreas, intestinum tenue, limb, muscle, nervus, etc; and of autoimmune, inflammatory, proliferative and hyperproliferative diseases (particularly of the skin), and of cutaneous manifestations of immunologically-mediated diseases, for example rheumatoid arthritis, lupus erythematosus, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, nephrotic syndrome, psoriasis (which is of particular interest), atopical dermatitis, contact dermatitis and further eczematous dermatitides, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne, Alopecia areata, eosinophilic fascitis, atherosclerosis and the like.

The compounds of the invention are also indicated in the treatment or prophylaxis of respiratory diseases, for example sarcoidosis, fibroid lung, idiopathic interstitial pneumonia and reversible obstructive airways diseases which latter term includes conditions such as asthma (e.g. bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (e.g. late asthma and airway hyper-responsiveness), bronchitis and the like. The prophylaxis of asthma is of particular interest.

The compounds of the invention are also indicated in certain eye diseases such as keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's disease, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leucoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' ophthalmopathy and the like.

The compounds of the invention are also indicated in the treatment of inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischaemic diseases and thrombosis, ischaemic bowel disease, inflammatory bowel disease, necrotizing enterocolitis, intestinal lesions associated with thermal burns and leukotriene B4-mediated diseases.

The compounds of the invention are also indicated in the treatment of renal diseases including interstitial nephritis, Goodpasture's syndrome, haemolytic-uraemic syndrome and diabetic nephropathy; nervous diseases including multiple myositis, Guillain-Barré syndrome, Ménière's disease and radiculopathy; endocrine diseases including hyperthyroidism and Basedow's disease; haematic diseases including pure red cell aplasia, aplastic anaemia, hypoplastic anaemia, idiopathic thrombocytopenic purpura, autoimmune haemolytic anaemia, agranulocytosis and anerythroplasia; bone diseases such as osteoporosis; skin diseases including dermatomyositis, leucoderma vulgaris, ichthyosis vulgaris, photoallergic sensitivity and cutaneous T-cell lymphoma; circulatory diseases selected from arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa and myocardosis; collagen diseases including scleroderma, Wegener's granuloma and Sjogren's syndrome; adiposis; periodontal disease; nephrotic syndrome; haemolytic-uraemic syndrome; and male pattern alopecia and alopecia senilis.

Further, the compounds of the invention are indicated in the treatment of diseases including intestinal inflammations/allergies such as Coeliac disease, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis; and food related allergic diseases which have symptomatic manifestation remote from the gastro-intestinal tract, for example migraine, rhinitis and eczema.

The compounds of the invention may also have liver regenerating activity and/or activity in stimulating hypertrophy and hyperplasia of hepatocytes. Therefore, they are indicated in the treatment and prevention of hepatic diseases such as immunogenic diseases (e.g. chronic autoimmune liver diseases including autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g. necrosis caused by toxins, viral hepatitis, shock or anoxia), B-virus hepatitis, non-A/non-B hepatitis and cirrhosis.

The compounds of the invention are also indicated for use as antimicrobial agents, and thus may be used in the treatment of diseases caused by pathogenic microorganisms and the like.

We therefore provide the use of the compounds of the invention as pharmaceuticals.

Further, we provide the use of a compound of the invention in the manufacture of a medicament for use as an immunosuppressive agent, in the manufacture of a medicament for the treatment of reversible obstructive airways diseases, and in the manufacture of a medicament for the treatment of inflammatory or hyperproliferative skin diseases or of cutaneous manifestations of immunologically-mediated illnesses.

Administration of a compound of the invention may be topical [for example by inhalation to the lung (particularly in the treatment of reversible obstructive airways diseases) or application to the skin (particularly in the treatment of inflammatory or hyperproliferative skin diseases or of cutaneous manifestations of immunologically-mediated illnesses)], or systemic (for example by oral administration to the gastrointestinal tract).

Dealing first with inhalation to the lung, the compounds of the invention may be inhaled as a dry powder formulation which may be pressurized or non-pressurized. In non-pressurized powder formulations, a compound of the invention in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier, for example crystalline lactose. Pressurized formulations may contain a compressed gas, eg nitrogen, or a liquefied gas propellant. Formulations of these types are described in detail in International Patent Application WO 90/14826. A suitable dose of active ingredient for administration by inhalation is in the range from 0.001 to 0.1mg.kg$^{-1}$.day$^{-1}$, and preferably 0.01mg.kg$^{-1}$.day$^{-1}$.

For topical administration to the skin, the formulation may be in the form of a lotion, gel or cream, and preferably contains the compound of the invention in a concentration of 0.003-3% by weight. Such formulations may be prepared by the methods described in European Patent Application 423714. Suitable doses for such topical administration are in the range from 0.05 to 10mg.kg$^{-1}$.day$^{-1}$, for example 0.5mg.kg$^{-1}$.day$^{-1}$.

Turning now to systemic administration, the compound of the invention may be formulated together with known adjuvants, diluents or carriers using conventional techniques to produce tablets or capsules for oral administration to the gastrointestinal tract. Suitable doses for such oral administration are in the range from 0.003 to 0.3 $mg.kg^{-1}.day^{-1}$, for example $0.03mg.kg^{-1}.day^{-1}$. Such formulations may be prepared by the methods described by T Hondo *et al*, Transplantation Proceedings, 1987, XIX, Supp 6, 17-22.

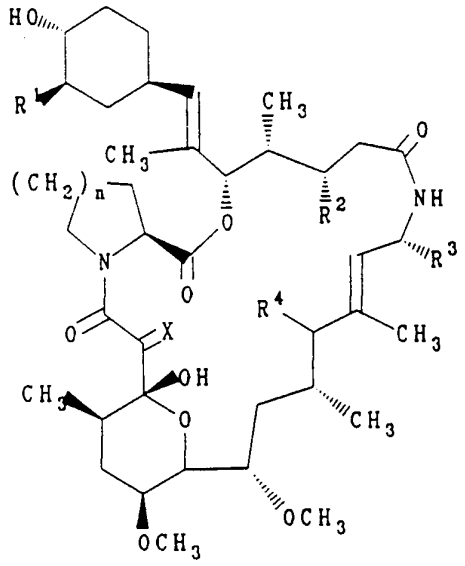
The dosage to be administered will of course vary within the above limits with the particular compound of the invention, the condition to be treated and with its severity.

The compound of the invention may be administered as divided doses from 1 to 6, and preferably 2 to 4, times per day. Each dose may comprise 1 or more unit doses.

The invention further provides a pharmaceutical formulation including preferably less than 80%, and more preferably less than 50% by weight, of a compound of the invention in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

According to a further aspect of the invention, there is provided a method of effecting immunosuppression, a method of treatment of reversible obstructive airways diseases, and a method of treatment of inflammatory or hyperproliferative skin diseases or of cutaneous manifestations of immunologically-mediated illnesses, each of which comprises administering a therapeutically effective amount of a compound of the invention to a patient.

The compounds of the invention have a number of chiral centres and may exist in a variety of stereoisomers. All optical and stereoisomers are provided, as well as mixtures thereof. The isomers may be resolved or separated by conventional techniques. The preferred stereochemistry of various chiral carbon atoms is shown in formula Ia,



Ia

wherein $R^{1-4}$, X and n are as defined above.

The compounds of the invention have the advantage that they are less toxic, more efficacious, longer acting, have a broader range of activity, are more potent, are more stable, produce fewer side effects, are more easily absorbed, are more soluble, or have other more useful physical or pharmacological properties, than the compounds of the prior art.

Test A

Murine Mixed Lymphocyte Reaction (MLR I)

The MLR test was performed in 96-well flat bottomed microtitre plates. The test compound was dissolved in ethanol at 10 times the desired concentration, and 20μl of the resulting solution added to each well. The ethanol was then allowed to evaporate. Into each well was then placed $3x10^5$ C57BL/6 responder cells ($H\text{-}2^b$), $3x10^5$ mitomycin C-treated (25μg/ml mitomycin C at 37°C for 30 minutes and washed three times with RPMI 1640 medium) BALB/C stimulator cells ($H\text{-}2^d$) in 0·2ml RPMI 1640 medium supplemented with 5% fetal calf serum, 2mM L-glutamine, $5x10^{-5}$M 2-mercaptoethanol, penicillin (50μg/ml) and streptomycin (50μg/ml). The cells were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% of air for 96 hours and pulsed with $^3$H-thymidine (0·5μCi) 6 hours before the cells were collected. The level of radio-

activity incorporated by the cells was then determined, which is a measure of T-cell proliferation.

Test B

Human Mixed Lymphocyte Reaction (MLR II)

The MLR test was performed in 96-well flat bottomed microtitre plates. The compound under test was dissolved in ethanol to 10 times the desired concentration and 20μl of the resulting solution placed in each well. The ethanol was then allowed to evaporate. Into each well was then placed $1{\cdot}5x10^5$ cells from each of two responding donors in a final volume of 0·2ml RPMI 1640 medium supplemented with 10% human serum, 2mM L-glutamine and penicillin/streptomycin. The cells were incubated at 37°C in a humidified atmosphere at 5% carbon dioxide for 120 hours. $^3$H-thymidine (0·5μCi) was added for the final 6 hours of the incubation. The level of radioactivity incorporated by the cells was then determined, which is a measure of T-cell proliferation.

Test C

Graft versus Host Assay (GVH)

Spleen cells from DA and DAxLewis F1 hybrid rats were prepared at approximately $10^8$ cells/ml. 0·1ml of these suspensions were injected respectively into the left and right rear footpads of DAxLewis F1 rats. Recipient animals were dosed with the compound under test, either orally or subcutaneously, on days 0-4. The assay is terminated on day 7 when the popliteal lymph nodes of the animals are removed and weighed. The increase in weight of the left node relative to the weight of the right is a measure of the GVH response.

Test D

Inhibition of Interleukin-2 (IL-2) secretion

The test was performed following the method of D J Henderson *et al*, Immunology 1991, Vol 73, pp316-327.

Test E

The compounds of the invention may be screened for their potential anti-psoriasis efficacy in the TPA-induced cutaneous inflammatory response screen described by R J

Griffiths, B E Wood, S Li and A Blackham in 'Effects of cyclosporin and protein synthesis inhibitors on cutaneous inflammation in mouse skin', Skin Pharmacology, 2, 30-37, 1989.

Test F

The compounds of the invention may be screened for their potential anti-asthma efficacy in the mast cell model described by E Wells, S T Harper, C G Jackson, J Mann and R P Eady in 'Characterization of primate bronchoalveolar mast cells: I. IgE-dependent release of histamine leukotrienes and prostaglandins', J Immunol 137(12): 3933-40, 1986; and 'Characterization of primate bronchoalveolar mast cells: II. Inhibition of histamine LTC4 and PGD2 release from primate bronchoalveolar mast cells and a comparison with rat peritoneal mast cells', J Immunol 137(12): 3941-3945, 1986.

The invention is illustrated by the following examples.

**Example 1**

**18-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-24,26-dimethoxy-13,20,22,28-tetramethyl-11,29-dioxa-4,17-diazatricyclo[23.3.1.$0^{4,9}$]nonaconta-19-ene-2,3,10,16-tetraone**

(a) 17-Allyl-14-'butyldimethylsilyloxy-1-hydroxy-12-[2-(4-hydroxy-3-methoxy cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone C16 E-oxime

To a stirred solution of 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone C16 E-oxime (as prepared in Example 25, WO 89/05304, and separated from the Z-oxime by chromatography on silica) (2.745g) in dry dichloromethane (100ml) and dry 2,6-lutidine (2.35ml) was added slowly 'butyldimethylsilyltrifluoromethanesulphonate (3.82ml). After 20 minutes at room temperature the solution was poured into 1N aqueous hydrochloric acid solution and the product was extracted with diethyl ether. The extracts were dried ($MgSO_4$) and concentrated *in vacuo* to a foam (4.32g).

The crude product was redissolved in methanol (75ml) and stirred with pyridinium toluenesulphonate (0.075g) for 2 days at room temperature and then for a further 3 days at 4°C. The mixture was poured into saturated sodium bicarbonate solution (150ml) and extracted with ethyl acetate. The extracts were washed with 1N aqueous hydrochloric acid followed by brine, dried ($MgSO_4$) and concentrated *in vacuo* to give the subtitle compound (2.488g) as a colourless foam.

(b) 18-Allyl-14-$^t$butyldimethylsilyloxy-1-hydroxy-12-[2-(4-hydroxy-3-methoxy-cyclohexyl)-1-methylvinyl-24,26-dimethoxy-13,20,22,28-tetramethyl-11,29-dioxa-4,17-diaza-tricyclo[23.3.1.$0^{4,9}$]nonaconta-19-ene-2,3,10,16-tetraone

To a stirred solution of the product of step (a) (2.485g) in dry dimethoxyethane (70ml) at 0°C under nitrogen, was added portionwise 1,3-dimethyl-2-fluoropyridinium 4-toluene-sulphonate (3.02g). Dry triethylamine (1.83ml) was added and the mixture stirred at 0°C for 30 minutes. Water (10ml) was added and stirring continued for 10 minutes. The mixture was poured into 1N aqueous hydrochloric acid and the product was extracted with diethyl ether. The extracts were washed with water then brine, dried ($MgSO_4$), concentrated *in vacuo* and chromatographed on silica gel eluting with acetone/hexane [2:5] to give the subtitle compound (0.154g).

(c) 18-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-24,26-dimethoxy-13,20,22,28-tetramethyl-11,29-dioxa-4,17-diazatricyclo[23.3.1.$0^{4,9}$]-nonaconta-19-ene-2,3,10,16-tetraone

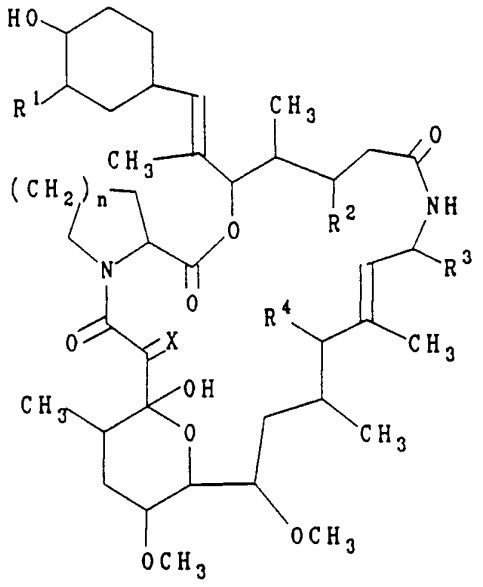
To a stirred solution of the product of step (b) (0.048g) in acetonitrile (5ml) was added a 40% aqueous solution of hydrofluoric acid (2 drops). The solution was stirred at room temperature for 30 minutes, then poured into saturated aqueous sodium bicarbonate solution and the product was extracted with diethyl ether. The extracts were dried ($MgSO_4$), concentrated *in vacuo* and chromatographed on silica gel eluting with acetone/hexane [3:4] to give the title compound (0.028g) as a colourless foam.

**Example 2**

The compound of Example 1 was tested in Test D above, and found to inhibit IL-2 secretion by 50% ($IC_{50}$) at a concentration of $4x10^{-9}M$.

**Claims:**

1. A compound of formula I,



I

wherein

$R^1$ represents OH or $OCH_3$;

$R^2$ represents H or OH;

$R^3$ represents methyl, ethyl, propyl, allyl or 2-oxopropyl;

$R^4$ represents H or OH;

X represents O, (H,OH) or (H,H);

n represents 1 or 2;

and pharmaceutically acceptable derivatives thereof.

2. A compound as claimed in claim 1, wherein $R^4$ represents H.

3. A compound as claimed in claim 1 or claim 2, which is 18-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-24,26-dimethoxy-13,20,22,28-tetramethyl-11,29-dioxa-4,17-diazatricyclo[23.3.1.$0^{4,9}$]nonaconta-19-ene-2,3,10,16-tetraone, or a pharmaceutically acceptable derivative thereof.

4. A pharmaceutical formulation including a compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

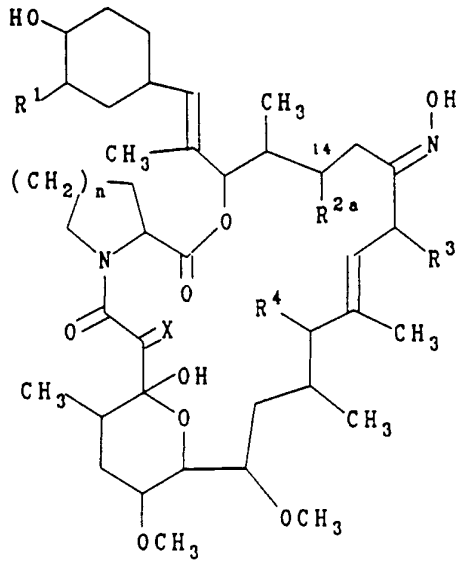
5. A compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, for use as a pharmaceutical.

6. A compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, for use in the manufacture of an immunosuppressive medicament.

7. A compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the treatment of reversible obstructive airways diseases.

8. A compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the treatment of inflammatory or hyperproliferative skin diseases or of cutaneous manifestations of immunologically-mediated illnesses.

9. A process for the production of a compound of formula I, or a pharmaceutically acceptable derivative thereof, which comprises a Beckmann rearrangement of an E-oxime of formula II,



II

wherein $R^1$, $R^3$, $R^4$, X and n are as defined in claim 1 and $R^{2a}$ represents H or protected hydroxy.

10. A method of effecting immunosuppression, which comprises administering a therapeutically effective amount of a compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, to a patient.

11. A method of treatment of reversible obstructive airways diseases, which comprises administering a therapeutically effective amount of a compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, to a patient.

12. A method of treatment of inflammatory or hyperproliferative skin diseases or of cutaneous manifestations of immunologically-mediated illnesses, which comprises administering a therapeutically effective amount of a compound of formula I, as defined in claim 1, or a pharmaceutically acceptable derivative thereof, to a patient.

# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 93/01850

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07H19/01 A61K31/71

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO,A,92 03441 (FISONS PLC) 5 March 1992<br>see page 5, line 36 - page 8, line 14;<br>claims 1,9<br>--- | 1-9 |
| Y | WO,A,89 05304 (FISONS PLC) 15 June 1989<br>see page 15, line 10 - line 25; claim 1<br>--- | 1-9 |
| P,X | TETRAHEDRON LETT.<br>vol. 34, no. 8 , 1993<br>pages 1351 - 1354<br>M. FURBER ET AL. 'Studies Relating to the Immunosuppressive Activity of FK506'<br>see the whole document<br>---<br>-/-- | 1-9 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

1

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 December 1993 | 14. 01. 94 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+31-70) 340-3016 | Brennan, J |

Form PCT/ISA/210 (second sheet) (July 1992)

Interr ·nal Application No
PC1/GB 93/01850

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | TRANSPLANTATION<br>vol. 53, no. 5 , May 1992<br>pages 1150 - 1153<br>R.V. BUNDICK ET AL. 'FK506 as an Agonist to Induce Inhibition of Interleukin 2 Production'<br>see the whole document<br>----- | 1-9 |

INTERNATIONAL SEARCH REPORT

:ernational application No.
PCT/GB93/01850

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 10-12 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

...information on patent family members

Inter nal Application No
PCT/GB 93/01850

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| WO-A-9203441 | 05-03-92 | AU-A- | 8394291 | 17-03-92 |
| | | CN-A- | 1060846 | 06-05-92 |
| | | EP-A- | 0543879 | 02-06-93 |
| WO-A-8905304 | 15-06-89 | AU-A- | 2822889 | 05-07-89 |
| | | EP-A- | 0323042 | 05-07-89 |
| | | EP-A- | 0346427 | 20-12-89 |
| | | JP-T- | 2502463 | 09-08-90 |