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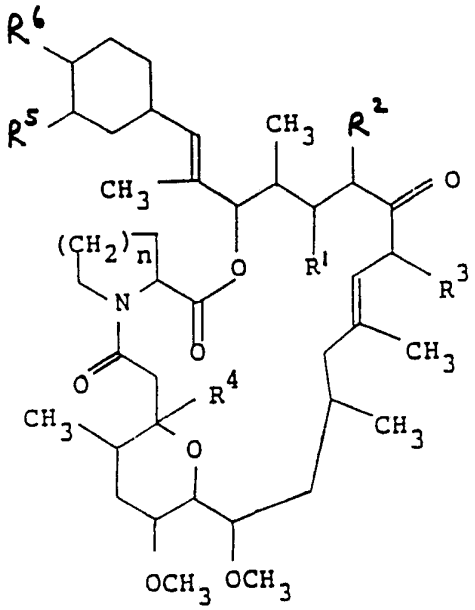

(I)

(57) Abstract

Compounds of formula (I) are described, wherein R^1 represents H, OH, alkoxy or R^7COO-; R^2 represents H; in addition, R^1 and R^2 may together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^3 represents methyl, ethyl, propyl or allyl; R^4 represents OH or alkoxy; R^5 represents OH or methoxy; R^6 represents OH, alkoxy or R^8COO-; in wich R^7 and R^8 have various significances including alkyl, aryl, NH_2, arylamino and alkylamino; and n represents 1 or 2; provided that when n is 1, then R^3 is allyl or propyl. Processes for their production and compositions containing them, e.g. for use as immunosuppressive agents, are also described.

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MACROCYCLIC COMPOUNDS

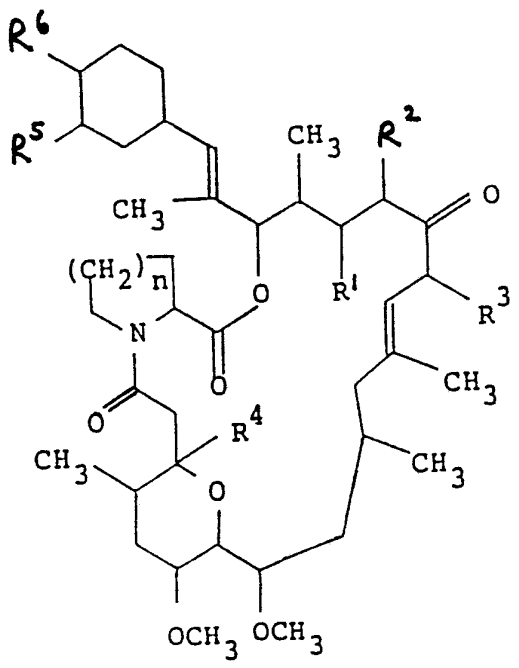
This invention relates to novel macrocyclic compounds, more particularly to novel macrocyclic immunosuppressive compounds, processes for their preparation, their use as medicaments, and compositions containing them.

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

International Patent Application WO 89/05304 (to Fisons plc), European Patent Application 353678 (to Fujisawa Pharmaceuticals Co Ltd), European Patent Applications 349049 and 349061 (to Merck & Co Inc) and European Patent Application 356399 (to Sandoz AG) also disclose a number of macrocyclic immunosuppressant compounds.

We have now found a novel group of compounds which possess certain advantageous properties over those disclosed previously.

Thus, according to the invention, we provide a compound of formula I,



I

wherein

R^1 represents H, OH, alkoxy or R^7COO-;

R^2 represents H;

in addition, R^1 and R^2 may together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

R^3 represents methyl, ethyl, propyl or allyl;

R^4 represents OH or alkoxy;

R^5 represents OH or methoxy;

R^6 represents OH, alkoxy or R^8COO-;

in which R^7 and R^8 independently represent alkyl; aryl; NH_2; a 5- or 6-membered heterocyclic ring optionally substituted by alkyl or aryl; arylamino; alkylamino; N,N-dialkylamino; N,N-diarylamino; or N-alkyl-N-arylamino; each alkyl group optionally being substituted by aryl, OH, NO_2 or halogen; and each aryl group optionally being substituted by alkyl, OH, NO_2 or halogen; and

n represents 1 or 2;

provided that when n is 1, then R^3 is allyl or propyl.

When any one of R^1, R^4, R^5 and R^6 represents a carbon containing group, that group preferably contains from 1 to 10 carbon atoms, more preferably from 1 to 6.

The term "alkyl" as used herein includes cyclic and branched alkyl groups, as well as straight chain alkyl groups.

Preferably, R^3 is ethyl.

We prefer at least one of R^1 and R^6 to represent OH.

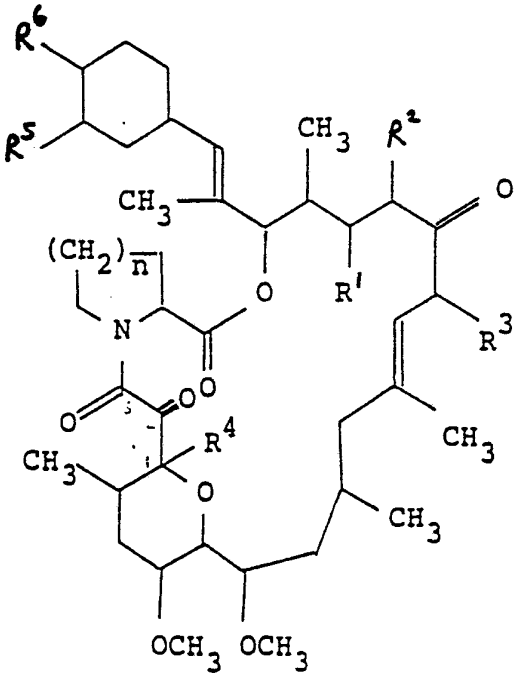
When R^7 or R^8 is present, we prefer those groups to be selected from alkyl; NH_2; piperidino; morpholino; aryl optionally substituted by halogen or OH; arylamino optionally substituted by halogen or OH; or alkylamino optionally substituted by OH; for example methyl or phenylamino.

Alkoxy groups which R^1, R^4 or R^6 may represent include methoxy.

Aryl groups which R^7 or R^8 may comprise include phenyl.

According to the invention, we also provide a process for the production of a compound of formula I, which comprises:

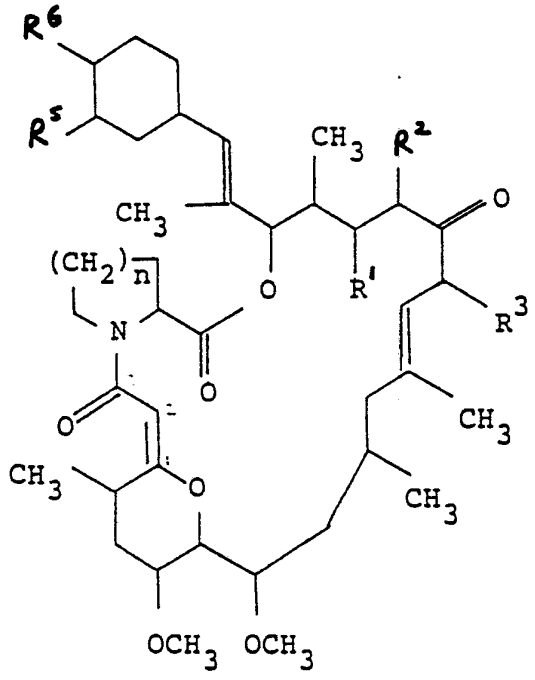
a) selective reduction of the C2-carbonyl group in a compound of formula II,



II

wherein R^1 to R^6 and n are as defined above, or

b) addition of a compound of formula R^4-H, wherein R^4 is as defined above, across the C1 alkene group in a compound of formula III,



III

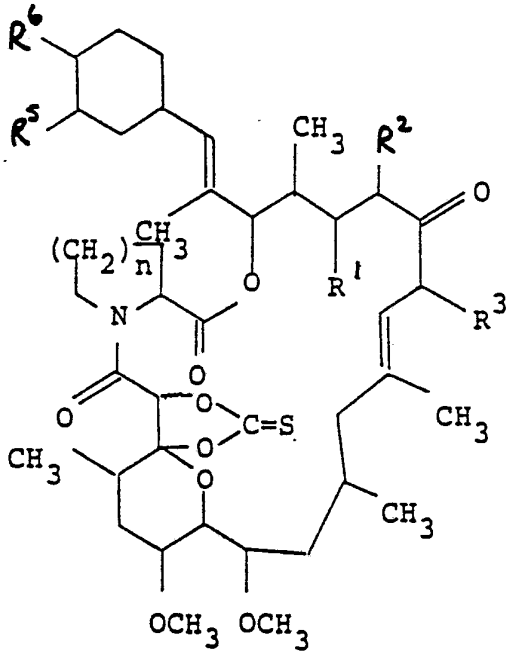
wherein R^1 to R^3, R^5, R^6 and n are as defined above.

In process (a), the reduction may be achieved by the action of H_2S, preferably in the presence of pyridine or an amine (for example morpholine), in a solvent which does not adversely affect the reaction (for example dimethylformamide, pyridine or methanol), at or around room temperature.

The preparation of many compounds of formula II is fully described in the patent applications mentioned above. Alternatively, the total synthesis of FR-900506 disclosed in European Patent Application 378318 (to Merck & Co Inc) may be modified where necessary to produce compounds of formula II. The teaching of the documents mentioned above is herein incorporated by reference.

In process (b), the addition of water across the C1-alkene group may be achieved by the action of dilute aqueous acid (for example dilute hydrochloric acid), in a solvent which does not adversely affect the reaction (for example water, methanol, ethanol, pyridine, ethyl acetate, dimethylformamide, dichloromethane or a mixture thereof), at or around room temperature. The addition of an alcohol may be achieved in the presence of a small amount of acid (for example p-toluenesulphonic acid), in a solvent which does not adversely affect the reaction (for example the alcohol to be added, pyridine, ethyl acetate, dimethylformamide, dichloromethane or a mixture thereof), at or around room temperature.

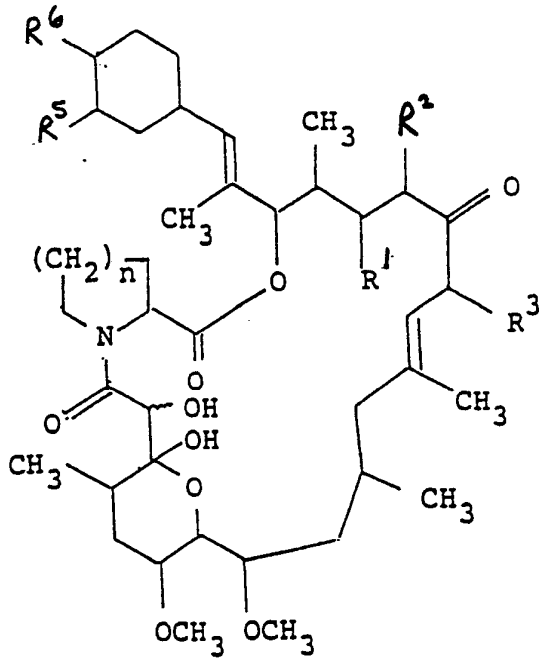
Compounds of formula III may be prepared from compounds of formula IV,



IV

wherein R^1 to R^3, R^5, R^6 and n are as defined above, by reduction, which may be achieved using tributyltin hydride, preferably in the presence of a catalytic amount of 2,2'-azobisisobutyronitrile, in a solvent which does not adversely affect the reaction conditions, for example anhydrous toluene, at a temperature of from room temperature to solvent reflux temperature.

Compounds of formula IV may be prepared from compounds of formula V,



V

wherein R^1 to R^3, R^5, R^6 and n are as defined above, by reaction with O-phenyl chlorothioformate, in a solvent which does not adversely affect the reaction (for example acetonitrile), optionally in the presence of dimethylaminopyridine, at or around room temperature.

Compounds of formula V may be prepared from compounds of formula II, as defined above, by reduction. The reduction may be achieved using zinc powder in acetic acid at or around room temperature.

When R^6 represents or comprises an OH group in the desired compound of formula I, we prefer to use process (a) to produce it.

The group R^8COO- may be formed in a starting compound of formula II in which R^6 represents OH using conventional techniques, for example:

i) when R^8 represents alkyl or aryl, an esterification reaction with an appropriate alkanoic acid or aromatic

carboxylic acid may be employed, or a derivative thereof such as an acid chloride or acid anhydride;

ii) when R^8 represents alkylamino or arylamino, reaction with an appropriate alkyl or aryl isocyanate; alternatively a reactive intermediate may first be formed with a compound such as p-nitrophenyl chloroformate, followed by reaction with the appropriate amine compound. This latter method may be employed when R^8 is NH_2.

Similarly, R^7COO- groups may be formed in a starting compound of formula II in which R^1 represents OH. This reaction may occur simultaneously with the formation of R^8COO- groups as described above, in which case R^7 and R^8 will be the same. Of course, where necessary, the OH group that R^1 or R^6 represents may be protected using conventional protecting group chemistry [as described in "Protective Groups in Organic Chemistry", ed: J W F McOmie, Plenum Press (1973), and "Protective Groups in Organic Synthesis", T W Greene, Wiley-Interscience (1981)], to ensure that R^7 and R^8 are different, or to allow one or other of R^1 and R^6 to be deprotected to OH after formation of the C2-methylene group or formation of the R^7COO- or R^8COO- group.

When process (a) is employed, R^7COO- or R^8COO- groups may be introduced before or after the reduction step.

In order to produce a compound of formula I in which R^1 and R^2 together represent a second carbon-carbon bond between the carbon atoms to which they are attached,

the double bond may be introduced by dehydration of a corresponding compound of formula I in which R^1 represents OH and R^2 represents H, or a starting comound may be used which already contains the group. Such a dehydration may be carried out in a solvent which does not adversely affect the reaction (eg toluene), in the presence of a trace amount of acid (eg p-toluenesulphonic acid), at a temperature of from 50 to 100°C.

The compounds of formula I may be isolated from their reaction mixtures using conventional techniques.

The compounds of formula I are useful because they possess pharmacological activity in animals; in particular they are useful because they possess immunosuppressive activity, eg in the tests set out in Tests A, B and C. Thus the compounds are indicated for use in the treatment or prevention of resistance to transplanted organs or tissues, such as kidney, heart, lung, bone marrow, skin, cornea, etc; and of autoimmune, inflammatory, proliferative and hyperproliferative diseases, 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, atopical dermatitis, contact dermatitis and further eczematous dermatitides, seborrheic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Alopecia

areata, etc.

The compounds of the invention are also indicated in the treatment of reversible obstructive airways disease.

Further, the compounds of the invention are indicated in the treatment of a disease selected from 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 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 compounds of formula I as pharmaceuticals.

Further, we provide the use of a compound of formula I in the manufacture of a medicament for use as an immunosuppressive agent.

For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired (eg topical, parenteral or oral) and the disease indicated. However, in general, satisfactory results are obtained when the compounds are administered at a daily dosage of from 0.001 to 20mg per kg of animal body weight.

For man the indicated total daily dosage is in the

range of from 0.01mg to 1000mg and preferably from 0.5mg to 100mg, which may be administered, for example twice weekly, or in divided doses from 1 to 6 times a day or in sustained release form. Thus unit dosage forms suitable for administration, eg oesophageally, comprise from 0.01mg to 500mg, and preferably 0.5mg to 100mg of the compound preferably admixed with a solid or liquid pharmaceutically acceptable diluent, carrier or adjuvant.

According to our invention we also provide a pharmaceutical composition comprising preferably less than 80%, and more preferably less than 50% by weight, of a compound of fomula I in combination with a pharmaceutically acceptable adjuvant, diluent or carrier. Examples of suitable adjuvants, diluents or carriers are: for tablets, capsules and dragees - microcrystalline cellulose, calcium phosphate, diatomaceous earth, a sugar such as lactose, dextrose or mannitol, talc, stearic acid, starch, sodium bicarbonate and/or gelatin; for suppositories - natural or hardened oils or waxes; and for inhalation compositions - coarse lactose. The compound of formula I preferably is in a form having a mass median diameter of from 0.01 to 10μm. The compositions may also contain suitable preserving, stabilising and wetting agents, solubilisers (eg a water-soluble cellulose polymer such as hydroxypropyl methylcellulose, or a water-soluble glycol such as propylene glycol), sweetening and colouring agents and flavourings. The compositions may, if desired, be formulated in sustained release form.

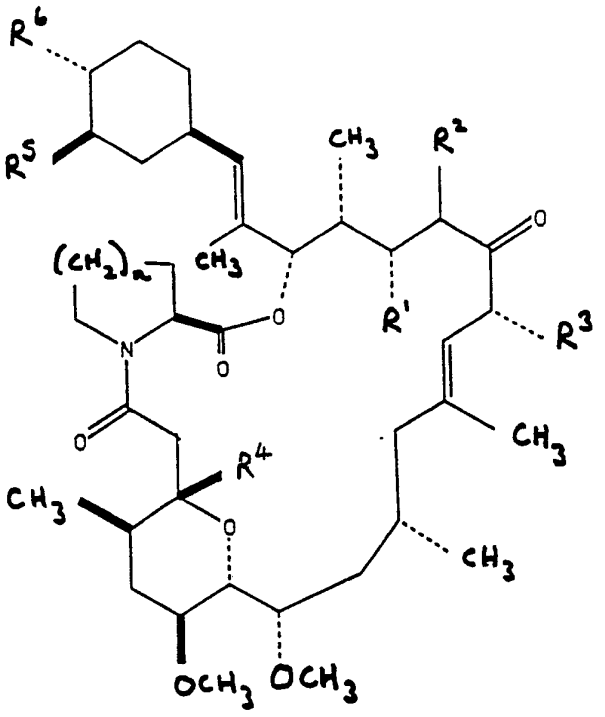
For the treatment of reversible obstructive airways disease, we prefer the compound of formula I to be administered by inhalation to the lung, especially in the form of a powder.

According to a further aspect of the invention, there is provided a method of effecting immunosuppression which comprises administering a therapeutically effective amount of a compound of formula I, as defined above, to a patient.

The compounds of formula I have the advantage that they are less toxic, more efficacious, are longer acting, have a broader range of activity, are more potent, are more stable, produce fewer side effects, are more easily absorbed or have other useful pharmacological properties, than compounds previously used in the therapeutic fields mentioned above.

The compounds of formula I have a number of chiral centres and may exist in a variety of stereoisomers. The invention provides all optical and stereoisomers, as well as racemic mixtures. The isomers may be resolved or separated by conventional techniques.

However, the preferred stereochemistry of various chiral carbon atoms are shown in formula Ia,



Ia

wherein R^1 to R^6 and n are as first defined above.

Test A

Mixed Lymphocyte Reaction (MLR) I

The MLR test was performed in microtitre plates, with each well containing 5 x 10^5 C57BL/6 responder cells (H-2^b), 5 x 10^5 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-2^d) in 0.2ml RPMI 1640 medium supplemented with 10% fetal calf serum, 2mM sodium hydrogen carbonate, 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 68 hours and pulsed with ^{3}H-thymidine (0.5μCi) 4 hours before the cells were collected. The object compound of this invention was dissolved in ethanol and further diluted in RPMI 1640

medium and added to the cultures to give final concentrations of 0.1μg/ml or less.

Test B

Mixed Lymphocyte Reaction (MLR) II

The MLR test was performed in 96-well microtitre plates with each well containing 3 x 10^5 cells from each of two responding donors in a final volume of 0.2ml RPMI 1640 medium supplemented with 10% human serum, L-glutamine and penicillin/streptomycin. The compound under test was dissolved at 10mg/ml in ethanol and further diluted in RPMI 1640. The cells were incubated at 37°C in a humidified atmosphere at 5% carbon dioxide for 96 hours.

3H-thymidine (0.5μCi) was added for the final 24 hours of the incubation to provide a measure of 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 into the rear footpads of DAxLewis F1 rats (left and right respectively). Recipient animals aere 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 wieght of the right is a measure of the GVH response.

The invention is illustrated, but in no way limited by, the following Examples.

Example 1

14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

a) 14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1,2-dihydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

To a solution of 14-acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-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 (the first compound of Example 6, EP 184162)(5.4g) in acetic acid (120ml) was added zinc powder (25g) portionwise and the suspension was vigorously stirred for 13 hours at ambient temperature. The reaction mixture was filtered and the filtrate was concentrated in vacuo to give a pale yellow powder (4.48g). 1g of the powder was purified by silica gel column chromatography eluting first with ethyl acetate/hexane [1:1] then ethyl acetate to give the subtitle compound (486mg) as white powder.

MS (FAB): 912 $(M+Na)^+$

mp: 93-96°C

^{13}C NMR ($CDCl_3$) δ: 207.7, 206.3, 172.9, 170.9, 170.1, 170.0, 169.9, 169.4, 169.3, 99.1, 97.1, 68.1

b) 14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-23,25-dimethoxy-13,19,21,27-

tetramethyl-1,2-thiooxomethylenedioxy-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

To a mixture of the product of step (a)(243mg) and dimethylaminopyridine (333mg) in anhydrous acetonitrile (5ml) was added O-phenyl chlorothioformate (68.7mg) and the reaction was stirred for 15 minutes at ambient temperature. The solution was diluted with diethyl ether (15ml) and washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with ethyl acetate/hexane [1:1] to give the subtitle compound (240 mg).

MS (FAB): 954 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 207.6, 188.4, 170.3, 169.6, 168.9, 161.4, 111.7, 54.7, 52.4

c) 14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-1,18-diene-3,10,16-trione

To a mixture of the product of step (b)(486mg) and 2,2'-azobisisobutyronitrile (catalytic amount) in anhydrous toluene (9ml) was added tributyltin hydride (0.8ml) and the reaction mixture was heated at reflux for 15 minutes. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography eluting with ethyl acetate/hexane [1:1] to give the subtitle compound (188mg).

MS (FAB): 973 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 207.3, 172.0, 170.3, 170.2,

169.5, 168.0, 95.7, 55.8, 36.2

d) 14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The product of step (c)(100mg) was dissolved in a mixture of 1N aqueous hydrochloric acid (0.2ml) and methanol (0.5ml). The solution was allowed to stand at ambient temperature for 16 hours and then the solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel eluting with ethyl acetate/hexane [1:2] to give the title compound (67mg).

MS (FAB): 896 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 208.1, 173.8, 170.4, 169.8, 169.2, 98.3, 52.9, 52.6, 37.1

Example 2

12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

a) 12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1,2-dihydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Following the method of Example 1(a) above, the subtitle compound (246mg) was prepared from 12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo

[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone (the second compound of Example 6, EP 184162)(1g).

MS (FAB): 854 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 208.5, 171.5, 171.0, 170.3, 98.8, 82.9, 80.3, 76.4, 75.4, 73.8, 71.7, 67.9

b) 12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-23,25-dimethoxy-13,19,21,27-tetramethyl-1,2-thiooxomethylenedioxy-11,28-dioxa-4-azatricyclo-[22,3,1,$0^{4,9}$]octacos-18-ene-3,10,16-trione

The subtitle compound (168mg) was prepared from the product of step (a) following the method of Example 1(b).

MS (FAB): 896 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 210.0, 188.6, 170.4, 169.0, 162.0, 111.5, 85.4, 80.4, 78.5, 76.0, 75.7, 74.1, 71.9

c) (1E)-12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacosa-1,18-diene-3,10,16-trione and

(1Z)-12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacosa-1,18-diene-3,10,16-trione

The subtitle compounds (88mg and 33mg respectively) were prepared from the product of step (b)(160mg) following the method of Example 1(c).

MS (FAB): 820 $(M+Na)^+$ (both compounds)

^{13}C NMR ($CDCl_3$) δ:

(1E)-compound 210.2, 171.8, 170.3, 167.6, 95.8,

80.4, 76.6, 76.2, 75.6, 75.2, 73.2, 55.9 (C9), 36.8 (C5)

(1Z)-compound 210.3, 170.3, 170.1, 166.2, 163.6, 100.3, 80.6, 80.4, 80.1, 76.8, 75.6, 73.5, 51.3 (C9), 43.5 (C5)

d) 12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (31mg) was prepared from the (1E)-compound of step (c) (60mg) following the method of Example 1(d). Similarly, the title compound was also prepared from the (1Z)-compound of step (c).

MS (FAB): 838 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 211.3, 173.7, 170.4, 169.8, 98.2, 81.0, 80.4, 76.4, 75.6, 74.2, 70.3

Example 3

14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

To a solution of the product of Example 1(c) (80mg) in anhydrous methanol (1ml) was added a 0.1M solution of p-toluenesulphonic acid monohydrate in methanol (0.25ml). After being stirred for 30 minutes, the solvent was evaporated and the residue was purified by preparative thin layer chromatography eluted with ethyl acetate/hexane [1:1] to give the title compound (38mg).

MS (FAB): 910 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 208.3, 171.2, 170.0, 169.6, 100.0, 55.7, 53.2, 47.2, 39.4

Example 4

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-3,10,16-trione

Hydrogen sulphide was bubbled through a 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 (FR-900506, EP 184162) (160mg) in dimethylformamide (5ml) and pyridine (1ml) for 4 hours at room temperature. After standing overnight elemental sulphur had precipitated. Dilute hydrochloric acid and ethyl acetate were then added, and the organic extract was separated, dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with ethyl acetate gave the title compound (120mg) as a foam.

^{13}C NMR δ: (major rotamer) 214.0 (C16); 173.9 (C3); 169.2 (C10); 141.05 (C19); 135.36 (C41); 132.33 (C29); 128.7 (C31); 121.25 (C18); 116.4 (C42); 98.39 (C1); 84.1 (C34); 70.54 (C24); 69.32 (C14); 53.3 (C17); 52.5 (C9); 48.26 (C20); 42.53 (C15); 42.23 (C5); 40.33 (C13); 38.35 (C27); 37.17 (C2); 35.75 (C40); 36.17 (C22); 32.49 (C26); 31.21 (C36); 30.60 (C37); 26.51 (C8); 25.67 (C21); 24.34 (C6); 20.90 (C7); 18.57 (C44); 16.78 (C47); 15.64 (C43);

14.39 (C30); 9.73 (C39)

MS (FAB): 790 $[M+H]^+$; 812 $[M+Na]^+$; 874 $[M+Rb]^+$

Example 5

17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

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

To a mixture of 17-allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone (the second compound of Example 17, EP 184162) (1g) and pyridine (1.77g) in anhydrous dichloromethane (10ml) was added phenyl isocyanate (1.28g), and the mixture was stirred for 16 hours at ambient temperature. The reaction mixture was washed with 1N aqueous hydrochloric acid solution, water, aqueous sodium bicarbonate solution and brine successively, and dried over magnesium sulphate. The solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography, eluting with a mixture of dichloromethane and diethyl ether [2:1] to give the subtitle compound (1.01g).

MS (FAB): 927 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 200.1, 198.7, 195.8, 191.2,

169.0, 168.6, 165.7, 164.8, 152.9, 147.8, 146.4, 137.8, 128.8, 127.5, 123.1, 118.5, 98.6, 97.7

b) 17-Allyl-1,2-dihydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Following the method of Example 1(a), the subtitle compound (263mg) was obtained from the product of step (a) (900mg).

MS (FAB): 931 $(M+Na)^+$

c) 17-Allyl-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-1,2-(thiooxomethylenedioxy)-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Following the method of Example 1(b), the subtitle compound (156mg) was obtained from the product of step (b) (233 mg).

MS (FAB): 973 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 210.0, 188.5, 138.0, 128.4, 123.6, 118.4, 111.6, 54.7, 52.7

d) (1E)-17-Allyl-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-1,18-diene-3,10,16-trione and

(1Z)-17-Allyl-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-1,18-diene-3,10,16-trione

Following the method of Example 1(c), the subtitle compounds (86mg and 23mg respectively) were prepared from

the product of step (c) (140mg).

MS (FAB): (both compounds) 897 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ:

(1E)-compound 210.0, 171.9, 170.4, 167.7, 152.9, 137.9, 128.0, 123.0, 118.5, 95.8 (C2), 55.9 (C9), 36.9 (C5)

(1Z)-compound 210.0, 170.1, 166.2, 163.6, 152.9, 137.8, 128.8, 123.1, 118.5, 100.0 (C2); 51.3 (C9); 43.6 (C5)

e) 17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Following the method of Example 1(d), the title compound (44mg) was obtained from the (1E)-compound of step (d) (60mg). Similarly, the title compound was also prepared from the (1Z)-compound of step (d).

MS (FAB): 915 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 211.1, 173.7, 169.8, 137.8, 128.8, 123.1, 118.5, 52.6, 52.5, 37.8

Example 6

17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

a) 17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

The subtitle compound (1.18g) was prepared from 17-allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone (the second compound of Example 17, EP 184162) (1.1g) and 4-fluorophenyl isocyanate following the method of Example 5(a).

MS (FAB): 946 $(M+Na)^+$

b) 17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1,2-dihydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Following the method of Example 1(a), the subtitle compound (0.58g) was obtained from the product of step (a) (1.0g).

MS (FAB): 950 $(M+Na)^+$

c) 17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-1,2-(thiooxomethylenedioxy)-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Following the method of Example 1(b), the subtitle compound (430mg) was obtained from the product of step (b) (580mg).

MS (FAB): 992 $(M+Na)^+$

d) (1E)-17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-1,18-diene-3,10,16-trione and

(1Z)-17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-

3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacosa-1,18-diene-3,10,16-trione

The subtitle compounds (101mg and 31mg respectively) were prepared from the product of step (c) (330 mg) following the method of Example 1(c).

mp: **(1E)-compound** 103-104°C

(1Z)-compound 94-95°C

MS (FAB): (both compounds) 915 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: [(1E)-compound] 210.0, 171.9, 170.3, 167.7, 161.0, 156.2, 153.3, 138.0, 135.8, 134.0, 131.4, 127.9, 124.3, 120.1, 116.0, 115.5, 115.0, 95.7, 55.9, 52.0, 36.8

e) 17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (24mg) was prepared from the (1E)-compound of step (d) (50mg) following the method of Example 1(d). Similarly, the title compound was also prepared from the (1Z)-compound of step (c).

MS (FAB): 933 $(M+Na)^+$

Example 7

1-Hydroxy-12-[2-[4-[(2R)-2-hydroxypropyl-carbamoyloxy]-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

a) 17-Allyl-1-hydroxy-12-[2-[4-[(2R)-2-

hydroxypropylcarbamoyloxy]-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

To a mixture 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 (FR-900506, EP 184162) (400mg) and pyridine (420mg) in anhydrous dichloromethane (8ml) was added p-nitrophenyl chloroformate (400mg), and the mixture was stirred for one hour at ambient temperature. (2R)-3-amino-2-propanol (375mg) was then added and after stirring for an additional hour at ambient temperature, an additional portion of (2R)-3-amino-2-propanol (150 mg) was added. After stirring for 30 minutes, the mixture was washed with brine, dried over magnesium sulphate, and evaporated in vacuo. The residue was purified by silica gel column chromatography, eluting with a mixture of ethyl acetate and n-hexane [4:1] to give the subtitle compound (188mg).

MS (FAB): 909 $(M+Na)^+$

mp: 94-96°C

^{13}C NMR ($CDCl_3$) δ: 200.0, 198.7, 195.8, 191.6, 169.0, 166.6, 165.7, 164.9, 156.7, 147.9, 146.5, 128.6, 127.2, 98.4, 97.6, 66.8

b) 1-hydroxy-12-[2-[4-[(2R)-2-hydroxypropylcarbamoyloxy]-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo

[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of step (a) (70mg) in acetic acid (1ml) was suspended with 5% palladium-on-carbon (10mg), and the reaction mixture was stirred for 4 hours under a hydrogen atmosphere at one atmosphere pressure. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography eluting with ethyl acetate to give the subtitle compound (28mg).

MS (FAB): 913 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 212.2, 210.9, 196.5, 193.4, 170.3, 169.1, 156.7, 98.1, 97.0, 66.9

c) 1-Hydroxy-12-[2-[4-[(2R)-2-hydroxypropyl-carbamoyloxy]-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (72mg) was prepared from the product of step (b) (140mg) following the method of Example 4.

MS (FAB): 899 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 212.2, 174.0, 173.7, 169.9, 169.8, 156.8, 98.2, 97.9, 70.3, 70.0, 38.4, 37.8

Example 8

17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

a) 17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-

tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

The subtitle compound (1.59g) was prepared from 17-allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone (the second compound of Example 17, EP 184162) (2.0g) and morpholine following the method of Example 7(a).

MS (FAB): 921 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 200.0, 198.7, 195.7, 191.3, 169.0, 168.6, 165.7, 164.8, 154.9, 147.8, 146.4, 128.7, 127.4, 98.5, 97.6, 66.4

b) 17-Allyl-1,2-dihydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The subtitle compound (1.59g) was prepared from the product of step (a) (1.59g) following the method of Example 1(a).

MS (FAB): 925 $(M+Na)^+$

c) 17-Allyl-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-1,2-(thiooxomethylenedioxy)-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The subtitle compound (0.771g) was obtained from the product of step (b) following the method of Example 1(b).

MS (FAB): 968 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 211.4, 210.0, 188.4, 170.8, 169.6, 169.0, 168.8, 162.0, 154.9, 111.5, 97.9

d) (1E)-17-Allyl-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-1,18-diene-3,10,16-trione and

(1Z)-17-Allyl-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-1,18-diene-3,10,16-trione

The subtitle compounds (190mg and 66mg respectively) were prepared from the product of step (c) (771mg) following the method of Example 1(c) (190 mg).

mp: **(1E)-compound** 82-83°C

(1Z)-compound 62-63°C

MS (FAB): (both compounds) 891 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ:

(1E)-compound 210.0, 170.7, 170.3, 167.6, 154.9, 95.8, 66.3, 55.9, 36.2

(1Z)-compound 210.0, 170.1, 166.2, 163.6, 154.9, 100.3, 66.4, 51.3, 38.7

e) 17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (116mg) was prepared from the (1E)-compound of step (d) (178mg) following the method of Example 1(d). Similarly, the title compound was also prepared from the (1Z)-compound.

MS (FAB): 909 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 211.2, 173.7, 169.7, 154.9, 98.1, 66.4, 52.6, 52.4, 37.8

Example 9

1-Hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Hydrogen sulphide gas was bubbled through a previously degassed solution of 1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (the compound of Example 11, WO 89/05304) (1g) in dry pyridine (5ml) for 3 hours at room temperature. After a further 3 hours at room temperature, the reaction mixture was poured into dilute aqueous hydrochloric acid (1M, 100ml) and this was extracted with diethyl ether (100ml). The ether extracts were then washed with water (20ml) and brine (20ml) before being dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with dichloromethane in an increasing acetone gradient then gave the title compound as a foam (683mg).

MS (FAB): 860 $(M+Rb)^+$; 798 $(M+Na)^+$; 776 $(M+H)^+$

^{13}C NMR ($CDCl_3$) δ: 98.4 (C1); 170 (C3); 173.9 (C10); 81.2 (C12); 212.5 (C16); 122.8 (C18); 130.2 (C19); 140.5 (C29); 131.1 (C31); 84.1 (C34)

Example 10

12-[2-(4-Carbamoyloxy-3-methoxycyclohexyl)-1-methylvinyl]-1-

hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (68mg) was prepared from the title compound of Example 9 (166mg) and ammonia following the method of Example 7(a).

MS (FAB): 841 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 211.9, 173.7, 169.8, 156.5, 98.2, 52.8, 52.4, 37.9

Example 11

1-Hydroxy-12-[2-[4-(3-hydroxypropylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (26mg) was prepared from the title compound of Example 9 (94mg) and 3-hydroxypropylamine following the method of Example 7(a).

MS (FAB): 899 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 212.4, 174.2, 173.8, 170.0, 169.8, 157.2, 98.3, 98.0

Example 12

17-Allyl-14-(4-chlorophenylcarbamoyloxy)-12-[2-[4-(4-chlorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

The title compound (16mg) was prepared from the title compound of Example 4 (1.0g) and 4-chlorophenylamine

following the method of Example 7(a).

MS (FAB): 1120 $(M+Na)^+$

^{13}C NMR ($CDCl_3$) δ: 208.2, 174.3, 169.5, 152.8, 152.2, 140.5, 136.4, 135.7, 132.0, 138.8, 128.1, 121.3, 119.7, 116.1, 98.3

Example 13

17-Allyl-1,14-dihydroxy-12-[2-(3,4-dihydroxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

Hydrogen sulphide gas was bubbled through a solution of 17-allyl-1,14-dihydroxy-12-[2-(3,4-dihydroxycyclohexyl)-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 (FR-901154, EP 353678) (40mg) in pyridine (2ml) and dimethylformamide (0.1ml) for 2 hours at room temperature. After standing for 4 hours at room temperature, dilute aqueous hydrochloric acid was added and the reaction mixture was extracted with ethyl acetate. The ethyl acetate extract was then dried ($MgSO_4$), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with ethyl acetate then gave the title compound as a foam (20mg).

MS (FAB): 860 $(M+Rb)^+$; 798 $(M+Na)^+$; 776 $(M+H)^+$; 758 $(M-OH)^+$

^{13}C NMR ($CDCl_3$) δ: 214.1 (C16); 174 (C3); 169.3 (C10); 141.9 (C19); 135.4 (C41); 132.5 (C29); 128.8 (C31); 121.3 (C18); 116.5 (C42); 98.5 (C1); 48.4 (C20); 20.6 (C7);

9.7 (C39)

Example 14

(17S)-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-3,10,16-trione

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 (the compound of Example 2, EP 356399) (50mg) was heated in tetrahydrofuran containing diethylamine and triethylamine (0.1ml of each) under reflux for 30 hours in a nitrogen atmosphere. The cooled reaction mixture was then poured into dilute aqueous hydrochloric acid (10ml) and this was extracted with diethyl ether (20ml). After washing with water (10ml) and brine (10ml), the extracts were dried ($MgSO_4$), filtered, and evaporated to an oil in vacuo. Chromatography on silica eluting with dichloromethane/acetonitrile [2:1] then gave the title compound as a foam (15mg).

MS (FAB): 875 $[M+Rb]^+$; 813 $[M+Na]^+$; 791 $[M+H]^+$; 773 $[M-OH]^+$; 755 $[M+H-2H_2O]^+$

^{13}C NMR ($CDCl_3$) δ: 214.4 (C16); 174.5 (C3); 169.7 (C10); 139.2 (C19); 135.6 (C41); 132.9 (C29); 127.7 (C31); 121.8 (C18); 116.7 (C42); 98.2 (C1); 84.2 (C34); 70.3 (C24); 70 (C14); 53.1 (C17); 52.4 (C9); 46.9 (C20); 42.6 (C5); 41.4 (C15); 30.2 (C21); 26.7 (C8); 24.5 (C6); 20.6

(C7); 20.4 (C44); 17.7 (C43); 16.9 (C47); 14.8 (C30); 9.5 (C39)

Example 15

17-Ethyl-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-3,10,16-trione

Hydrogen sulphide gas was bubbled through a solution of 17-ethyl-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 (FR-900520 from EP 184162, also known as 'ascomycin') (100mg) and dimethylformamide (0.1ml) in pyridine (3ml) for one hour at room temperature. The reaction mixture was then stored for 60 hours at room temperature before being poured into a mixture of dilute aqueous hydrochloric acid (1N) and ethyl acetate. The ethyl acetate layer was separated, dried ($MgSO_4$), filtered and evaporated in vacuo to an oil. Chromatography on silica eluting with ethyl acetate then gave the title compound as a foam (35mg).

MS (FAB): 862 $[M+Rb]^+$; 760 $[M+H]^+$

^{13}C NMR ($CDCl_3$) δ: 215.1 (C16); 173.9 (C10); 169.3 (C3); 141 (C19); 132.4 (C29); 128.6 (C31); 121.9 (C18); 98.4 (C1); 84.1 (C34); 55 (C9); 52.5 (C17); 48.4 (C20); 38.3 (C13); 34.6 (C27); 25.6 (C21); 11.7 (C30); 9.6 (C39)

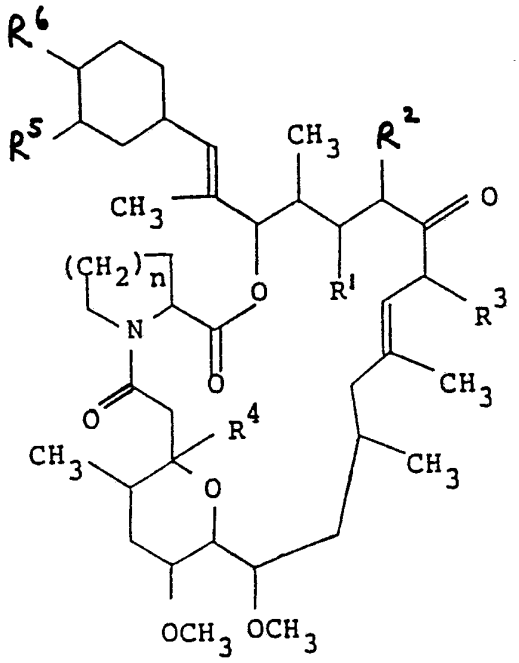
Example 16

The compound of Example 1 was tested according to Test A

above, and found to suppress the mixed lymphocyte reaction by 50% (IC_{50}) at a concentration of $2.4x10^{-8}M$.

CLAIMS:

1. A compound of formula I,



I

wherein

R^1 represents H, OH, alkoxy or R^7COO-;

R^2 represents H;

in addition, R^1 and R^2 may together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

R^3 represents methyl, ethyl, propyl or allyl;

R^4 represents OH or alkoxy;

R^5 represents OH or methoxy;

R^6 represents OH, alkoxy or R^8COO-;

in which R^7 and R^8 independently represent alkyl; aryl; NH_2; a 5- or 6-membered heterocyclic ring optionally substituted by alkyl or aryl; arylamino; alkylamino; N,N-dialkylamino; N,N-diarylamino; or N-alkyl-N-arylamino; each alkyl group optionally being substituted by aryl, OH,

NO_2 or halogen; and each aryl group optionally being substituted by alkyl, OH, NO_2 or halogen; and

n represents 1 or 2;

provided that when n is 1, then R^3 is allyl or propyl.

2. A compound of formula I as defined in claim 1, wherein R^3 is ethyl.

3. A compound of formula I as defined in claim 1, wherein at least one of R^1 and R^6 is OH.

4. A compound of formula I as defined in claim 1, wherein R^7 and R^8 are selected from alkyl; NH_2; piperidino; morpholino; aryl optionally substituted by halogen or OH; arylamino optionally substituted by halogen or OH; or alkylamino optionally substituted by OH.

5. A compound of formula I as defined in claim 1, which is

14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

12-[2-(4-Acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

14-Acetoxy-12-[2-(4-acetoxy-3-methoxycyclohexyl)-1-methylvinyl]-17-allyl-1,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

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-3,10,16-trione,

17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-phenylcarbamoyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

17-Allyl-12-[2-[4-(4-fluorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

1-Hydroxy-12-[2-[4-[(2R)-2-hydroxypropyl-carbamoyloxy]-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

17-Allyl-1-hydroxy-23,25-dimethoxy-12-[2-(3-methoxy-4-morpholinocarbonyloxycyclohexyl)-1-methylvinyl]-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

1-Hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

12-[2-(4-Carbamoyloxy-3-methoxycyclohexyl)-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

1-Hydroxy-12-[2-[4-(3-hydroxypropylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-17-propyl-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

17-Allyl-14-(4-chlorophenylcarbamoyloxy)-12-[2-[4-(4-chlorophenylcarbamoyloxy)-3-methoxycyclohexyl]-1-methylvinyl]-1-hydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

17-Allyl-1,14-dihydroxy-12-[2-(3,4-dihydroxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

(17S)-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-3,10,16-trione, or

17-Ethyl-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-3,10,16-trione.

6. The use of a compound of formula I, as defined in claim 1, as a pharmaceutical.

7. A pharmaceutical composition comprising a compound of formula I, as defined in claim 1, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

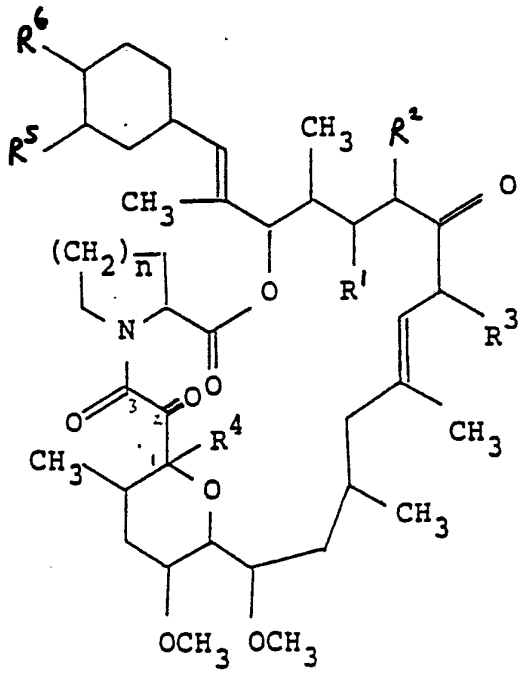
8. The use of a compound of formula I, as defined in claim 1, in the manufacture of a medicament for use as an

immunosuppressive agent.

9. A method of effecting immunosuppression which comprises administering a therapeutically effective amount of a compound of formula I, as defined in claim 1, to a patient.

10. A process for the production of a compound of formula I as defined in claim 1, which comprises:

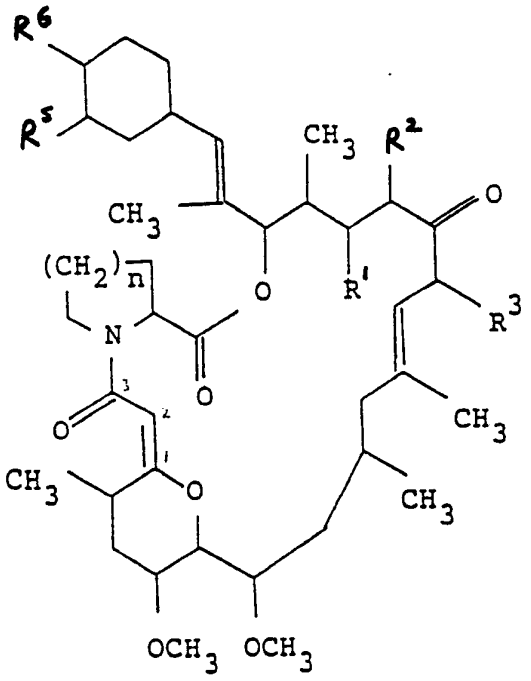
a) selective reduction of the C2-carbonyl group in a compound of formula II,



II

wherein R^1 to R^6 and n are as defined above, or

b) addition of a compound of formula R^4-H, wherein R^4 is as defined above, across the C1 alkene group in a compound of formula III,



III

wherein R^1 to R^3, R^5, R^6 and n are as defined above.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01262

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 D 498/18, A 61 K 31/33//(C 07 D 498/18, 311:00, 273:00 221:00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 D; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP, A3, 0184162 (FUJISAWA PHARMACEUTICAL CO., LTD.) 11 June 1986, see the whole document	1-5,7,8, 10
P,X	EP, A2, 0349049 (MERCK & CO. INC.) 3 January 1990, see the whole document	1-5,7,8, 10
P,X	EP, A2, 0349061 (MERCK & CO. INC.) 3 January 1990, see the whole document	1-5,7,8, 10

* Special categories of cited documents: [10]

"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

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27th November 1990	- 7. 12. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	H. Peis M. PEIS

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

P,X	EP, A2, 0353678 (FUJISAWA PHARMACEUTICAL CO., LTD.) 7 February 1990, see the whole document --	1-5,7,8, 10
P,X,	EP, A2, 0356399 (SANDOZ AG) 28 February 1990, see the whole document --	1-5,7,8, 10
X	WO, A1, 8905304 (FISONS PLC) 15 June 1989, see the whole document --	1-5,7,8, 10

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. [X] Claim numbers *), because they relate to subject matter not required to be searched by this Authority, namely:

*) 6 and 9

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods /see PCT Rule 39(iv)7.

2. [ ] Claim numbers....., 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. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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 of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] 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 claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- [ ] The additional search fees were accompanied by applicant's protest.
- [ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 90/01262

SA 39680

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 01/11/90
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A3- 0184162	11/06/86	AU-B-	592067	04/01/90
		AU-D-	5059685	12/06/86
		JP-A-	61148181	05/07/86
		US-A-	4894366	16/01/90
		US-A-	4929611	29/05/90
		US-A-	4956352	11/09/90
EP-A2- 0349049	03/01/90	NONE		
EP-A2- 0349061	03/01/90	NONE		
EP-A2- 0353678	07/02/90	JP-A-	2131590	21/05/90
EP-A2- 0356399	28/02/90	AU-D-	4024689	01/03/90
WO-A1- 8905304	15/06/89	AU-D-	2822889	05/07/89
		EP-A-	0323042	05/07/89
		EP-A-	0346427	20/12/89