RTMP's copy (1)

REPLACED SHEETS SG
☑ Description : paged 1 to paged 20
☑ Claim(s) : paged 21 to paged 24
☐ Drawing(s) : paged — to paged —
☑ Abstract : paged 25 to paged 25

Title of the Invention

A pharmaceutical solution

Field of the Invention

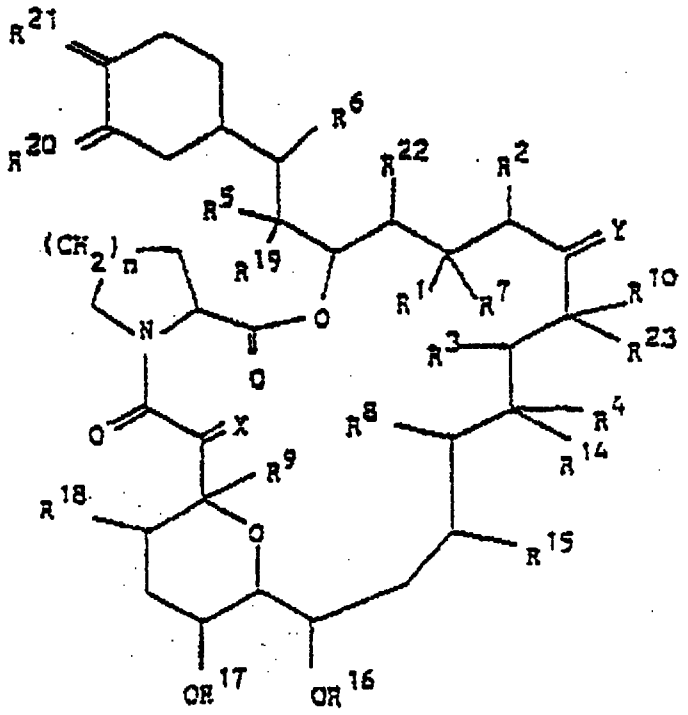
The present invention relates to a pharmaceutical solution which contains the compound (I) or a pharmaceutically acceptable salt thereof described later that is known as showing immunosuppressive activity.

In detail, the present invention relates to the solution which shows long term storage stability in a nonaqueous solution and may be diluted with such as physiological saline, glucose solution for injection, water, fruit juice, and the like without occurence of any precipitations of the compound(I).

Accordingly, the present invention relates to the above pharmaceutical solution which can be applied for various form of medicine such as intravenous injection, oral administrating liquidous medicine, or the like.

Prior Arts

The compound(I) used in the present invention is shown as follows.



(I)

This Sheet Has Been Replaced

(17)

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached ;

in addition to its significance above, R^2 may represent an alkyl group ;

R^7 represents H, OH, protected hydroxy or O-alkyl, or in conjunction with R^1 it may represent =O ;

R^8 and R^9 independently represent H or OH ;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by = O ;

X represents O, (H,OH), (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H) or $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl ;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl ;

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively ; $R^{20}a$ and $R^{21}a$ independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$ or $R^{21}a$ is protected hydroxy ;

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atoms in an epoxide ring ;

n is 1, 2 or 3 ;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O-containing heterocyclic ring, which may be saturated or unsaturated, and which may be substistuted by one or more groups selected from alkyl, hydroxy, alkyl substituted by one or more hydroxyl groups, O-alkyl,

This Sheet Has Been Replaced

benzyl and $-CH_2Se(C_6H_5)$.

The compound (I) and its pharmaceutically acceptable salt have remarkable immunosuppressive, antimicrobial and other pharmacologic activities and are known to be of value in the treatment and prevention of resistance to organ or tissue transplantation, graft-versus-host disease, various autoimmune disease and infectious disease (Japanese Kokai Patent Publication No. 61-148181/1986 and European Patent Publication No.0323042).

Such compound(I) and its pharmaceutically acceptable salt are prepared in the same manner as the one described in the above-mentioned two patent applications. Particularly, the macrolides, which are produced by fermentation of Streptmyces tsukubaensis No.9993 (FERM BP-927) or Streptmyces hygroscopicus subsp. yakushimaensis No.7238 (FERM BP-928), are numbered FR-900506, FR-900520, FR-900523 and FR-900525.

It is considered to prepare various kind forms of medicine such as powder, suspension, pharmaceutical solution which contains the compound (I) and pharmaceutically acceptable salt thereof (hereinafter the term "compound (I)" is representatively used to show them). However it is difficult to prepare stable pharmaceutical solution of the compound(I), which causes a difficulty in applying the compound (I) for clinical use where it is desired to prepare pharmaceutical solution e.g., injection, oral administrating liquid, local scattering solution, dropping lotion in the eye, and the like.

Object of the Invention

It is the object of the present invention to prepare pharmaceutical solution containing the compound (I).

In more detail it is the object of the present invention to prepare the above pharmaceutical solution which shows clear aqueous solution state that is especially desired for intravenous injection.

This Sheet Has Been Replaced

Summary of the Invention

Pharmaceutical solution of the present invention comprises of the above compound (I) as an active ingredient, a pharmaceutically acceptable surface active agent and nonaqueous solvent.

Detailed Despcription of the Invention

In the above and subsequent descriptions on the present invention, suitable examples and illustrations of the various definitions which the present invention includes within the scope thereof are explained in detail as follows.

The term " lower " as used in this specification means, unless otherwise indicated, any number of carbon atoms between 1 and 6, inclusive.

Suitable " alkyl " means straight or branched saturated aliphatic hydrocarbon residue and may include lower alkyl such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, neopentyl, hexyl, and the like.

Suitable " alkenyl " means straight or branched unsaturated aliphatic hydrocarbon residue having one double bond and may include lower alkenyl such as vinyl, propenyl, butenyl, methylpropenyl, pentenyl, hexenyl, and the like.

Suitable " aryl " may include phenyl, tolyl, xylyl, cumenyl, mesityl, naphthyl, and the like.

Suitable examples of the protective group in the " protected hydroxyl group " may include:
1-(lower alkylthio)(lower)alkyl groups such as lower alkylthiomethyl groups (e.g. methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), more desirably C_1-C_4 alkylthiomethyl groups, and most desirably methylthiomethyl;
tri-substituted silyl groups such as tri(lower)-alkylsilyl groups (e.g. trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyl-dimethylsilyl, tri-tert-butylsilyl, etc.);
lower alkyl-diarylsilyl groups (e.g. methyldiphenyl-silyl, ethyldiphenylsilyl, propyldiphenylsilyl, tert-butyl-

This Sheet Has Been Replaced

diphenylsilyl, etc.), more desirably tri(C_1-C_4)alkylsilyl and C_1-C_4 alkyldiphenylsilyl groups and most desirably tert-butyldimethylsilyl and tert-butyldiphenylsilyl; and acyl groups such as aliphatic acyl groups, aromatic acyl groups and aliphatic acyl groups substituted by aromatic groups, which are derived from carboxylic acids, sulfonic acids or carbamic acids.

The aliphatic acyl group may includes lower alkanoyl groups which may optionally have one or more suitable substituents such as carboxy (e.g. formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, carboxyacetyl, carboxypropionyl, carboxybutyryl, carboxyhexanoyl, etc.), cyclo(lower)alkoxy-(lower)alkanoyl groups which may optionally have one or more appropriate substituents such as lower alkyl (e.g. cyclopropyloxyacetyl, cyclobutyloxypropionyl, cycloheptyloxybutyryl, menthyloxyacetyl, menthyloxy-propionyl, menthyloxybutyryl, menthyloxypentanoyl, menthyloxyhexanoyl, etc.), camphorsulfonyl, lower alkylcarbamoyl groups having one or more suitable substituents such as carboxy or protected carboxy, for example carboxy(lower)alkylcarbamoyl groups(e.g. carboxymethylcarbamoyl, carboxyethylcarbamoyl, carboxypropylcarbamoyl, carboxybutylcarbamoyl, carboxypentylcarbamoyl, carboxyhexylcarbamoyl, etc.), protected carboxy(lower)alkylcarbamoyl groups such as tri(lower)alkylsilyl(lower)alkoxycarbonyl(lower)alkylc-arbamoyl groups(e.g. trimethylsilylmethoxycarbonyl-ethylcarbamoyl, trimethylsilylethoxycarbonylpropyl carbamoyl, triethylsilylethoxycarbonylpropylcarbamoyl, tert-butyldimethylsilylethoxycarbonylpropylcarbamoyl, trimethylsilylpropoxycarbonylbutylcarbamoyl, etc.) and so on.

The aromatic acyl group may include aroyl groups which may optionally have one or more suitable substituents such as nitro (e.g. benzoyl, toluoyl, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl, etc),

This Sheet Has Been Replaced

arenesulfonyl groups which may optionally have one or more suitable substituent(s) such as halogen (e.g. benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodobenzenesulfonyl, etc.), and so on.

The aromatic group-substituted aliphatic acyl group may include ar(lower)alkanoyl groups which may optionally have one or more suitable substituent(s) such as lower alkoxy and trihalo(lower)alkyl (e.g. phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl, etc.), and so on.

Among the above-mentioned acyl groups, the more desirable acyl groups are C_1-C_4 alkanoyl groups which may optionally be substituted by carboxy, cyclo(C_5-C_6)alkyloxy-(C_1-C_4)alkanoyl groups having two (C_1-C_4)alkyl groups in the cycloalkyl moiety, camphorsulfonyl, carboxy(C_1-C_4)alkyl-carbamoyl groups, tri(C_1-C_4)alkylsilyl(C_1-C_4)alkoxycarbonyl-(C_1-C_4)alkylcarbamoyl groups, benzoyl which may have one or two nitro groups, halogen-substituted benzenesulfonyl groups, phenyl(C_1-C_4)alkanoyl groups having C_1-C_4 alkoxy and trihalo(C_1-C_4)alkyl groups. Of these groups, the most desirable are acetyl, carboxypropionyl, menthyloxyacetyl, camphorsulfonyl, benzoyl, nitrobenzoyl, dinitrobenzoyl, iodobenzenesulfonyl and 2-trifluoromethyl-2-methoxy-2-phenylacetyl.

Suitable " 5- or 6-membered N-, S- or O-containing heterocyclic ring " may include pyrrolyl, tetrahydrofuryl, and the like.

The pharmaceutically acceptable salt of compound (I) is a nontoxic salt, which may be the corresponding salt with an inorganic or organic base such as alkali metal salts (e.g. sodium salt, potassium salt, etc.), alkaline earth metal salts (e.g. calcium salt, magnesium salt, etc.), ammonium salt and amine salts (e.g. triethylamine salt, N-benzyl-N-methylamine salt, etc.) and so on.

This Sheet Has Been Replaced

With regard to the compound (I) of the present invention, it is to be noted that there may be one or more than one conformers on stereoisomers such as optically or geometrically isomeric pairs due to the presence of one or more than one assymetric carbon atom or double bond, and these are included within a scope of the compound (I) of the present invention.

It will hereinafter be described in detail how the present invention has completed, especially relating to the important point of the present invention i.e., the reason why a mixture of surface active agent and nonaqueous solvent is selected in this invention.

A liquidous pharmaceutical containing the compound (I) should be offered as a stable liquid as it is for the purpose of administrating to human body and transferring the effective amount of the ingredient compound to human body. Further on considering a special use such as intravenous injection that is the main object of the present invention, should be offered a whole clear liquidous pharmaceutical which can maintain the clearity under long term storage.

From the above point of view, the inventors of the present invention studied, at first, the solubility of the compound (I) in water. As a test compound, the inventors selected the following compound as a free form which has an excellent immunosuprressive activity and is called FK 506 hereafter.

R^1, R^2, R^8, R^{23} = hydrogen
R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} = methyl
R^{20} = R^{20}_a,H (R^{20}_a = methoxy)
R^{21} = R^{21}_a,H (R^{21}_a = hydroxy)
R^3, R^4 = form a second bond between the vicinal carbon atoms to which they are attached
R^5, R^6 = form a second bond between the vicinal carbon atoms to which they are attached
R^7,R^9 = hydroxy
R^{10} = allyl
X,Y = oxygen
n = 2

This Sheet Has Been Replaced

The solubility of FK 506 in water is at most 3μ g/ml under ambient temperature. Accordingly, it is decided to add a surface active agent in order to increase the solubility of FK 506 in water to the level where the clinically effective amount of FK 506 is dissloved. Table 1 shows the solubility of FK 506 under the different condition e.g., the kind and the concentration of a surface active agent and temperature. As a surface active agent, a castor oil-surface active agent i.e., HCO-10, HCO-40, HCO-60 (trademark, prepared by Nikko Chemicals, respectively) are selected for examination.

It is conjectured from the result shown in Table 1 that the concentration of the surface active agent should be controlled at 1.43w/v% [about 150mg of the surface active agent against 1mg of FK506] to dissolve 0.1mg of FK 506 in 1ml of water, calculating based on the result that 0.035mg of FK 506 is dissolved in 1ml of 0.5w/v% HCO-60 aqueous solution at 20 ℃. Accordingly, if it is desired to prepare 5mg/ml aqueous solution FK 506, the concentration of the surface active agent is estimated to come up to 87w/v% from the calculation based on the result of HCO-60, 20w/v%, 20℃. Such tolerably high concentration of surface active agent in aqueous solution should not be realized in practice of clinical field.

This Sheet Has Been Replaced

Table 1

Concentration of surface active agent (w/v %)	Kind of surface active agent and solubility of FK506 (mg/ml)				
	HCO-40	HCO-60		Mixture of HCO-60 and HCO-10 (4:1)	
	20 ℃	20℃	30℃	20℃	30℃
0.1	—	0.005	—	—	—
0.3	—	0.019	—	—	—
0.5	—	0.035	—	—	—
5	0.40	0.28	0.29	0.26	0.27
10	0.78	0.61	0.57	0.56	0.56
20	1.52	1.15	1.14	1.13	1.14

HCO-60 : Polyoxyethylenehydrogenated Castor Oil 60
HCO-40 : Polyoxyethylenehydrogenated Castor Oil 40
HCO-10 : Polyoxyethylenehydrogenated Castor Oil 10

Table 2 shows a remaining percentage of FK 506 in aqueous solution wherein FK 506 is dissolved in water in the presence of large amount of surface active agent. From this table, it is found that the stability for long period storage is not expectable in the above prescription .

This Sheet Has Been Replaced

Table 2

Storage conditions	Remaining percentage of FK 506 (%) (prescription) FK 506 0.5mg HCO-60 100mg phosphoric acid buffer (pH 6) 1ml
Initial	100.0
After 3days (at 40 ℃)	52.7
After 3days (at 80 ℃)	42.2

From the results of the above experiment, it is recognized that the use of surface active agent is not profitable means for the purpose of dissolving the compound (I), for example FK 506, in water.

Table 3 shows solubility of FK 506 in several kinds of nonaqueous solvent such as PEG (polyethylene glycol) 400, ethanol and propylene glycol. From the consideration of the illustrated data, it is found that FK 506 is dissolved in the experimented solvent at the concentration of more than 40mg/ml.

This Sheet Has Been Replaced

Table 3

Solvent	Solubility (at ambient temperature)
Ethanol	>300
PEG 400	>40
Propylene glycol	>40

(mg/ml)

Nonaqueous solvent is, at the time of administrating into the human vascular tract, usually diluted with aqueous solvent such as physiological saline, since nonaqueous solvent has hemolysis action. Therefore, the inventors of the present invention diluted the experimental solution by using nonaqueous solution (1ml) described in the following prescriptions 1 & 2 with physiological saline (100ml), and it was found that the nonaqueous solution became turbid at once and fine crystal of FK 506 was precipitated in a mixed medium.

(Prescription 1)

FK 506		10mg
Ethanol	to	1ml

(Prescription 2)

FK 506		10mg
Propylene glycol	to	1ml

Standing on the above result, the inventors of the present invention have then studied on combination use of nonaqueous solvent and surface active agent.

The experimental solution (1ml) of the following prescription 3 consisting of FK 506, surface active agent and

This Sheet Has Been Replaced

nonaqueous solvent was diluted with physiological saline (100ml) to find that the clear appearance of prescription was kept unchange.

(Prescription 3)

FK 506		10mg
HCO-60		100mg
Ethanol	to	1ml

Then several prescriptions of clear solution were further prepared altering the concentration of FK 506, the kind and the concentration of surface active agent, and were tested how change the clearity of the solution and whether separate out the crystal or not under the several condition of different degree of dilution. The results thereof are shown in Table 4.

This Sheet Has Been Replaced

Table 4

Concentration of FK 506 (mg/ml)	Dilution times with physiological saline	Period till solution becomes turbid to crystallize FK506 (in days)						
		Kind of surface active agent/Ratio of ethanol to surface active agent						
		HCO-60			Cremophor®EL		HCO-40	
		35/65	60/40	80/20	35/65	80/20	40/60	80/20
5	2	≧7	≧7	≧7	≧7	≧7	≧7	≧7
	100	≧7	≧7	≧7	≧7	≧7	≧7	≧7
10	2	≧7	≧7	≧7	≧7	≧7	≧7	≧7
	100	≧7	≧7	≧7	≧7	≧7	≧7	≧7
25	2	≧7	≧7	≧7	≧7	≧7	≧7	≧7
	100	≧7	≧1	≧7	≧7	≧1	≧7	≧1
50	2	≧7	≧7	≧7	≧7	≧1	≧7	≧1

Cremophor®EL : trademark, prepared by BASF (Polyoxyethylene Castor Oil 35)

This Sheet Has Been Replaced

It is concluded from the result illustrated in Table 4 that a clear pharmaceutical solution which does not cause precipitation of the compound (I) on diluting with physiological saline can be prepared by controlling the ratio of the compound(I), surface active agent and nonaqueous solvent according to the kind of surface active agent under the condition that the concentration of the compound (I) is less than 50mg/ml.

Lastly it is tested the remaining percentage of FK 506 after storage in nonaqueous solvent containing both FK 506 and surface active agent. In the test solution, the concentration of FK 506 is adjusted to 5mg/ml, and, for comparative a nonaqueous solution which contain only FK 506 is prepared. The experimental result is shown in Table 5.

This Sheet Has Been Replaced

Table 5

Storage conditions		Remaining percentage of FK 506(%) (prescription) FK 506 5mg HCO-60 400mg ethanol to 1ml
Initial		100.0
80 ℃	1 day	95.2
	3 day	90.4
	5 day	86.4
	10 day	78.6
	17 day	68.0
60 ℃	5 day	96.4
	10 day	95.1
	17 day	92.4
	1 month	88.0
40 ℃	1 month	96.7
	3 month	96.6
	18 month	84.6

It is concluded from the result shown in table 5 that HCO-60 is the most preferable surface active agent in the view point of storage stability.

As considered from the above experiments, the compound (I) such as FK 506 has quite poor solubility in water and this is not so improved even in the presence of surface active agent.

This Sheet Has Been Replaced

(D)

and the storage stability especially at ambient temperature is quite inferior excepting that only frozen one can stand for some period of time.

In the mean time, it is found that the compound (I) is well dissolved in nonaqueous solvent. However it causes precipitation of the compound (I) on diluting with physiological saline to diminishing hemolysis action of nonaqueous solvent. Occurence of precipitation makes it impossible to use in the clinical field.

Under the condition of cooperative use of nonaqueous solvent and surface active agent, it is noted that the compound (I) is well dissolved and there is no problem after long pereiod storage, and further it does not happen to give any precipitate at the time of diluting with physiological saline.

The kind of nonaqueous solvent is not limited in the present invention, and any nonaqueous solvent may be used as much as it can dissolve the effective amount of the compound (I) and it may be acceptable in clinical use. The nonaqueous solvent may be used either alone or as a mixture thereof. Suitable examples thereof include ethanol, propylene glycol, glycerin, polyethylene glycol (e.g., PEG 400, PEG 300, PEG 200, etc.), or the mixture thereof from a view point of solubility and viscosity, etc. and most preferable one is ethanol. The representative examples of the surface active agent include, from a view point of storage stability for long term, a castor oil-surface active agents, and more preferable one is HCO (polyoxyethylene hardened oil)-surface active agents, and most preferable one is HCO-60, HCO-50 and the like. In addition to the above exemplified surface active agents, polyoxyethylene sorbitane fatty acid ester derivative (e.g., Polysorbate 80, etc.), glycerine fatty acid ester derivative (e.g., glycerine monocaprylate, etc.), polyethylene glycol fatty acid ester derivative (e.g., polyoxyethylene 40 monostearate, etc.), and the like may also be used.

The concentration of the compound (I) is determined from

This Sheet Has Been Replaced

the judgement including the kind and the concentration of nonaqueous solvent and surface active agent, the composition ratio thereof, the stability after being diluted with physiological saline, etc. and storage stability. The suitable range of thus determined concentration is usually in the range of 0.1~ 50mg/ml and more preferably 1 ~ 20mg/ml.

As for the amount of surface active agent, it is noted to be less than the calculated estimation. From the experimental calculation based on the result illustrated in Table 1, about 150mg of the surface active agent may be necessary to obtain a saturated aqueous solution containing 1 mg of the compound (I) as stated above. However in the present invention the compound (I) is dissolved in a mixed solution of nonaqueous solvent - surface active agent - water to form stable supersaturation state, and therefore the necessary amount ofsurface active agent becomes less than the calculated one. These specific action, i.e., the low precipitating speed of thecrystalline from the supersaturated solution, is based on the characteristic property of the compound (I). The range of the ratio of surface active agent to the compound (I) is preferably 1~ 100mg/1mg, and more preferably 30~ 60mg/1mg to prevent the occurence of precipitation at the time of diluting for clinical use.

The pharmaceutical solution of the present invention may further contain, if necessary, other agent such as stabilizing agent, anodyne, and the like.

The pharmaceutical solution of the present invention is stable during long term storage and does not occur the precipitation of crystalline at the time of dilution. Therefore, this is applicable to various kind of medicine form such as intravenous injection, dropping lotion in the eye, dropping lotion in the nose, intraenteric injection, percutaneous liniment, local scattering agent, oral administration agent (e.g., syrup, etc.), and the like.

This Sheet Has Been Replaced

(1)

Example

Following prescriptions are shown only for the purpose of the explanation of this invention.

Prescription 1

FK 506		10mg
HCO-60		400mg
Ethanol	to	1ml

The solution comprising the ingredients stated above is prepared by dissolving the FK 506 and HCO-60 in ethanol by a conventional manner.

The following solutions are also prepared a similar manner of the Prescription 1.

Prescription 2

FK 506		5mg
HCO-40		200mg
PEG 400	to	1ml

Prescription 3

FK 506		2mg
Polysorbate 80		50mg
Propylene glycol	to	1ml

Prescription 4

FK 506		2mg
Polysorbate 80		10mg
Glycerine		0.5ml
Ethanol	to	1ml

Prescription 5

FK 506		2mg
HCO-60		20mg
Propylene glycol	to	1ml

This Sheet Has Been Replaced

Prescription 6

FK 506		1mg
Polyoxyethylene (40) mono stearate		20mg
Propylene glycol	to	1ml

Prescription 7

FK 506		10mg
HCO-60		400mg
Ethanol	to	1ml

Prescription 8

FK 506		5mg
HCO-60		400mg
Ethanol	to	1ml

Prescription 9

FK 506		25mg
HCO-60		400mg
Ethanol	to	1ml

Prescription 10

FK 506		2mg
HCO-60		10mg
Glycerine		0.5ml
Ethanol	to	1ml

Effect of the Invention

Thus obtained nonaqueous pharmaceutical solution containing the compound (I) is stable during long term storage and does not occur any precipitation at the time of diluting with physiological saline, glucose solution for injection, water, fruit juice, milk, or the like for clinical use. Accordingly, the pharmaceutical solution of the present invention is applicable to various kind of medicine form such as intravenous injection, oral adminitration agent and the like, which could contribute

This Sheet Has Been Replaced

the compound (I) in clinical field wherein its immunosuppressive activity is intensely desired. Paticularly, the most preferable medicine form of the present nonaqueouspharmaceutical solution is the one for intravenous injection by diluting with the physiological saline.

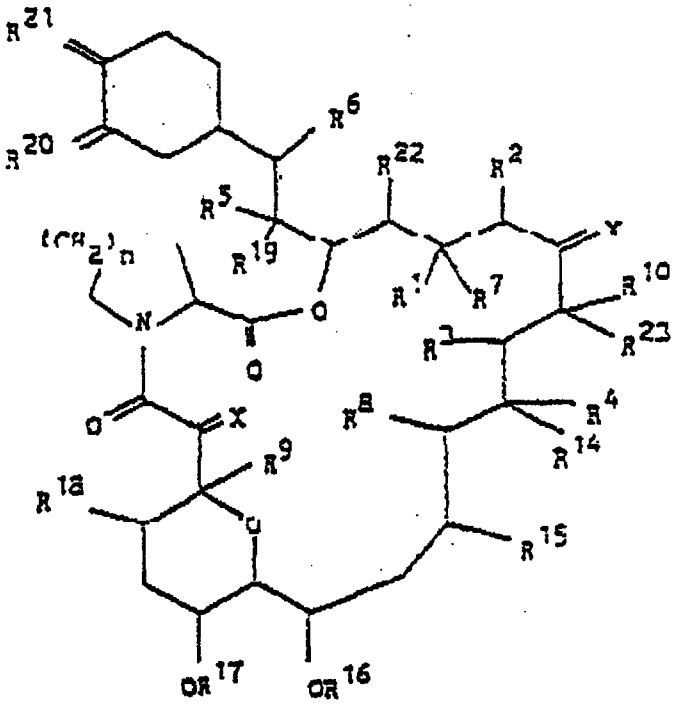
This Sheet Has Been Replaced

(1)

What we claim

1. A pharmaceutical solution comprises a compound of the general formula

This Sheet Has Been Replaced



(I)

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached ;

in addition to its significance above, R^2 may represent an alkyl group ;

R^7 represents H, OH, protected hydroxy or O-alkyl, or in conjunction with R^1 it may represent =O ;

R^8 and R^9 independently represent H or OH ;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by = O ;

* C L M S *

(1)

X represents O, (H,OH), (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H) or $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl ;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl ;

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively ; $R^{20}a$ and $R^{21}a$ independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$, or $R^{21}a$ is protected hydroxy ;

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atoms in an epoxide ring ;

n is 1, 2 or 3 ;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O-containing heterocyclic ring, which may be saturated or unsaturated, and which may be substistuted by one or more groups selected from alkyl, hydroxy, alkyl substituted by one or more hydroxyl groups, O-alkyl, benzyl and $-CH_2Se(C_6H_5)$.

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable surface active agent and a pharmaceutically acceptable nonaqueous solvent.

2. The pharmaceutical solution of claim 1, wherein the compound (I) or a pharmaceutically acceptable salt thereof and the pharmaceutically acceptable surface active agent are in the ratio of 1:1 to 1:100 by weight.

3. The pharmaceutical solution of claim 2, wherein the pharmaceutically acceptable surface active agent is a castor oil-surface active agent.

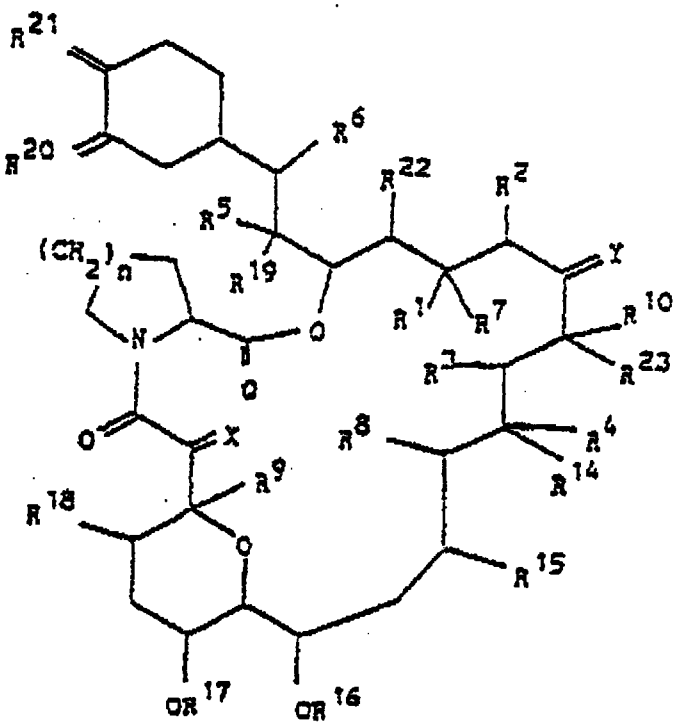
This Sheet Has Been Replaced

* C L M S *

4. The pharmaceutical solution of claim 3, wherein th pharmaceutically acceptable nonaqueous solvent is ethyl alcohol.

5. The pharmaceutical solution of claim 4, wherein the compound (I) is 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.

6. A process for preparing a pharmaceutical solution, which is characterizing by dissolving a compound of the formula :



(I)

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached ;

in addition to its significance above, R^2 may represent an alkyl group ;

This Sheet Has Been Replaced

* C L M S *

(1)

R^7 represents H, OH, protected hydroxy or O-alkyl, or in conjunction with R^1 it may represent =O ;

R^8 and R^9 independently represent H or OH ;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by = O ;

X represents O, (H,OH), (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H) or $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl ;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl ;

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively ; $R^{20}a$ and $R^{21}a$ independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$, or $R^{21}a$ is protected hydroxy ;

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atoms in an epoxide ring ;

n is 1, 2 or 3 ;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O-containing heterocyclic ring, which may be saturated or unsaturated, and which may be substistuted by one or more groups selected from alkyl, hydroxy, alkyl substituted by one or more hydroxyl groups, O-alkyl, benzyl and $-CH_2Se(C_6H_5)$.

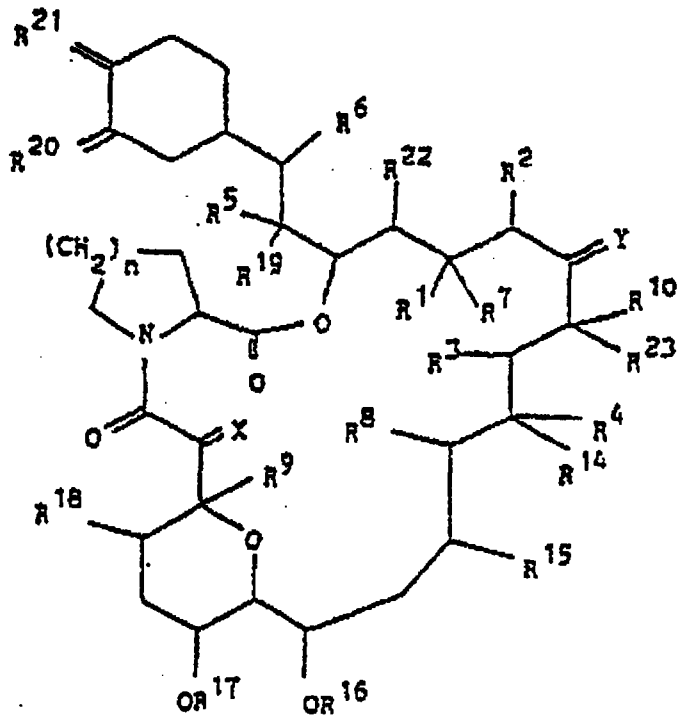
or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable surface active agent in a pharmaceutically acceptable nonaqueous solvent.

This Sheet Has Been Replaced

* C L M S *

Abstract of the Disclosure

A pharmaceutical solution which contains the compound of the general formula having the immusuppreseive activity is disclosed.



This Sheet Has Been Replaced

Title of the Invention

A pharmaceutical solution

Field of the Invention

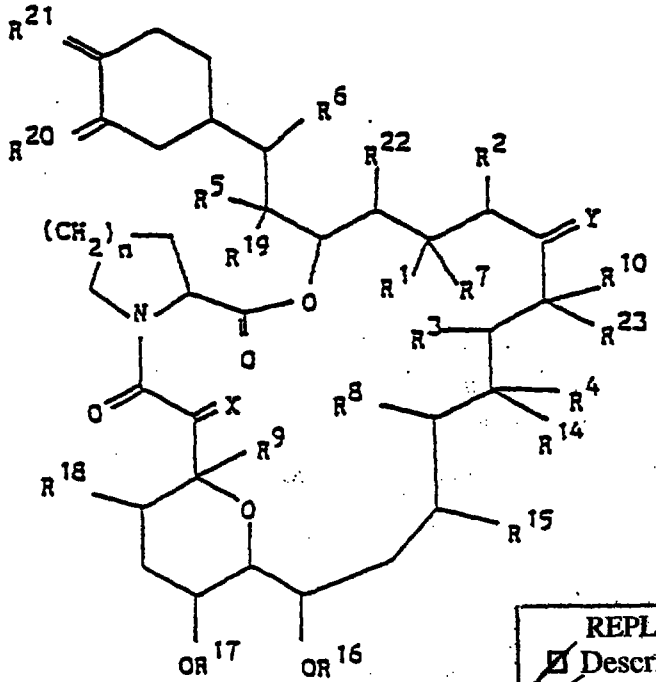
The present invention relates to a pharmaceutical solution which contains the compound (I) or a pharmaceutically acceptable salt thereof described later that is known as showing immunosuppressive activity.

In detail,the present invention relates to the solution which shows long term storage stability in a nonaqueous solution and may be diluted with such as physiological saline, glucose solution for injection, water, fruit juice, and the like without occurence of any precipitations of the compound(I).

Accordingly, the present invention relates to the above pharmaceutical solution which can be applied for various form of medicine such as intravenous injection, oral administrating liquidous medicine, or the like.

Prior Arts

The compound(I) used in the present invention is shown as follows.



(I)

REPLACED SHEETS SG
☐ Description : paged ..... to paged .....
☑ Claim(s) : paged 21 to paged 24
☐ Drawing(s) : paged ..... to paged .....
☐ Abstract : paged ..... to paged .....

REPLACEMENT SHEETS SG
☑ Description : paged 1 to paged 20
☑ Claim(s) : paged 21 to paged 24
☐ Drawing(s) : paged ..... to paged .....
☑ Abstract : paged ..... to paged .....
DATE OF RECEIPT 22 OCT 1996

This is the replacement sheet
Date: 22 OCT 1996

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached ;

in addition to its significance above, R^2 may represent an alkyl group ;

R^7 represents H, OH, protected hydroxy or O-alkyl, or in conjunction with R^1 it may represent =O ;

R^8 and R^9 independently represent H or OH ;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by = O ;

X represents O, (H,OH), (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H) or $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl ;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl ;

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively ; $R^{20}a$ and $R^{21}a$ independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$ or $R^{21}a$ is protected hydroxy ;

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atoms in an epoxide ring ;

n is 1, 2 or 3 ;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O-containing heterocyclic ring, which may be saturated or unsaturated, and which may be substistuted by one or more groups selected from alkyl, hydroxy, alkyl substituted by one or more hydroxyl groups, O-alkyl,

benzyl and $-CH_2Se(C_6H_5)$.

The compound (I) and its pharmaceutically acceptable salt have remarkable immunosuppressive, antimicrobial and other pharmacologic activities and are known to be of value in the treatment and prevention of resistance to organ or tissue transplantation, graft-versus-host disease, various autoimmune disease and infectious disease (Japanese Kokai Patent Publication No. 61-148181/1986 and European Patent Publication No.0323042).

Such compound(I) and its pharmaceutically acceptable salt are prepared in the same manner as the one described in the above-mentioned two patent applications. Particularly, the macrolides, which are produced by fermentation of Streptmyces tsukūbaensis No.9993 (FERM BP-927) or Streptmyces hygroscopicus subsp. yakushimaensis No.7238 (FERM BP-928), are numbered FR-900506, FR-900520, FR-900523 and FR-900525.

It is considered to prepare various kind forms of medicine such as powder, suspension, pharmaceutical solution which contains the compound (I) and pharmaceutically acceptable salt thereof (hereinafter the term "compound (I)" is representatively used to show them). However it is difficult to prepare stable pharmaceutical solution of the compound(I), which causes a difficulty in applying the compound (I) for clinical use where it is desired to prepare pharmaceutical solution e.g., injection, oral administrating liquid, local scattering solution, dropping lotion in the eye, and the like.

Object of the Invention

It is the object of the present invention to prepare pharmaceutical solution containing the compound (I).

In more detail it is the object of the present invention to prepare the above pharmaceutical solution which shows clear aqueous solution state that is especially desired for intravenous injection.

This is the replacement sheet

Date: 22 OCT 1996

Summary of the Invention

Pharmaceutical solution of the present invention comprises of the above compound (I) as an active ingredient, a pharmaceutically acceptable surface active agent and nonaqueous solvent.

Detailed Despcription of the Invention

In the above and subsequent descriptions on the present invention, suitable examples and illustrations of the various definitions which the present invention includes within the scope thereof are explained in detail as follows.

The term " lower " as used in this specification means, unless otherwise indicated, any number of carbon atoms between 1 and 6, inclusive.

Suitable " alkyl " means straight or branched saturated aliphatic hydrocarbon residue and may include lower alkyl such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, neopentyl, hexyl, and the like.

Suitable " alkenyl " means straight or branched unsaturated aliphatic hydrocarbon residue having one double bond and may include lower alkenyl such as vinyl, propenyl, butenyl, methylpropenyl, pentenyl, hexenyl, and the like.

Suitable " aryl " may include phenyl, tolyl, xylyl, cumenyl, mesityl, naphthyl, and the like.

Suitable examples of the protective group in the " protected hydroxyl group " may include:
1-(lower alkylthio)(lower)alkyl groups such as lower alkylthiomethyl groups (e.g. methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), more desirably C_1-C_4 alkylthiomethyl groups, and most desirably methylthiomethyl;
tri-substituted silyl groups such as tri(lower)-alkylsilyl groups (e.g. trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyl-dimethylsilyl, tri-tert-butylsilyl, etc.);
lower alkyl-diarylsilyl groups (e.g. methyldiphenyl-silyl, ethyldiphenylsilyl, propyldiphenylsilyl, tert-butyl-

This is the replacement sheet

Date: 22 OCT 1996

diphenylsilyl, etc.), more desirably tri(C_1-C_4)alkylsilyl and C_1-C_4 alkyldiphenylsilyl groups and most desirably tert-butyldimethylsilyl and tert-butyldiphenylsilyl; and acyl groups such as aliphatic acyl groups, aromatic acyl groups and aliphatic acyl groups substituted by aromatic groups, which are derived from carboxylic acids, sulfonic acids or carbamic acids.

The aliphatic acyl group may includes lower alkanoyl groups which may optionally have one or more suitable substituents such as carboxy (e.g. formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, carboxyacetyl, carboxypropionyl, carboxybutyryl, carboxyhexanoyl, etc.), cyclo(lower)alkoxy-(lower)alkanoyl groups which may optionally have one or more appropriate substituents such as lower alkyl (e.g. cyclopropyloxyacetyl, cyclobutyloxypropionyl, cycloheptyloxybutyryl, menthyloxyacetyl, menthyloxy-propionyl, menthyloxybutyryl, menthyloxypentanoyl, menthyloxyhexanoyl, etc.), camphorsulfonyl, lower alkylcarbamoyl groups having one or more suitable substituents such as carboxy or protected carboxy, for example carboxy(lower)alkylcarbamoyl groups(e.g. carboxymethylcarbamoyl, carboxyethylcarbamoyl, carboxypropylcarbamoyl, carboxybutylcarbamoyl, carboxypentylcarbamoyl, carboxyhexylcarbamoyl, etc.), protected carboxy(lower)alkylcarbamoyl groups such as tri(lower)alkylsilyl(lower)alkoxycarbonyl(lower)alkylc-arbamoyl groups(e.g. trimethylsilylmethoxycarbonyl-ethylcarbamoyl, trimethylsilylethoxycarbonylpropyl carbamoyl, triethylsilylethoxycarbonylpropylcarbamoyl, tert-butyldimethylsilylethoxycarbonylpropylcarbamoyl, trimethylsilylpropoxycarbonylbutylcarbamoyl, etc.) and so on.

The aromatic acyl group may include aroyl groups which may optionally have one or more suitable substituents such as nitro (e.g. benzoyl, toluoyl, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl, etc),

This is the replacement sheet

Date: 22 OCT 1996

arenesulfonyl groups which may optionally have one or more suitable substituent(s) such as halogen (e.g. benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodobenzenesulfonyl, etc.), and so on.

The aromatic group-substituted aliphatic acyl group may include ar(lower)alkanoyl groups which may optionally have one or more suitable substituent(s) such as lower alkoxy and trihalo(lower)alkyl (e.g. phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl, etc.), and so on.

Among the above-mentioned acyl groups, the more desirable acyl groups are C_1-C_4 alkanoyl groups which may optionally be substituted by carboxy, cyclo(C_5-C_6)alkyloxy-(C_1-C_4)alkanoyl groups having two (C_1-C_4)alkyl groups in the cycloalkyl moiety, camphorsulfonyl, carboxy(C_1-C_4)alkylcarbamoyl groups, tri(C_1-C_4)alkylsilyl(C_1-C_4)alkoxycarbonyl-(C_1-C_4)alkylcarbamoyl groups, benzoyl which may have one or two nitro groups, halogen-substituted benzenesulfonyl groups, phenyl(C_1-C_4)alkanoyl groups having C_1-C_4 alkoxy and trihalo(C_1-C_4)alkyl groups. Of these groups, the most desirable are acetyl, carboxypropionyl, menthyloxyacetyl, camphorsulfonyl, benzoyl, nitrobenzoyl, dinitrobenzoyl, iodobenzenesulfonyl and 2-trifluoromethyl-2-methoxy-2-phenylacetyl.

Suitable " 5- or 6-membered N-, S- or O-containing heterocyclic ring " may include pyrrolyl, tetrahydrofuryl, and the like.

The pharmaceutically acceptable salt of compound (I) is a nontoxic salt, which may be the corresponding salt with an inorganic or organic base such as alkali metal salts (e.g. sodium salt, potassium salt, etc.), alkaline earth metal salts (e.g. calcium salt, magnesium salt, etc.), ammonium salt and amine salts (e.g. triethylamine salt, N-benzyl-N-methylamine salt, etc.) and so on.

This is the replacement sheet

Date: 22 OCT 1996

With regard to the compound (I) of the present invention, it is to be noted that there may be one or more than one conformers on stereoisomers such as optically or geometrically isomeric pairs due to the presence of one or more than one assymetric carbon atom or double bond, and these are included within a scope of the compound (I) of the present invention.

It will hereinafter be described in detail how the present invention has completed, especially relating to the important point of the present invention i.e., the reason why a mixture of surface active agent and nonaqueous solvent is selected in this invention.

A liquidous pharmaceutical containing the compound (I) should be offered as a stable liquid as it is for the purpose of administrating to human body and transferring the effective amount of the ingredient compound to human body. Further on considering a special use such as intravenous injection that is the main object of the present invention, should be offered a whole clear liquidous pharmaceutical which can maintain the clearity under long term storage.

From the above point of view, the inventors of the present invention studied, at first, the solubility of the compound (I) in water. As a test compound, the inventors selected the following compound as a free form which has an excellent immunosuprressive activity and is called FK 506 hereafter.

R^1, R^2, R^8, R^{23} = hydrogen
R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} = methyl
R^{20} = $R^{20}a$,H ($R^{20}a$ = methoxy)
R^{21} = $R^{21}a$,H ($R^{21}a$ = hydroxy)
R^3, R^4 = form a second bond between the vicinal carbon atoms to which they are attached
R^5, R^6 = form a second bond between the vicinal carbon atoms to which they are attached
R^7,R^9 = hydroxy
R^{10} = allyl
X,Y = oxygen
n = 2

This is the replacement sheet

Date: 22 OCT 1996

The solubility of FK 506 in water is at most 3μg/ml under ambient temperature. Accordingly, it is decided to add a surface active agent in order to increase the solubility of FK 506 in water to the level where the clinically effective amount of FK 506 is dissloved. Table 1 shows the solubility of FK 506 under the different condition e.g., the kind and the concentration of a surface active agent and temperature. As a surface active agent, a castor oil-surface active agent i.e., HCO-10, HCO-40, HCO-60 (trademark, prepared by Nikko Chemicals, respectively) are selected for examination.

It is conjectured from the result shown in Table 1 that the concentration of the surface active agent should be controlled at 1.43w/v% [about 150mg of the surface active agent against 1mg of FK506] to dissolve 0.1mg of FK 506 in 1ml of water, calculating based on the result that 0.035mg of FK 506 is dissolved in 1ml of 0.5w/v% HCO-60 aqueous solution at 20 ℃. Accordingly, if it is desired to prepare 5mg/ml aqueous solution FK 506, the concentration of the surface active agent is estimated to come up to 87w/v% from the calculation based on the result of HCO-60, 20w/v%, 20℃. Such tolerably high concentration of surface active agent in aqueous solution should not be realized in practice of clinical field.

This is the replacement sheet

Date: 22 OCT 1996

Table 1

Concentration of surface active agent (w/v %)	Kind of surface active agent and solubility of FK506 (mg/ml)				
	HCO-40	HCO-60		Mixture of HCO-60 and HCO-10 (4:1)	
	20 ℃	20℃	30℃	20℃	30℃
0.1	—	0.005	—	—	—
0.3	—	0.019	—	—	—
0.5	—	0.035	—	—	—
5	0.40	0.28	0.29	0.26	0.27
10	0.78	0.61	0.57	0.56	0.56
20	1.52	1.15	1.14	1.13	1.14

HCO-60 : Polyoxyethylenehydrogenated Castor Oil 60
HCO-40 : Polyoxyethylenehydrogenated Castor Oil 40
HCO-10 : Polyoxyethylenehydrogenated Castor Oil 10

Table 2 shows a remaining percentage of FK 506 in aqueous solution wherein FK 506 is dissolved in water in the presence of large amount of surface active agent. From this table, it is found that the stability for long period storage is not expectable in the above prescription .

This is the replacement sheet

Date: 22 OCT 1996

Table 2

Storage conditions	Remaining percentage of FK 506 (%) (prescription) FK 506 0.5mg HCO-60 100mg phosphoric acid buffer (pH 6) 1ml
Initial	100.0
After 3days (at 40 ℃)	52.7
After 3days (at 80 ℃)	42.2

From the results of the above experiment, it is recognized that the use of surface active agent is not profitable means for the purpose of dissolving the compound (I), for example FK 506, in water.

Table 3 shows solubility of FK 506 in several kinds of nonaqueous solvent such as PEG (polyethylene glycol) 400, ethanol and propylene glycol. From the consideration of the illustrated data, it is found that FK 506 is dissolved in the experimented solvent at the concentration of more than 40mg/ml.

This is the replacement sheet

Date: 22 OCT 1996

Table 3

Solvent	Solubility (at ambient temperature)
Ethanol	>300
PEG 400	>40
Propylene glycol	>40

(mg/ml)

Nonaqueous solvent is, at the time of administrating into the human vascular tract, usually diluted with aqueous solvent such as physiological saline, since nonaqueous solvent has hemolysis action. Therefore, the inventors of the present invention diluted the experimental solution by using nonaqueous solution (1ml) described in the following prescriptions 1 & 2 with physiological saline (100ml), and it was found that the nonaquous solution became turbid at once and fine crystal of FK 506 was precipitated in a mixed medium.

(Prescription 1)

FK 506		10mg
Ethanol	to	1ml

(Prescription 2)

FK 506		10mg
Propylene glycol	to	1ml

Standing on the above result, the inventors of the present invention have then studied on combination use of nonaqueous solvent and surface active agent.

The experimental solution (1ml) of the following prescription 3 consisting of FK 506, surface active agent and

This is the replacement sheet

Date: 22 OCT 1996

nonaqueous solvent was diluted with physiological saline (100ml) to find that the clear appearance of prescription was kept unchange.

(Prescription 3)

FK 506		10mg
HCO-60		100mg
Ethanol	to	1ml

Then several prescriptions of clear solution were further prepared altering the concentration of FK 506, the kind and the concentration of surface active agent, and were tested how change the clearity of the solution and whether separate out the crystal or not under the several condition of different degree of dilution. The results thereof are shown in Table 4.

This is the replacement sheet

Date: 22 OCT 1996

Table 4

Concentration of FK 506 (mg/ml)	Dilution times with physiological saline	Period till solution becomes turbid to crystallize FK506 (in days)							
		Kind of surface active agent/Ratio of ethanol to surface active agent							
		HCO-60			Cremophor®EL		HCO-40		
		35/65	60/40	80/20	35/65	80/20	40/60	80/20
5	2	≧7	≧7	≧7	≧7	≧7	≧7	≧7
	100	≧7	≧7	≧7	≧7	≧7	≧7	≧7
10	2	≧7	≧7	≧7	≧7	≧7	≧7	≧7
	100	≧7	≧7	≧7	≧7	≧7	≧7	≧7
25	2	≧7	≧7	≧7	≧7	≧7	≧7	≧7
	100	≧7	≧1	≧7	≧7	≧1	≧7	≧1
50	2	≧7	≧7	≧7	≧7	≧1	≧7	≧1

Cremophor®EL : trademark, prepared by BASF (Polyoxyethylene Castor Oil 35)

This is the replacement sheet

Date: 22 OCT 1996

It is concluded from the result illustrated in Table 4 that a clear pharmaceutical solution which does not cause precipitation of the compound (I) on diluting with physiological saline can be prepared by controlling the ratio of the compound(I), surface active agent and nonaqueous solvent according to the kind of surface active agent under the condition that the concentration of the compound (I) is less than 50mg/ml.

Lastly it is tested the remaining percentage of FK 506 after storage in nonaqueous solvent containing both FK 506 and surface active agent. In the test solution, the concentration of FK 506 is adjusted to 5mg/ml, and, for comparative a nonaqueous solution which contain only FK 506 is prepared. The experimental result is shown in Table 5.

This is the replacement sheet

Date: 22 OCT 1996

Table 5

Storage conditions		Remaining percentage of FK 506(%) (prescription) FK 506 5mg HCO-60 400mg ethanol to 1ml
Initial		100.0
80 ℃	1 day	95.2
	3 day	90.4
	5 day	86.4
	10 day	78.6
	17 day	68.0
60 ℃	5 day	96.4
	10 day	95.1
	17 day	92.4
	1 month	88.0
40 ℃	1 month	96.7
	3 month	96.6
	18 month	84.6

It is concluded from the result shown in table 5 that HCO-60 is the most preferable surface active agent in the view point of storage stability.

As considered from the above experiments, the compound (I) such as FK 506 has quite poor solubility in water and this is not so improved even in the presence of surface active agent,

This is the replacement sheet

Date: 22 OCT 1996

and the storage stability especially at ambient temperature is quite inferior excepting that only frozen one can stand for some period of time.

In the mean time, it is found that the compound (I) is well dissolved in nonaqueous solvent. However it causes precipitation of the compound (I) on diluting with physiological saline to diminishing hemolysis action of nonaqueous solvent. Occurence of precipitation makes it impossible to use in the clinical field.

Under the condition of cooperative use of nonaqueous solvent and surface active agent, it is noted that the compound (I) is well dissolved and there is no problem after long pereiod storage, and further it does not happen to give any precipitate at the time of diluting with physiological saline.

The kind of nonaqueous solvent is not limited in the present invention, and any nonaqueous solvent may be used as much as it can dissolve the effective amount of the compound (I) and it may be acceptable in clinical use. The nonaqueous solvent may be used either alone or as a mixture thereof. Suitable examples thereof include ethanol, propylene glycol, glycerin, polyethylene glycol (e.g., PEG 400, PEG 300, PEG 200, etc.), or the mixture thereof from a view point of solubility and viscosity, etc. and most preferable one is ethanol. The representative examples of the surface active agent include, from a view point of storage stability for long term, a castor oil-surface active agents, and more preferable one is HCO (polyoxyethylene hardened oil)-surface active agents, and most preferable one is HCO-60, HCO-50 and the like. In addition to the above exemplified surface active agents, polyoxyethylene sorbitane fatty acid ester derivative (e.g., Polysorbate 80, etc.,), glycerine fatty acid ester derivative (e.g., glycerine monocaprylate, etc.), polyethylene glycol fatty acid ester derivative (e.g., polyoxyethylene 40 monostearate, etc.), and the like may also be used.

The concentration of the compound (I) is determined from

This is the replacement sheet

Date: 22 OCT 1996

the judgement including the kind and the concentration of nonaqueous solvent and surface active agent, the composition ratio thereof, the stability after being diluted with physiological saline, etc. and storage stability. The suitable range of thus determined concentration is usually in the range of 0.1～50mg/ml and more preferably 1 ～20mg/ml.

As for the amount of surface active agent, it is noted to be less than the calculated estimation. From the experimental calculation based on the result illustrated in Table 1, about 150mg of the surface active agent may be necessary to obtain a saturated aqueous solution containing 1 mg of the compound (I) as stated above. However in the present invention the compound (I) is dissolved in a mixed solution of nonaqueous solvent - surface active agent - water to form stable supersaturation state, and therefore the necessary amount ofsurface active agent becomes less than the calculated one. These specific action, i.e., the low precipitating speed of thecrystalline from the supersaturated solution, is based on the characteristic property of the compound (I). The range of the ratio of surface active agent to the compound (I) is preferably 1～100mg/1mg, and more preferably 30～60mg/1mg to prevent the occurence of precipitation at the time of diluting for clinical use.

The pharmaceutical solution of the present invention may further contain, if necessary, other agent such as stabilizing agent, anodyne, and the like.

The pharmaceutical solution of the present invention is stable during long term storage and does not occur the precipitation of crystalline at the time of dilution. Therefore, this is applicable to various kind of medicine form such as intravenous injection, dropping lotion in the eye, dropping lotion in the nose, intraenteric injection, percutaneous liniment, local scattering agent, oral administration agent (e.g., syrup, etc.), and the like.

This is the replacement sheet

Date: 2 2 OCT 1996

Example

Following prescriptions are shown only for the purpose of the explanation of this invention.

Prescription 1

FK 506		10mg
HCO-60		400mg
Ethanol	to	1ml

The solution comprising the ingredients stated above is prepared by dissolving the FK 506 and HCO-60 in ethanol by a conventional manner.

The following solutions are also prepared a similar manner of the Prescription 1.

Prescription 2

FK 506		5mg
HCO-40		200mg
PEG 400	to	1ml

Prescription 3

FK 506		2mg
Polysorbate 80		50mg
Propylene glycol	to	1ml

Prescription 4

FK 506		2mg
Polysorbate 80		10mg
Glycerine		0.5ml
Ethanol	to	1ml

Prescription 5

FK 506		2mg
HCO-60		20mg
Propylene glycol	to	1ml

This is the replacement sheet

Date: 22 OCT 1996

Prescription 6

FK 506		1mg
Polyoxyethylene (40) mono stearate		20mg
Propylene glycol	to	1ml

Prescription 7

FK 506		10mg
HCO-60		400mg
Ethanol	to	1ml

Prescription 8

FK 506		5mg
HCO-60		400mg
Ethanol	to	1ml

Prescription 9

FK 506		25mg
HCO-60		400mg
Ethanol	to	1ml

Prescription 10

FK 506		2mg
HCO-60		10mg
Glycerine		0.5ml
Ethanol	to	1ml

Effect of the Invention

Thus obtained nonaqueous pharmaceutical solution containing the compound (I) is stable during long term storage and does not occur any precipitation at the time of diluting with physiological saline, glucose solution for injection, water, fruit juice, milk, or the like for clinical use. Accordingly, the pharmaceutical solution of the present invention is applicable to various kind of medicine form such as intravenous injection, oral adminitration agent and the like, which could contribute

This is the replacement sheet

Date: 22 OCT 1996

the compound (I) in clinical field wherein its immunosuppressive activity is intensely desired. Paticularly, the most preferable medicine form of the present nonaqueouspharmaceutical solution is the one for intravenous injection by diluting with the physiological saline.

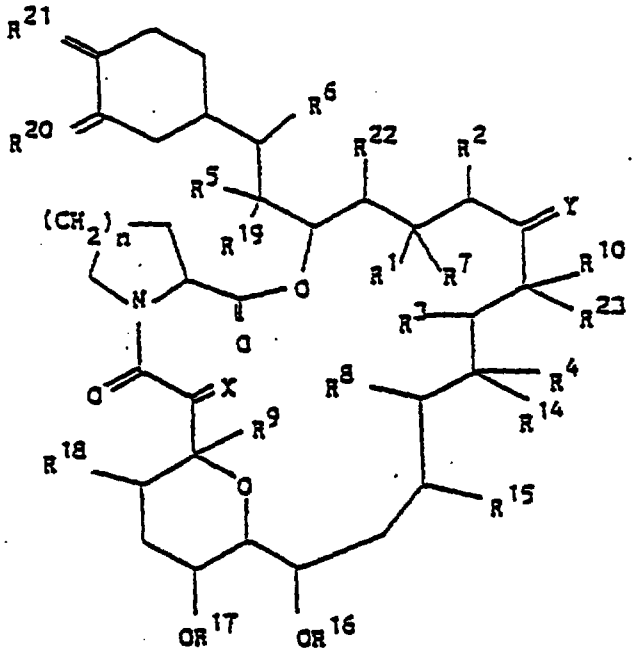
This is the replacement sheet

Date: 22 OCT 1996

This Sheet Has Been Replaced

What we claim

1. A pharmaceutical solution comprises a compound of the general formula



(I)

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached ;

in addition to its significance above, R^2 may represent an alkyl group ;

R^7 represents H, OH, protected hydroxy or O-alkyl, or in conjunction with R^1 it may represent =O ;

R^8 and R^9 independently represent H or OH ;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by = O ;

* C L M S *

This is the replacement sheet

Date: 22 OCT 1996

This Sheet Has Been Replaced

X represents O, (H,OH), (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H) or $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl ;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl ;

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively ; $R^{20}a$ and $R^{21}a$ independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$ or $R^{21}a$ is protected hydroxy ;

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atoms in an epoxide ring ;

n is 1, 2 or 3 ;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O-containing heterocyclic ring, which may be saturated or unsaturated, and which may be substistuted by one or more groups selected from alkyl, hydroxy, alkyl substituted by one or more hydroxyl groups, O-alkyl, benzyl and $-CH_2Se(C_6H_5)$.

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable surface active agent and a pharmaceutically acceptable nonaqueous solvent.

2. The pharmaceutical solution of claim 1, wherein the compound (I) or a pharmaceutically acceptable salt thereof and the pharmaceutically acceptable surface active agent are in the ratio of 1:1 to 1:100 by weight.

3. The pharmaceutical solution of claim 2, wherein the pharmaceutically acceptable surface active agent is a castor oil-surface active agent.

* C L M S *

This is the replacement sheet

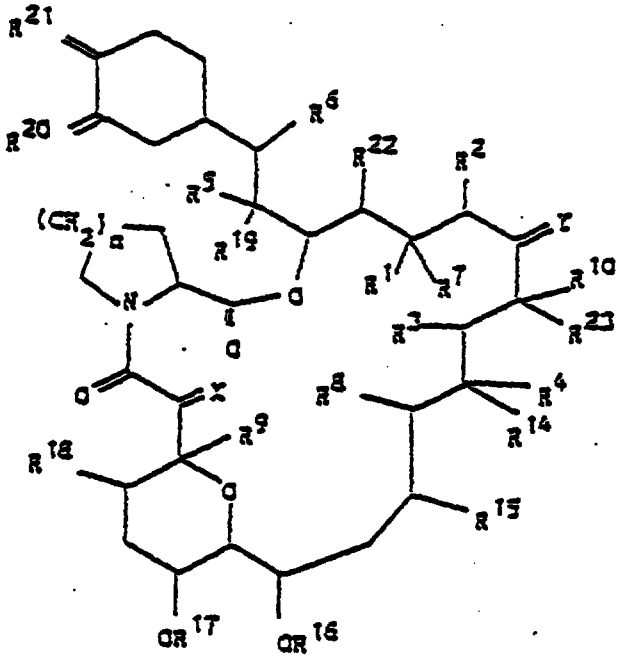
Date: 22 OCT 1996

This Sheet Has Been Replaced

4. The pharmaceutical solution of claim 3, wherein the pharmaceutically acceptable nonaqueous solvent is ethyl alcohol.

5. The pharmaceutical solution of claim 4, wherein the compound (I) is 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.

6. A process for preparing a pharmaceutical solution, which is characterizing by dissolving a compound of the formula :



(I)

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached ;

in addition to its significance above, R^1 may represent an alkyl group ;

This is the replacement sheet

Date: 22 OCT 1996

* C L M S *

This Sheet Has Been Replaced

R^7 represents H, OH, protected hydroxy or O-alkyl, or in conjunction with R^1 it may represent =O ;

R^8 and R^9 independently represent H or OH ;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by = O ;

X represents O, (H,OH), (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H) or $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl ;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl ;

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively ; $R^{20}a$ and $R^{21}a$ independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$ or $R^{21}a$ is protected hydroxy ;

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atoms in an epoxide ring ;

n is 1, 2 or 3 ;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O-containing heterocyclic ring, which may be saturated or unsaturated, and which may be substistuted by one or more groups selected from alkyl, hydroxy, alkyl substituted by one or more hydroxyl groups, O-alkyl, benzyl and $-CH_2Se(C_6H_5)$.

or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable surface active agent in a pharmaceutically acceptable nonaqueous solvent.

* C L M S *

This is the replacement sheet

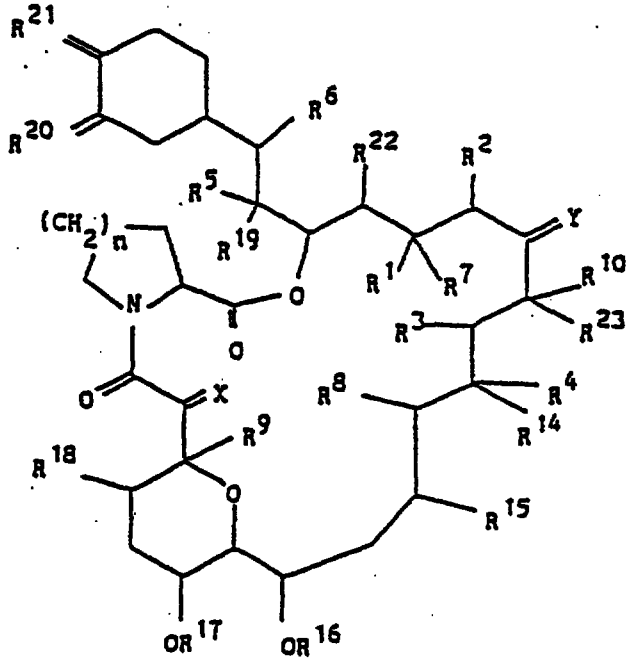
Date: 22 OCT 1996

Abstract of the Disclosure

Title of the Invention

A pharmaceutical solution

A pharmaceutical solution which contains the compound of the general formula having the immusuppreseive activity is disclosed.



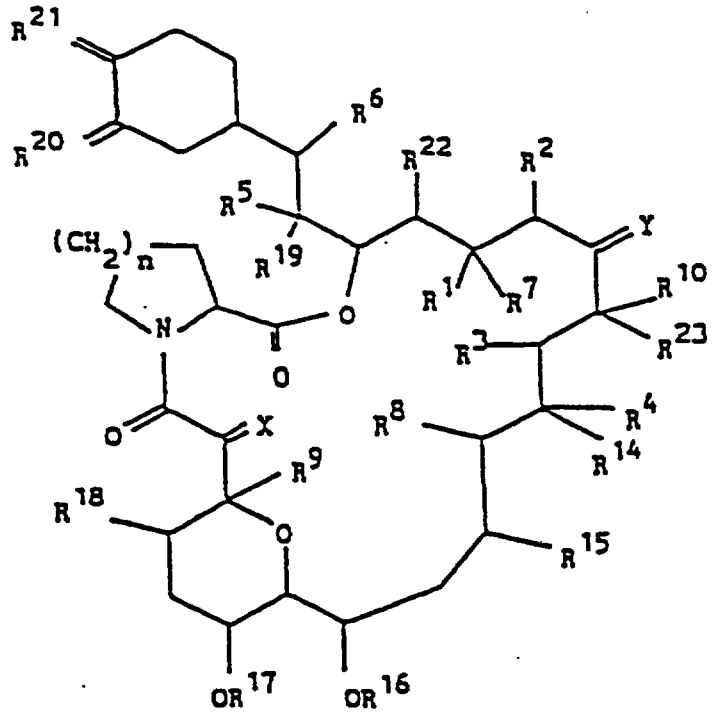
This is the replacement sheet

Date: 22 OCT 1996

REPLACEMENT SHEETS SG
☐ Description : paged ..... to paged .....
☑ Claim(s) : paged 21 to paged 27
☐ Drawing(s) : paged ..... to paged .....
☐ Abstract : paged ..... to paged .....
DATE OF RECEIPT - 8 APR 1998

What we claim :-

1. A pharmaceutical solution comprising a compound of the general formula



(I)

wherein each vicinal pair of substitutents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6], independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached;

in addition to its significance above, R^2 may represent a C_1-C_6-alkyl group;

* C L M S *

This is the replacement sheet

Date: - 8 APR 1998

R^7	represents H, OH, protected hydroxy selected from 1-(C_1-C_6-alkylthio)(C_1-C_6)alkyloxy, tri(C_1-C_6)alkylsilyloxy, C_1-C_6-alkyl-diphenyl-silyloxy and acyloxy, or O-(C_1-C_6)alkyl,
R^8 and R^9	independently represent H or OH;
R^{10}	represents H, C_1-C_6-alkyl, C_1-C_6-alkyl substituted by one or more hydroxyl groups, C_2-C_6-alkenyl, C_2-C_6-alkenyl substituted by one or more hydroxyl groups, or C_1-C_6-alkyl substituted by = O;
X	represents O, (H,OH), (H,H) or -CH_2O-;
Y	represents O, (H,OH), (H,H) or N-$NR^{11}R^{12}$ or N-OR^{13};
R^{11} and R^{12}	independently represent H, C_1-C_6-alkyl, aryl selected from phenyl, tolyl, xylyl, cumenyl, mesityl and naphthyl or tosyl;
R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23}	independently represent H or C_1-C_6-alkyl;

* C L M S *

This is the replacement sheet

Date: - 8 APR 1998

R^{20} and R^{21} independently represent O, or they may independently represent ($R^{20}a$, H) and ($R^{21}a$, H) respectively; $R^{20}a$ and $R^{21}a$ independently represent OH, O-(C_1-C_6)-alkyl or $OCH_2OCH_2CH_2OCH_3$ or R^{21} a is protected hydroxy selected from 1-(C_1-C_6-alkylthio)(C_1-C_6)alkyloxy, tri(C_1-C_6)alkylsilyloxy, C_1-C_6-alkyl-diphenyl-silyloxy and acyloxy,

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atom in an epoxide ring;

n is 1 or 2;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a heterocyclic ring selected from pyrrolyl and tetrahydrofuryl, and which may be substituted by one or more groups selected from C_1-C_6-alkyl, hydroxy, C_1-C_6-alkyl substituted by one more more hydroxyl groups, O-(C_1-C_6)alkyl, benzyl and $-CH_2Se(C_6H_5)$;

or a pharmaceutically acceptable salt thereof,

a pharmaceutically acceptable surface active agent and a pharmaceutically acceptable nonaqueous solvent.

CLMS

This is the replacement sheet

Date: - 8 APR 1998

2. The pharmaceutical solution of claim 1, wherein the compound (I) or a pharmaceutically acceptable salt thereof and the pharmaceutically acceptable surface active agent are present in a weight ratio 1:1 to 1:100.

3. The pharmaceutical solution of claim 2, wherein the pharmaceutically acceptable surface active agent is a castor oil-surface active agent.

4. The pharmaceutical solution of claim 3, wherein the pharmaceutically acceptable nonaqueous solvent is ethyl alcohol.

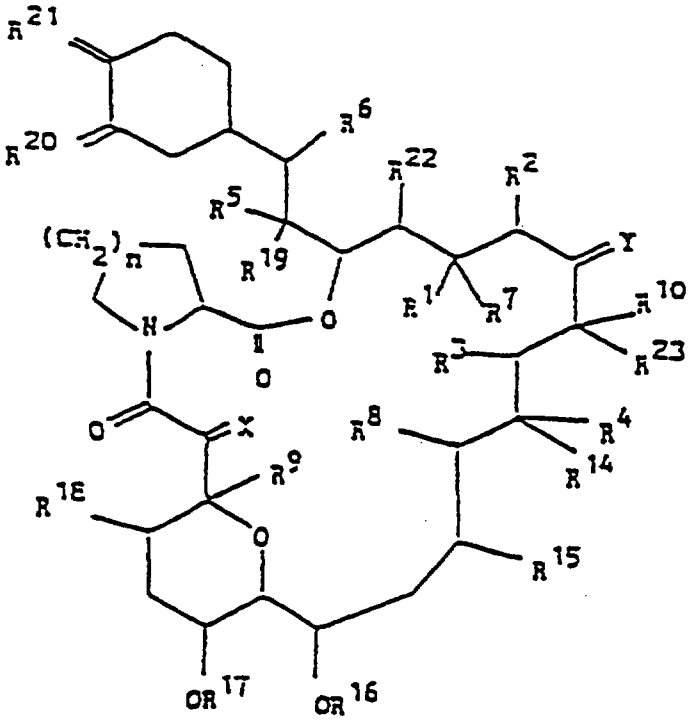
5. The pharmaceutical solution of claim 4, wherein the compound (I) is 17-allyl-1, 14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23, 25-dimethoxy-13,19,21,27-tetramethyl-11, 28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$] octacos-18-ene-2,3,10,16-tetraone.

6. A process for preparing a pharmaceutical solution, which is characterized by dissolving a compound of the formula:

* C L M S *

This is the replacement sheet

Date: - 8 APR 1998



(I)

wherein each vicinal pair of substitutents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached;

in addition to its significance above, R^2 may represent a C_1-C_6-alkyl group;

R^7 represents H, OH, protected hydroxy selected from 1-(C_1-C_6-alkylthio)(C_1-C_6)alkyloxy, tri(C_1-C_6)alkylsilyloxy, C_1-C_6-alkyl-diphenyl-silyloxy and acyloxy, or O-(C_1-C_6)alkyl,

* C L M S *

This is the replacement sheet

Date: - 8 APR 1998

R^8 and R^9	independently represent H or OH;
R^{10}	represents H, C_1-C_6-alkyl, C_1-C_6-alkyl substituted by one or more hydroxyl groups, C_2-C_6-alkenyl, C_2-C_6-alkenyl substituted by one or more hydroxyl groups, or C_1-C_6-alkyl substituted by = O;
X	represents O, (H,OH), (H,H) or $-CH_2O-$;
Y	represents O, (H,OH), (H,H) or N-$NR^{11}R^{12}$ or N-OR^{13};
R^{11} and R^{12}	independently represent H, C_1-C_6-alkyl, aryl selected from phenyl, tolyl, xylyl, cumenyl, mesityl and naphthyl or tosyl;
R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23}	independently represent H or C_1-C_6-alkyl;
R^{20} and R^{21}	independently represent O, or they may independently represent (R^{20}a, H) and (R^{21}a, H) respectively; R^{20}a and R^{21}a independently represent OH, O-(C_1-C_6)-alkyl or $OCH_2OCH_2CH_2OCH_3$ or R^{21}a is protected hydroxy selected from 1-(C_1-C_6-

* C L M S *

This is the replacement sheet

Date: - 8 APR 1998

alkylthio)(C_1-C_6)alkyloxy, tri(C_1-C_6)alkylsilyloxy, C_1-C_6-alkyl-diphenyl-silyloxy and acyloxy,

in addition, $R^{20}a$ and $R^{21}a$ may together represent an oxygen atom in an epoxide ring;

n is 1 or 2;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a heterocyclic ring selected from pyrrolyl and tetrahydrofuryl, and which may be substituted by one or more groups selected from C_1-C_6-alkyl, hydroxy, C_1-C_6-alkyl substituted by one more more hydroxyl groups, O-(C_1-C_6)alkyl, benzyl and $-CH_2Se(C_6H_5)$;

or a pharmaceutically acceptable salt thereof,

a pharmaceutically acceptable surface active agent and a pharmaceutically acceptable nonaqueous solvent.

* C L M S *

This is the replacement sheet

Date: - 8 APR 1998