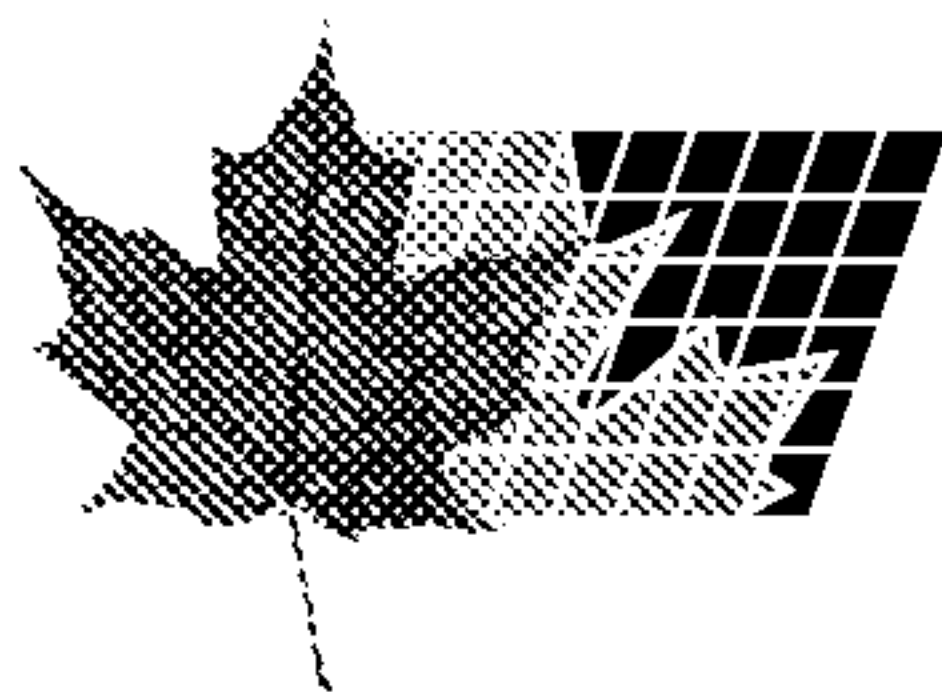




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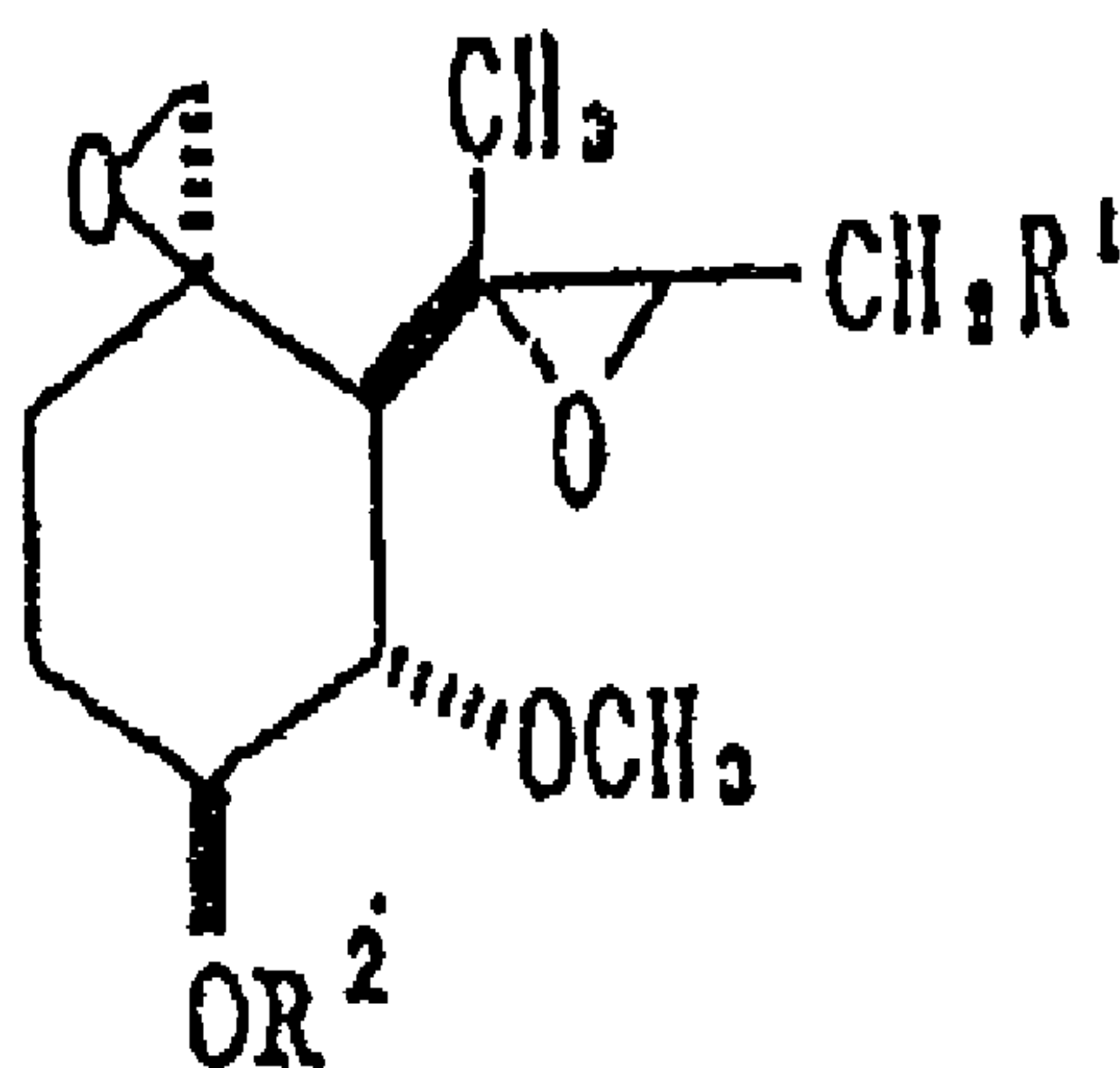
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(54) **6-EPIFUMAGILLOLS, PRODUCTION ET UTILISATION**

(54) **6-EPIFUMAGILLOLS, PRODUCTION AND USE THEREOF**

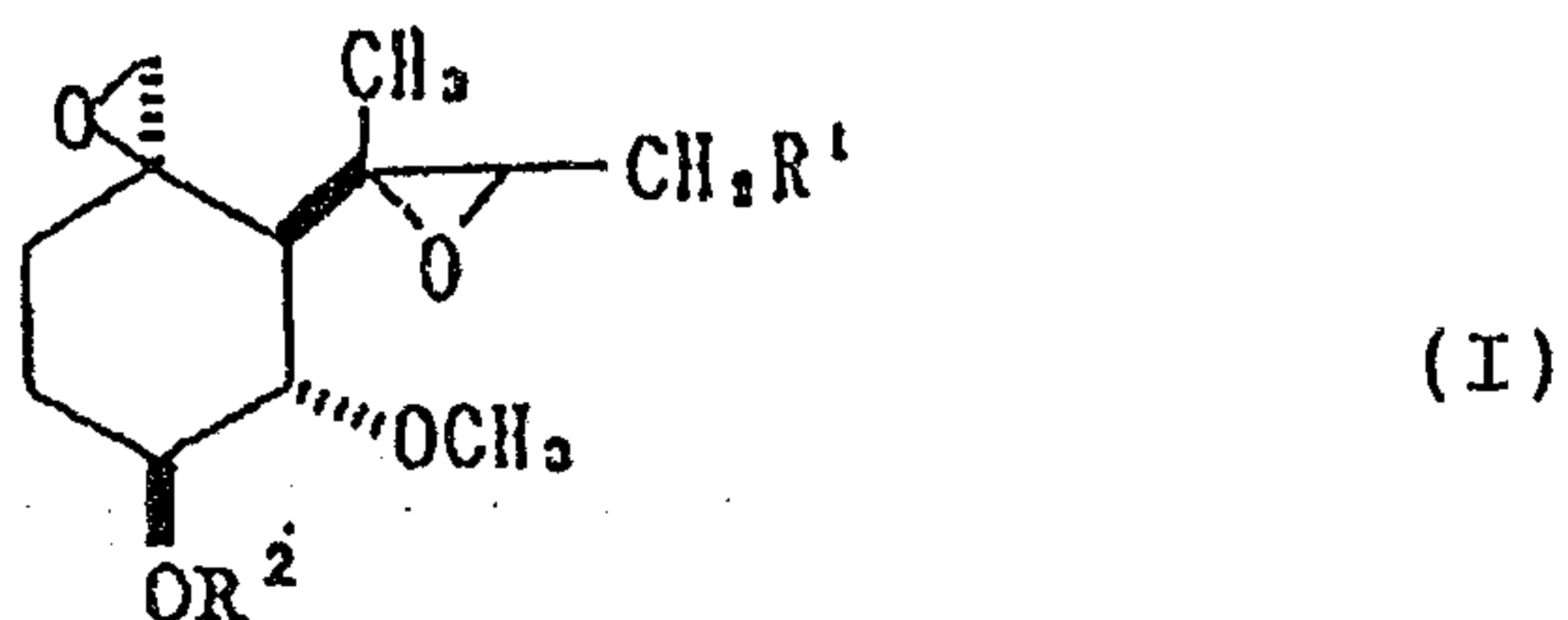


(I)

(57) The present invention relates to a compound of the formula: (see formula I) wherein R¹ is 2-methyl-1-propenyl group or isobutyl group; R² is hydrogen atom, an optionally substituted aliphatic hydrocarbon residue or an optionally substituted acyl group or a salt thereof. The compound (I) of the invention has, among others, angiogenesis inhibiting activity, cell-proliferation inhibiting activity and immune reaction inhibiting activity, thus being useful as medicines, etc.

Abstract of the Disclosure

The present invention relates to a compound of the formula:



wherein R^1 is 2-methyl-1-propenyl group or isobutyl group; R^2 is hydrogen atom, an optionally substituted aliphatic hydrocarbon residue or an optionally substituted acyl group or a salt thereof.

The compound (I) of the invention has, among others, angiogenesis inhibiting activity, cell-proliferation inhibiting activity and immune reaction inhibiting activity, thus being useful as medicines, etc.

6-Epifumagillols, Production and Use Thereof

TECHNICAL FIELD

This invention relates to novel 6-epifumagillols having an activities of inhibiting, among others, angiogenesis, cell-proliferation and immune reactions, and having therapeutic and prophylactic activities against, for example, various inflammatory diseases (rheumatic diseases, psoriasis, etc.), diabetic retinopathy, arteriosclerosis, tumors and rejection symptoms in the case of internal organ transplantation, or salts thereof, and production, and use thereof.

BACKGROUND TECHNOLOGY

Angiogenesis is deeply concerned with occurrence or pathological processes of various inflammatory diseases (rheumatic diseases, psoriasis, etc.), diabetic retinopathy, tumors, etc. Therefore, it has been considered that inhibition of angiogenesis has a connection with therapy and prophylaxis of these diseases and several research groups have searched for substances capable of inhibiting angiogenesis. For example, mention is made of research works for application of Protamine by Taylor [Taylor, S. et al., Nature, 297, 307 (1982)] and for use of heparin in the presence of cortisone by Folkman et al. [Folkman, J. et al., Science, 221, 719 (1983)]. Furthermore, patent applications have been filed directed to ascorbic acid ether and its related compounds (JP-A-131978/1983) or polysaccharide sulfate DS4152 (JP-A-119500/1988) as compounds showing activity of inhibiting angiogenesis. However, the activities of those compounds are not sufficiently satisfactory, and compounds having more excellent activity are desired to come out.

Cell-proliferation is a function indispensable for living bodies to grow and maintain their lives. In the higher animals, various tissues or organs have respectively specific proliferation mechanisms which

are controlled with various controlling mechanisms. In recent years, several dozen kinds of substances positively controlling cell-proliferation, i.e., "cell-proliferation factors", have been isolated and purified. And, it has been clarified that these factors perform an important role in ontogeny and maintenance of life. On the other hand, there are many reports disclosing that abnormal cell-proliferation, especially such proliferation as being out of the control, is related with various diseases. Tumors and arteriosclerosis, for example, are typical ones of those diseases.

And, it has been elucidated that various cell-proliferation factors participate in activation of immunocompetent cells, especially lymphocytes. Excess production or excess response of these cell-proliferation factors are considered as one of the factors of aggravating autoimmune diseases or allergic diseases. Therefore, exploitation of medicines showing actions of selectively inhibiting cell-proliferation factors, controlling responses and of immunosuppression is considered to provide effective means of prophylaxis and therapy of these diseases, and also of suppressing graft rejection in internal organ transplantation.

OBJECT OF THE INVENTION

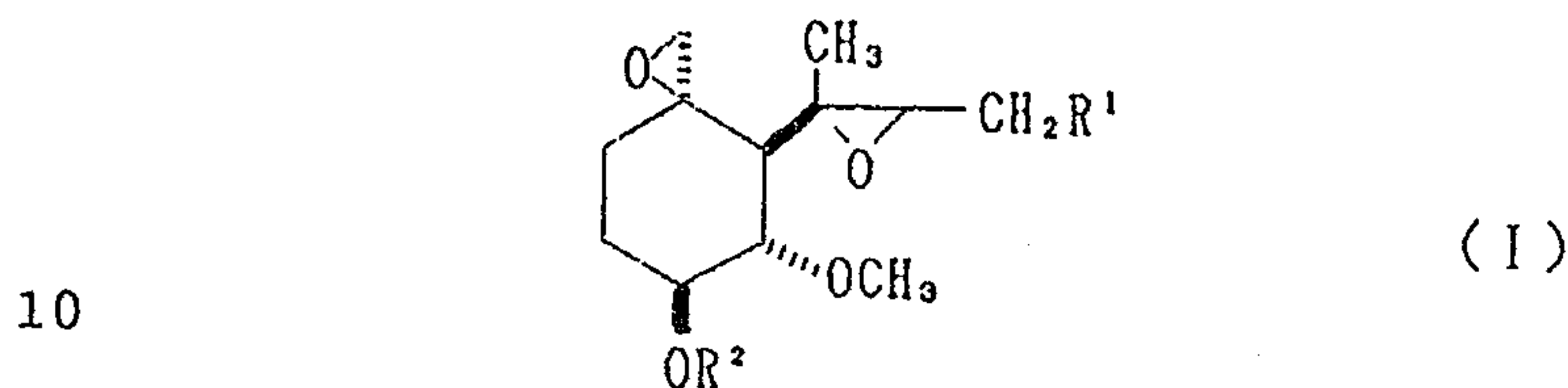
The object of this invention lies in providing novel compounds having, among others, actions of inhibiting angiogenesis, suppressing cell-proliferation and immunosuppression.

For attaining the above-mentioned object, the present inventors have conducted searches for various compounds and evaluation of them. As a result, they found that 6-epifumagillol chemically derived from fumagillin which has been known as an antibiotic agent and an antiprotozoal agent, and its related compounds have excellent actions of inhibiting angiogenesis,

suppressing cell-proliferation and immnosuppression,
thus the present invention has been accomplished.

SUMMARY OF THE INVENTION

5 The present invention relates to 6-epifumagillols
represented by the formula,



15 wherein R¹ stands for 2-methyl-1-propenyl group or
isobutyl group; R² stands for hydrogen atom, an
optionally substituted aliphatic hydrocarbon residue or
an optionally substituted acyl group, and salts
thereof.

DETAILED DESCRIPTION OF THE INVENTION

20 Examples of the aliphatic hydrocarbon residues of
the optionally substituted aliphatic hydrocarbon
residues shown by R² include straight-chained or
branched alkyl groups, alkenyl groups, alkynyl groups
or cycloaliphatic hydrocarbon residues, and, among
others, alkyl groups are especially preferable.

25 Examples of the optionally substituted acyl groups
shown by R² include acyl groups derived from carboxylic
acid or its amide groups (e.g. alkanoyl group, aroyl
group, aromatic heterocyclic carbonyl group, carbamoyl
group, alkoxy carbonyl, phenoxy carbonyl group, etc.)
30 or acyl groups derived from sulfonic acid or its amido
groups, (e.g. benzenesulfonic group, sulfamoyl group,
etc.).

35 Preferable embodiments of the above-mentioned R²
are as follows. Examples of optionally substituted
alkyl groups shown by R² include, C₁₋₂₀ straight-chain or
branched alkyl groups optionally having 1-3

substituents. These alkyl groups may be epoxidated at optional positions. Among them, preferable is C_{1-6} alkyl optionally substituted with phenyl, such as methyl, ethyl, benzyl, etc.

Examples of substituents of optionally substituted alkyl groups shown by R^2 include amino, lower alkyl amino (e.g. methylamino, ethylamino, isopropylamino, etc.), di-lower alkyl amino (e.g. dimethylamino, diethylamino, etc.), nitro, halogen (e.g. fluorine, chlorine, bromine, iodine, etc.), hydroxyl, lower alkoxy (e.g. methoxy, ethoxy, etc.), cyano, carbamoyl, carboxyl, lower alkoxy-carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, etc.), carboxy lower alkoxy (carboxymethoxy, 2-carboxyethoxy, etc.), optionally substituted phenyl, aromatic heterocyclic groups (preferably 5-6 membered aromatic heterocyclic groups containing 1-4 hetero-atoms such as nitrogen, oxygen, sulfur, etc., such as 2-furyl, 2-thienyl, 4-thiazolyl, 4-imidazolyl, 4-pyridyl, etc.).

Examples of the optionally substituted alkenyl groups, alkynyl groups and cycloaliphatic hydrocarbon residues are C_{2-20} alkenyl groups, C_{2-20} alkynyl groups and C_{3-8} cycloalkyl groups.

Examples of the optionally substituted alkanoyl groups shown by R^2 include alkanoyl groups optionally substituted with 1 to 3 substituents similar to those of the above-mentioned optionally substituted alkyl groups (preferably C_{1-20} unsubstituted alkanoyl groups, e.g. formyl, acetyl, propionyl, isopropionyl, butyryl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, lauroyl, undecanoyl, myristoyl, palmitoyl, stearoyl, arachinoyl, etc.). Among them, acetyl, butyryl, octanoyl, 3-carboxylpropionyl

and 4-carboxybutyryl are preferable.

Examples of the optionally substituted aroyl groups shown by R^2 include benzoyl, 1-naphthoyl and 2-naphthoyl which may be substituted with C_{2-6} lower alkyl such as ethyl, propyl, etc., amino, halogen (e.g. fluorine, chlorine, bromine, etc.), hydroxyl, lower alkoxy (e.g. methoxy, ethoxy, etc.), cyano, carbamoyl, carboxyl, etc., preferably benzoyl optionally having 1 to 3 substituents, 1-naphthoyl, 2-naphthoyl, etc. Among them, benzoyl and 2-carboxybenzoyl are preferable.

10 Examples of the substituents in the optionally substituted aromatic heterocyclic carbonyl groups shown by R^2 include the same substituents as those of the above-mentioned substituted aroyl group. As the aromatic heterocyclic carbonyl groups, use is made of 5- or 6-membered ones containing 1 to 4 hetero atoms such as nitrogen, oxygen, sulfur, etc., and, among them, 2-furoyl, 2-thenoyl, nicotinoyl, isonicotinoyl, imidazole-1-carbonyl, etc. are preferable.

20 The optionally substituted carbamoyl groups shown by R^2 include carbamoyl group, mono-substituted carbamoyl group and di-substituted carbamoyl group, and substituents of them are exemplified by lower alkyl (e.g. methyl, ethyl, propyl, butyl, etc.) which may further be substituted by for example mono- or di-lower alkylamino, lower alkanoyl (preferably C_{1-6} , e.g. formyl acetyl, propionyl, etc.), chloroacetyl, dichloroacetyl, trichloroacetyl, lower alkoxy carbonyl methyl (e.g. methoxy carbonyl methyl, ethoxy carbonyl methyl, etc.), carboxy methyl, optionally substituted phenyl, naphthyl, benzoyl, and substituents forming

cyclic amino group (e.g. pyrrolidino, piperidino, morpholino, piperazino, 4-methylpiperazino, 4-phenylpiperazino, etc.), taken together with the nitrogen atom of the carbamoyl group. The cyclic amino groups may optionally be substituted with up to three substituents such as lower alkyl, phenyl and oxo. Examples of the substituted cyclic amino group are 4-methylpiperazino, 4-phenylpiperazino and 4-ethyl-2,3-dioxopiperazino. Among the substituents of the carbamoyl groups chloroacetyl, phenyl and benzoyl are preferable.

10

As the optionally substituted alkoxycarbonyl groups shown by R^2 , mention is made of, for example, straight-chain or branched lower alkoxycarbonyl groups which may have 1 to 3 substituents which are the same as those of the above-mentioned optionally substituted alkanoyl groups. Among them, are preferable methoxy carbonyl, ethoxy carbonyl, propoxy carbonyl, butoxy carbonyl, isobutoxy carbonyl and 1-chloroethoxy carbonyl.

Examples of substituents of the optionally

substituted benzenesulfonyl group shown by R^2 include lower alkyl (e.g. methyl, ethyl, etc.), halogen (fluorine, chlorine, bromine, etc.), and one to three of these substituents may be located at optional positions of the benzene ring.

Examples of the substituents of optionally substituted phenoxycarbonyl groups shown by R^2 include the same substituents of the above-mentioned optionally substituted benzenesulfonyl groups, and one to three of these substituents may be substituted at optionally positions of phenoxy group.

Examples of optionally substituted alkylsulfonyl groups shown by R^2 include C_{1-6} lower alkyl sulfonyl groups optionally having one to three of the same substituents as those of the above-mentioned optionally substituted alkanoyl groups. Among them, methyl sulfonyl and ethyl sulfonyl are preferable.

Examples of the optionally substituted sulfamoyl groups shown by R^2 include a lower alkyl (e.g. methyl, ethyl, propyl, isopropyl, isobutyl, etc.) and optionally substituted phenyl, and these substituents may be one or two which may be the same as or different from each other.

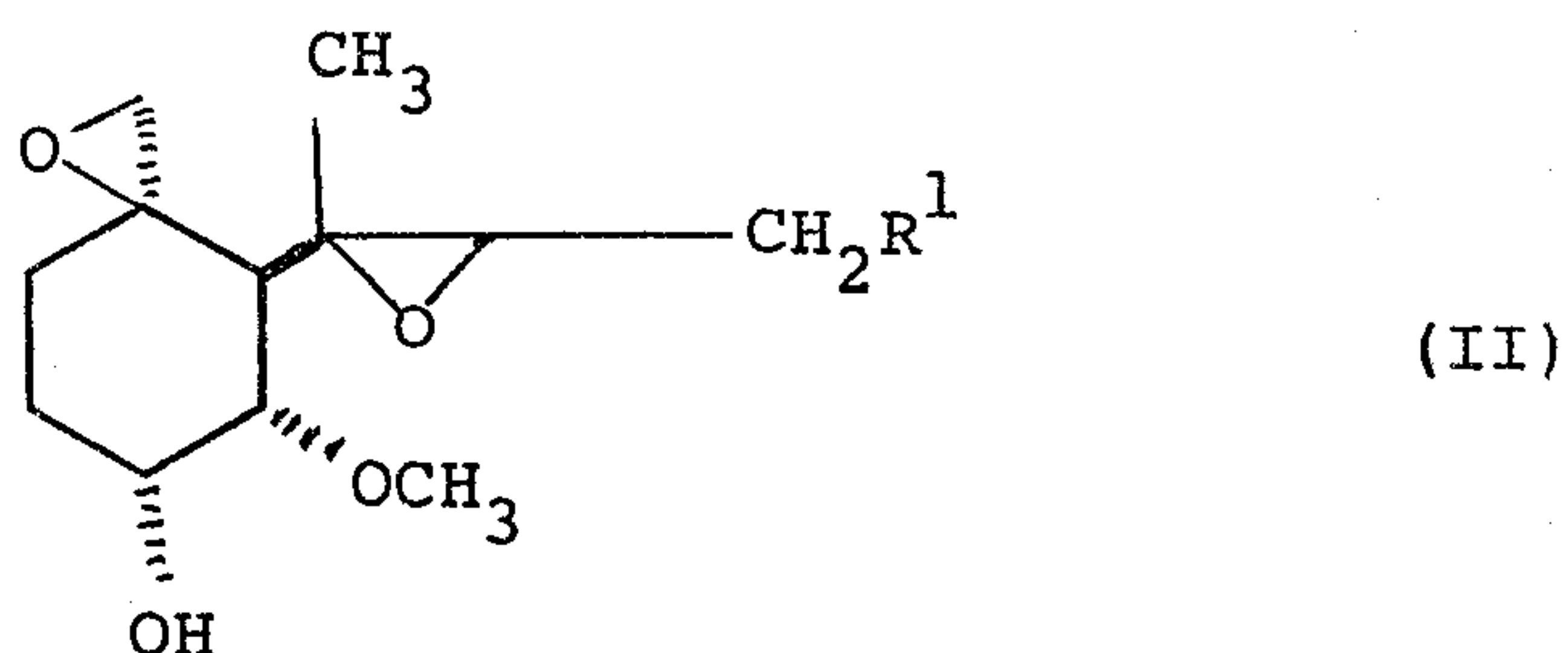
In the present specification, examples of substituents of optionally substituted phenyl groups include lower alkyl (e.g. methyl, ethyl, propyl, butyl, etc.), lower alkoxy (e.g. methoxy, ethoxy, propoxy, etc.), halogen (e.g. fluorine, chlorine, bromine, etc.), halogenated alkyl (e.g. trifluoromethyl, chloromethyl, etc.), nitro, etc., and one to five of these substituents may be substituted at optional positions of the phenyl ring.

And, in the present specification, unless otherwise specified, the lower alkyl group means C_{1-6} straight-chain or branched alkyl groups, and the lower alkoxy group means C_{1-6} alkoxy groups.

When the compound (I) of this invention has in its molecule an acidic substituent (e.g. carboxyl or the like) or a basic substituent (e.g. amino, lower alkylamino, di-lower alkylamino or the like), it can be used as a pharmacologically acceptable salt. As the pharmacologically acceptable salts, use is made of salts with inorganic bases, salts with organic bases, salts with basic or acid amino acid, or the like. As inorganic bases capable of forming these salts, use is made of, for example, an alkali metal (e.g. sodium, potassium, etc.), an alkaline earth metal (e.g. calcium, magnesium, etc.), etc.; as organic bases, use is made of trimethylamine, triethylamine, pyridine, picoline, N,N-dibenzylethylenediamine, ethanolamine, diethanolamine, tris-hydroxymethylaminomethane, dicyclohexylamine, etc.; as inorganic acids, use is made of, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, etc.; as organic acids, use is made of, for example, formic acid, acetic acid, trifluoroacetic acid, oxalic acid, tartaric acid, fumaric acid, maleic acid, methanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, etc.; and. as basic or acid amino acids, use is made of, for example, arginine, lysine, ornithine, aspartic acid, glutamic acid, etc. Of these salts, those with bases (i.e. salts with inorganic bases, salts with organic bases, salts with basic amino acids) mean salts which can be formed with the carboxyl group in the substituents of compound (I), and, salts with acids (i.e. salts with inorganic acids, salts with organic acids, salts with acid amino acids) mean salts which can be formed with the amino group, lower alkylamino group, di-lower alkylamino group, etc. in the substituents of the compound (I).

Another aspect of the invention provides a process of producing the compound of the formula (I) defined above, which comprises:

epimerizing the hydroxyl group in a compound of the formula:



(wherein R^1 is as defined above), thereby obtaining a compound (I) in which R^2 is hydrogen and, where required, treating the resulting compound with a reagent to introduce the aliphatic hydrocarbon residue or the acyl, thereby obtaining a compound (I) in which R^2 is other than hydrogen.

A compound of the formula (I), wherein R^1 is 2-methyl-1-propenyl group, and R^2 is hydrogen atom, i.e.

6-epifumagillol is the compound derived from fumagillol which is the hydrolysate of fumagillin produced by a microorganism [Tarbell, D. S. et al., Journal of American Chemical Society, 83, 3096 (1961)] by
5 subjecting to Mitsunobu reaction using diethyl azocarboxylate, triphenyl phosphine and a carboxylic acid such as formic acid or benzoic acid [Mitsunobu, O., Synthesis, 1981, p.1], followed by hydrolysis.

Production of the compound (I), wherein R^1 is
10 isobutyl group, R^2 is hydrogen atom, i.e. 6-epidihydrofumagillol can be accomplished by subjecting 6-epifumagillol to catalytic reduction under usual conditions (e.g. using 5% palladium-carbon in a methanol solution). This compound can also be obtained
15 by subjecting fumagillol to catalytic reduction, followed by subjecting to Mitsunobu reaction and hydrolysis.

The compound (I), wherein R^2 is a substituent other than hydrogen atom, can be produced by subjecting 6-
20 epifumagillol or 6-epidihydrofumagillol to alkylation or acylation (e.g. carbamoylation, sulfonylation) by, for example, the method described below, or by isolating the intermediates in those reactions. And, when R^2 is a group which does not change by catalytic
25 reduction, 6-O-substituted-6-epifumagillol is subjected to catalytic reduction to convert into 6-O-substituted-6-epidihydrofumagillol. When the alkylating agent, acylating agent (carbamoylating agent, sulfonylating agent, etc.) have a substituent such as amino,
30 hydroxyl, carboxyl etc., these substituents are preferably protected, and the protecting groups are selected in accordance with the stability of the product. Preferable examples of the protecting groups are, in the case of amino, 4-nitrobenzyloxycarbonyl, 2-trimethylsilylethoxycarbonyl, etc., and in case of
35 hydroxyl, are 4-nitrobenzyl, t-butyl dimethylsilyl,

etc., and, in case of carboxyl, are 4-nitrobenzyl, etc.
For deprotection, a conventional means such as
catalytic reduction or use of fluoride ion is employed.
Additionally stating, in cases of carbamoylation and
5 alkylation, it is possible that a lower alkyl such as
methyl, ethyl, etc. is used as the protecting group of
the carboxyl group, then, after the reaction, the
protecting group is removed by hydrolysis under mild
alkaline conditions.

10 1) Acylation by carboxylic acid

This acylation is conducted by allowing a reactive
derivative of activated carboxylic acid such as acid
anhydride or acid halide (acid chloride, acid bromide,
etc.) to react with 6-epifumagillol or 6-
15 epidihydrofumagillol (hereinafter referred to simply as
starting alcohol).

Namely, the acylation is usually conducted by such
reaction as shown by the following scheme:

Reactive derivative of R^3OH + starting alcohol-
20 Compound(I)



(wherein R^3 stands for optionally substituted alkanoyl
group, optionally substituted aroyl group and
optionally substituted aromatic heterocyclic carbonyl
25 group, etc. defined for R^2).

Said reactive derivative of carboxylic acid is
used usually in an amount of about 1 to 10 times mol.,
preferably 1 to 5 times mol., relative to 1 mol. of the
starting alcohol.

30 This reaction is carried out usually in the
presence of a base. Examples of the base include
tertiary amine such as diisopropylethylamine,
triethylamine, pyridine, N,N-dimethylaminopyridine,
etc., alkali metal hydrogencarbonates such as sodium
35 hydrogencarbonate, potassium hydrogencarbonate, etc.,
alkali metal carbonates such as potassium carbonate,

5 sodium carbonate, etc., alkali metal hydrides such as sodium hydride, potassium hydride, etc., organic metals such as butyl lithium, lithium diisopropylamide, etc., and the amount of the base to be added usually ranges from about 1 mol. to 10 times mol. relative to 1 mol. of the starting alcohol.

10 This reaction is conducted usually in an organic solvent which does not exert undesirable effects on the reaction. Examples of the organic solvent which does not exert undesirable effects on the reaction include amides such as dimethylformamide, dimethylacetamide, etc., halogenated hydrocarbons such as dichloromethane, chloroform, 1,2-dichloroethane, etc., ethers such as diethylether, tetrahydrofuran, dioxane, etc., esters
15 such as methyl acetate, ethyl acetate, isobutyl acetate, methyl propionate, etc., nitriles such as acetonitrile, propionitrile, etc., nitro compounds such as nitromethane, nitroethane, etc., ketones such as acetone, methyl ethyl ketone, etc., aromatic
20 hydrocarbons such as benzene, toluene, etc., and these may be used singly or as a mixture of two or more species in a suitable ratio. And, the tertiary amine employed as the base may be used as the solvent simultaneously.

25 The reaction temperature varies with the amounts, kinds, etc. of carboxylic derivatives, bases and solvents, and ranges from -80 C to 100 C, preferably from 0 C to room temperatures (in this specification, room temperatures mean temperatures ranging from about
30 20 to about 35 C, unless otherwise specified). The reaction time ranges from about 30 minutes to about 5 days.

2) Alkylation

35 This alkylation is conducted by allowing a starting alcohol to react with an alkylating agent, for

example, alkyl halide represented by the formula R^4Y
[wherein R^4 stands for, among the definition of R^2 , an
optionally substituted alkyl groups, and Y stands for a
leaving group (e.g. halogen (chlorine, bromine, iodine,
etc.))], alkyl sulfate (e.g. methyl sulfate, ethyl
sulfate, etc.). This alkylating agent is used in an
amount of usually about 1 to 5 times mol. relative to
the starting alcohol.

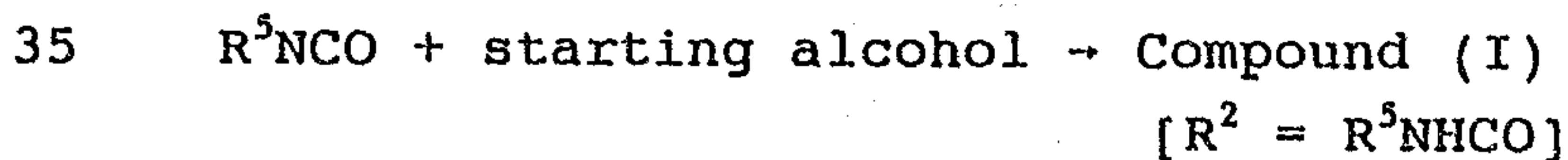
This reaction is conducted usually in the presence
of a base. As the base, use is made of afore-mentioned
alkali metal hydrogencarbonates, alkali metal
carbonates, alkali metal hydrides, organic metals,
etc., and the amount to be added ranges from about 1 to
5 times mol. relative to the starting alcohol.

This reaction is carried out usually in an organic
solvent which does not exert undesirable influence on
the reaction. Examples of such organic solvents as
above include afore-mentioned amides, halogenated
hydrocarbons, ethers, esters, nitriles, nitro-
compounds, ketones and aromatic hydrocarbons, and these
solvents can be used singly or as a mixture of two or
more species of them in a suitable ratio.

The reaction temperature varies with the amounts,
kinds etc., of alkylating agents, bases and solvents,
and it ranges from -80 to 100 C, preferably from 0 C to
room temperatures. The reaction time ranges from about
20 minutes to about 5 days.

3) Carbamoylation

Carbamoylation for introducing a mono-substituted
carbamoyl group is carried out by usually allowing
isocyanate to react with the starting amine, as, for
example, shown by the following reaction scheme.



(wherein R^5 stands for a substituent of the optionally substituted carbamoyl group shown by R^2 such as lower alkyl, lower alkanoyl chloroacetyl, etc.). The
5 isocyanate is used in an amount of usually about 1 mol to 5 times mol. relative to 1 mol. of the starting alcohol.

This reaction is carried out usually in the presence of a base. As the base, use is made of above-
10 mentioned tertiary amine, alkali metal hydrogencarbonates, alkali metal carbonates, alkali metal hydrides, organic metals, etc., and the amount of such a base as above to be added ranges from about 1 mol. to 5 times mol. relative to the starting alcohol.

15 This reaction is carried out usually in an organic solvent which does not exert undesirable influence on the reaction. Examples of such organic solvents as above include afore-mentioned amides, halogenated hydrocarbons, ethers, esters, nitriles, nitro-
20 compounds, ketones and aromatic hydrocarbons, and these solvents can be used singly or as a mixture of two or more species of them in a suitable ratio. The tertiary amine employed as the base may be used as the solvent simultaneously.

25 The reaction temperature varies with the amounts and kinds of isocyanate, the base and the solvent then employed, and usually ranges from about -80°C to 100°C , preferably from 0°C to room temperatures. The reaction time ranges from about one hour to about five days.

30 Among the compounds having mono-substituted carbamoyl group thus obtained, compounds having, for example, chloroacetylcarbamoyl, trichloroacetylcarbamoyl, etc., can be converted to compounds having carbamoyl group by removing
35 chloroacetyl group or trichloroacetyl group by a conventional process (e.g. at room temperatures or an

elevated temperatures under basic conditions).

The said carbamoylation can also be conducted by allowing the starting alcohol to react with carbamoyl halide.

5 The said carbamoyl halide is used in an amount of usually 1 mol. to 5 times mol. relative to 1 mol. of the starting alcohol.

10 This reaction is carried out usually in the presence of a base. As the base, use is made of the above-mentioned tertiary amine, alkali metal hydrogencarboantes, alkali metal carbonates, alkali metal hydrides, organic alkali metals, etc., and the amount of the base to be added ranges from about 1 mol. to 5 times mol. relative to the starting alcohol.

15 This reaction is carried out usually in an organic solvent which does not exert undesirable influence on the reaction. Examples of such organic solvents as above include afore-mentioned amides, halogenated hydrocarbons, ethers, esters, nitriles, nitro-
20 compounds, ketones and aromatic hydrocarbons, and these solvents can be used singly or as a mixture of two or more species of them in a suitable ratio. The tertiary amine employed as the base may be used as the solvent simultaneously.

25 The reaction temperature varies with the amounts and kinds of carbamoyl halide, bases and solvents, and it ranges from about 0°C to around reflux temperatures of the reaction medium, preferably from about 25°C to reflux temperature.

30 The said carbamoylation can also be carried out by allowing the starting alcohol to react with chloroformic ester (e.g. phenyl chloroformate, ethyl chloroformate, isobutyl chloroformate, chloroformic acid 1-chloro-ethyl, etc.) or 1,1'-carbonyl diimidazole
35 to give an active ester, followed by allowing the ester to react with primary or secondary amine. The said

chloroformic esters or 1,1'-carbonyl diimidazole and amines are used in an amount usually ranging from 1 mol. to 5 times mol. relative to one mol. of the starting alcohol.

5 In this reaction, the reaction between the starting alcohol and chloroformic ester is carried out in the presence of a base. As the said base, use is made of the above-mentioned tertiary amine, alkali metal hydrogencarbonates, alkali metal carbonates,
10 alkali metal hydrides, organic alkali metals, etc. The amount of the base to be added ranges usually from about 1 mol. to 5 times mol. relative to 1 mol. of the starting alcohol.

15 This reaction is carried out usually in an organic solvent which does not exert undesirable influence on the reaction. Examples of such organic solvents as above include afore-mentioned amides, halogenated hydrocarbons, ethers, esters, nitriles, nitro-
20 compounds, ketones and aromatic hydrocarbons, and these solvents can be used singly or as a mixture of two or more species of them in a suitable ratio. The reaction temperature varies with the amounts and kinds of the chloroformic esters, bases, amines and solvents, and it ranges from -20°C to the reflux temperature of the
25 reaction medium, preferably from 0°C to 50°C. Incidentally, the active esters obtained as intermediates are also included in the compounds (I) which are the object compounds of the present application.

30

4) Sulfonylation

35 The sulfonylation is conducted by allowing an activated sulfonic acid derivative, for example, sulfonic anhydride, sulfonic halide (e.g. sulfonyl chloride, sulfonyl bromide, etc.) or sulfamoyl halide

(e.g. sulfamoyl chloride, sulfamoyl bromide, etc.) to react with the starting alcohol.

More specifically, the reaction is performed as shown by the following scheme.

5

Reactive derivative of R^6OH + starting alcohol $\rightarrow$
Compound (I)
[$R^2 = R^6$]

10

[wherein R^6 stands for an optionally substituted benzene sulfonyl group, among the definition of R^2 , or an optionally substituted alkyl sulfonyl group, or an optionally substituted sulfamoyl group].

15

The reactive derivative of the sulfonic acid is, generally, used in an amount of about 1 to 5 times mol. relative to 1 mol. of the starting alcohol.

20

This reaction is usually conducted in the presence of a base. As the base, use is made of the aforementioned tertiary amine, alkali metal hydrogencarbonates, alkali metal carbonates, alkali metal hydrides, organic metals, etc., and the amount thereof to be added is, generally, about 1 to 10 times mol. relative to 1 mol. of the starting alcohol.

25

This reaction is conducted usually in an organic solvent which does not exert an undesirable effect on the reaction. Examples of organic solvents exerting no undesirable effect on the reaction include the aforementioned amides, halogenated hydrocarbons, ethers, esters, nitriles, nitro compounds, ketones, and aromatic hydrocarbons, and these can be employed singly or as a mixture of two or more species of them in a suitable ratio. And, the tertiary amine employed as the base can be used also as the solvent.

30

35

The reaction temperature varies with amounts and kinds of the sulfonic acid or sulfamic acid derivatives, bases and solvents then employed, but it

usually ranges from -80°C to 100°C , preferably from 0°C to room temperatures. The reaction time ranges from ten minutes to about 5 days.

Thus-produced 6-epifumagillol and related
5 compounds (I) can be isolated by per se known separating and refining means (e.g. chromatography, crystallization), etc.

The compound (I) has asymmetric center in the molecule and is possessed of optical activity, and its
10 absolute structure is based on the starting fumagillol, which means that it is in agreement with the absolute structure of fumagillol.

The compounds of this invention show actions of, among others, inhibiting angiogenesis, cell-
15 proliferation and immune reactions, and are useful as therapeutic and prophylactic agents of various inflammatory diseases (rheumatic diseases, psoriasis), diabetic retinopathy, arteriosclerosis, tumors and rejection symptoms in the case of internal organ
20 transplantation. And, they can be safely administered orally or non-orally as they are or a pharmaceutical composition prepared by mixing with per se known pharmaceutically acceptable carriers, excipients, etc. [e.g. tablets, capsules (including soft capsules,
25 microcapsules), liquids, injections, suppositories]. The dosage varies with, among others, subjects, routes and symptoms, but, usually, it ranges, in adults, from about 0.1 mg/kg to about 40 mg/kg body weight, preferably from about 0.5 mg/kg to about 20 mg/kg body
30 weight per day.

Experimental Example 1

The object compounds (I) obtained in the Examples given below were evaluated for angiogenesis inhibitory
35 activity by the rat cornea micropocket method. The data obtained are summarized in Table 1.

Method of Evaluation :

Essentially the same method of Gimbrone et al. [J. National Cancer Institute, 52, 413-419 (1974)] was followed. Thus, adult male Sprague-Dawley rats (11 to 16 week old) were anesthetized with nembutal and locally anesthetized by instillation of xylocaine eyedrops onto the eyeball. The cornea was incised to a length of about 2 mm at about 2 mm inside from the corneal circumference by means of an injection needle, and a basic fibroblast growth factor (bFGF; bovine brain-derived, purified product; R & D Inc.) and a sustained release pellet containing the test sample were inserted side by side into the incision so that the bFGF pellet was located on the central side in the cornea. In the control group, the bFGF pellet and a sample-free pellet were inserted into the cornea. After 10 days, the cornea was observed under a stereoscopic microscope. When the sample administration resulted in retardation or reduction of bFGF-induced angiogenesis, the sample was judged to have inhibitory activity.

The sustained release pellets were prepared in the following manner. An ethylene-vinyl acetate copolymer (Takeda Chemical Industries, Ltd.) was dissolved in dichloromethane to a concentration of 8%. A 3 μ l portion of the solution was air-dried on a glass dish, an aqueous solution of bFGF (250 ng) was then placed thereon and air-dried and, finally 3 μ l of the above ethylene-vinyl acetate copolymer solution was placed further thereon and air-dried to give a sandwich sheet. This sandwich sheet was made round into a bFGF pellet. The test sample pellets were prepared by dissolving each sample in ethanol in a concentration of 20 ug/2 μ l, mixing the solution with 6 μ l of an ethylene-vinyl acetate copolymer solution, air-drying the mixed solution in a glass dish and making the thus-obtained

sheet round.

Table 1 Angiogenesis inhibitory activity

5	Example No.	Inhibitory Rate	Judgment
	1	3/7	±
	2	3/7	±
	5	4/8	±
	6	6/6	+
10	7	5/6	+
	8	5/7	+
	12	3/4	+

15 In the Table 1 above, the inhibitory rate means the number of rats on which angiogenesis inhibitory activity was observed relative to the number of rats tested.

20 Experimental Example 2: Evaluation of inhibition of human umbilical vein endothelial cell growth

Human umbilical vein endothelial cells were isolated by perfusion of an umbilical vein with a trypsin-containing medium. The cells were cultured in sequence in GIT medium (Daigo Eiyo Kagaku) supplemented with 2.5% fetal bovine serum and 2.0 ng/ml or recombinant human fibroblast growth factor (hereinafter simply referred to rFGF, prepared at Biotechnology Research Laboratories, Takeda Chemical Industries, Ltd.).

30 A suspension of human vein endothelial cells at the cell density of 2×10^3 (100 μ l) was seeded on 96-well incubation plate (Nunc, 1-67008), and incubation was conducted in a gas-controlled thermostat vessel.

35 The following day, 100 μ l of medium containing rFGF (2 ng/ml at the final concentration) and samples of

various concentrations were added. The samples were dissolved in dimethylsulfoxide (DMSO) and then diluted with culture medium so that the final DMSO concentration does not exceed 0.25%. After 5-day culture, the culture solution containing samples was removed by suction, 100 μ l of 1 mg/ml of MTT solution [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide was dissolved in the culture solution] was added and kept warming for 4 hours. Then, 100 μ l of a 10% SDS solution (aqueous solution of sodium dodecyl sulfate) was added, and the mixture was kept warming for 5-6 hours. The cells and MTT pigment were solubilized, and the OD₅₉₀ value was measured using a spectrophotometer. The OD value of the control group to which no test sample was added was set as 100%, and the activity of each test sample for inhibiting endothelial cell growth was shown in Table 2 by the concentration of the test compound giving 50% OD value, i.e. IC₅₀ value.

Table 2 Activity of inhibiting endothelial cell growth

	Example No.	IC ₅₀ (ng/ml)
5	1	<0.08
	2	<0.08
	3	9.82
	5	1.91
	6	2.05
10	7	8.71
	8	0.166
	9	0.1
	11	4.68
	12	6.5

15

Experimental Example 3:

Immunosuppressive effects derivatives

20

in vitro experiment

The effects of compound 5 (Example 5) and compound 8 (Example 8) on the ³H-thymidine uptake induced by the mixed lymphocyte reaction of spleen cells from BALB/c and C57BL/6 mice were examined. Compounds 5 and 8 showed 50% inhibition of ³H-thymidine uptake at concentrations of 0.3 and 0.01μM, respectively.

25

The effects of compounds 5 and 8 on the antibody production induced by pokeweed mitogen, a T cell dependent B cell mitogen, and Epstein Barr virus, a T cell independent B cell mitogen, were examined.

30

Compounds 5 and 8 showed 50% suppression of T cell dependent immunoglobulin production at concentrations of 7 and 4μM, respectively. Also, compounds 5 and 8 showed 50% suppression of T cell independent antibody production at as low concentrations as 0.03 and 0.001μM, respectively.

35

in vivo experiment

BALB/c mice were immunized with 50µg of bovine gamma globulin mixed with Freund's complete adjuvant, and then 20 mg/kg of compounds 5 and 8, respectively, were intraperitoneally administered to the mice every 2 days after the immunization. Anti-bovine gamma globulin antibody titer was assayed in sera obtained 3 weeks after the immunization. The administration of compounds 5 and 8 induced significant suppression of anti-bovine gamma globulin antibody titer.

BALB/c mice were immunized with 10% sheep red blood cells, and then 100 mg/kg of compounds 5 and 8, respectively, were intraperitoneally administered to the mice once a day for 4 days after the immunization. The administrations of compounds 5 and 8 suppressed the formation of germinal center and the induction of plasma cells in the spleen on day 6 after the immunization. Such an obvious change was not observed in the cyclosporin A treated group.

Furthermore, the reduction of spleen weight was observed in the compounds 5 and 8 treated groups, but in the cyclosporin a treated group only the reduction of thymus weight was observed.

Table 3

Method	Compound		
	Compound 5	Compound 8	Cyclosporin A
5			
in vitro ¹⁾			
Mixed lymphocyte reaction			
	0.3 μ M	0.4 μ M	0.04 μ M
10	7.0 μ M	4.0 μ M	0.004 μ M
Ab production			
T independent			
	0.03 μ M	0.001 μ M	>10 μ M
Ab production			
in vivo ²⁾			
15	13%	9%	9%
Anti-bovine gamma globulin Ab titer			
Histological study in the spleen			
Inhibition of germinal center formation			
	+	+	-
20	+	+	-
Inhibition of plasma cell induction			
Thymus weight			
	105%	116%	46%
Spleen weight			
	57%	60%	101%
25	1) The concentrations which showed 50% inhibitions were represented.		
30	2) Anti-bovine gamma globulin antibody titer and the weights of thymus and spleen were represented as percentages against those of a reference group which was injected with saline.		

Examples

By the following examples, the present invention will be described in more detail, but the present invention is by no means limited to these examples.

The elution in the column chromatography in the following examples (bracketed terms are solvents used

for elution) is conducted under observation by means of thin layer chromatography (TLC). In the TLC observation, as the TLC plate, Kieselgel* 60F₂₅₀ (70 to 230 mesh, Merck) was employed, as the method of detection, a UV detector, a color-development method with phosphorus molybdate, etc. were employed. As the silica gel for the column, Kieselgel* 60 (70 to 230 mesh, Merck) was employed. NMR spectrum shows proton NMR(¹H-NMR), and, as interior or exterior standard, tetramethylsilane was employed, and the measurement was carried out by using Gemini* 200 (VARIAN) showing the δ value in terms of ppm.

Abbreviations used in examples have the following significances.

s : singlet, br : broad, d : doublet, dd : double doublet, ddd : doublet doublet doublet, t : triplet, q : quartet, m : multiplet, ABq : AB quartet, J : coupling constant, Hz : Hertz, CDCl₃ : heavy chloroform, d₆-DMSO : heavy dimethyl sulfoxide, % : weight %

And, "room temperatures" appearing in the following working examples means temperatures ranging from about 15 to 25°C. Melting points and temperatures are all shown by centigrade.

Example 1

Synthesis of 6-O-formyl-6-epifumagillol (1)

In tetrahydrofuran (100 ml) were dissolved fumagillol (4.0 g), triphenylphosphine (11.2 g) and formic acid (1.1 ml). To the solution was added dropwise a solution of diethyl azodicarboxylate (7.4 g) in tetrahydrofuran (20 ml). The mixture was stirred overnight, which was diluted with ethyl acetate (300 ml), followed by washing with a saturated aqueous solution of sodium chloride, then with a saturated aqueous solution of sodium hydrogencarbonate and

*Trade-mark

further with a saturated aqueous solution of sodium hydrogencarbonate. The reaction mixture was dried over anhydrous magnesium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified by means of a silica gel chromatography (developing solvent : ethyl acetate - hexane = 1:3) to afford 6-O-formyl-6-epifumagillol (2.6 g).

NMR spectrum (δ value; CDCl_3) : 1.21(1H,m), 1.27(3H,s), 1.61(1H,d,11Hz), 1.66(3H,s), 1.75(3H,s), 1.70 to 2.25(4H,m), 2.56(1H,m), 2.59(1H,d,4Hz), 2.98(1H,d,4Hz), 3.56(3H,s), 3.83(1H,dd,9Hz,11Hz), 5.00(1H,m), 5.20(1H,m), 8.17(1H,s).

Example 2

Synthesis of 6-epifumagillol (2)

In methanol (20 ml) was dissolved the compound 1 (2.5 g), to which was added a conc. ammonia water (5 ml). The mixture was stirred for 15 minutes. The solvent was distilled under reduced pressure. The residue was dissolved in ethyl acetate (100 ml) and washed with a saturated aqueous solution of sodium chloride, which was dried over anhydrous magnesium sulfate. The solvent was then distilled off under reduced pressure. The residue was purified by means of silica gel chromatography (developing solvent : ethyl acetate - hexane = 2:1) to afford 6-epifumagillol (1.8 g).

NMR spectrum (δ value; CDCl_3) : 1.22(1H,m), 1.30(3H,s), 1.54(1H,d,11Hz), 1.66(3H,s), 1.75(3H,s), 1.70 to 2.25(4H,m), 2.38(1H,m), 2.54(1H,d,4Hz), 2.57(1H,t,7Hz), 2.91(1H,d,4Hz), 3.54 to 3.80(2H,m), 3.61(3H,s), 5.20(1H,m).

Example 3

Synthesis of 6-O-methyl-6-epifumagillol (3)

In a mixture of tetrahydrofuran (1 ml) and dimethylformamide (1 ml) were dissolved the compound 2 (0.19 g) and methyl iodide (1 ml). To the solution was
5 added under ice-cooling sodium hydride (0.2 g). The mixture was stirred for one hour at room temperature, to which was added water, followed by dilution with ether (30 ml). The solution was washed with water and a saturated aqueous solution of sodium chloride, which
10 was dried over magnesium sulfate, followed by distilling off the solvent under reduced pressure. The residue was purified by means of a silica gel chromatography (developing solvent : ethyl acetate - hexane = 1.2) to afford 6-O-methyl-6-epifumagillol
15 (0.18 g).

NMR spectrum (δ value; CDCl_3) : 1.10(1H,m), 1.24(3H,s), 1.51(1H,d,11Hz), 1.66(3H,s), 1.74(3H,s), 1.5 to 2.45(5H,m), 2.54(2H,m), 2.93(1H,d,4Hz), 3.28(1H,m), 3.48(3H,s), 3.58(1H,dd,9Hz,11Hz),
20 3.64(3H,s), 5.21(1H,m).

Example 4

Synthesis of 6-O-benzyl-6-epifumagillol (4)

In a mixture of tetrahydrofuran (1 ml) and
25 dimethylformamide (1 ml) were dissolved the compound 2 (0.17 g) and benzyl bromide (0.16 g). To the solution was added sodium hydride (0.2 g) under ice-cooling. The mixture was stirred for 30 minutes for 30 minutes, to which was added water, followed by dilution with
30 ether (30 ml). The solution was washed with water and a saturated aqueous solution of sodium chloride, which was then dried over magnesium sulfate. The solvent was distilled off under reduced pressure. The residue was purified by means of silica gel chromatography
35 (developing solvent : ethyl acetate - hexane = 1:5) to afford 6-O-benzyl-6-epifumagillol (0.21 g).

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NMR spectrum (δ value ; CDCl_3) : 1.08(1H,m),
1.25(3H,s), 1.51(1H,d,11Hz), 1.65(3H,s), 1.74(3H,s),
1.5 to 2.5(5H,m), 2.51(2H,m), 2.92(1H,d,4Hz), 3.4 to
3.75(2H,m), 3.69(3H,s), 4.69(2H,s), 5.20(1H,m), 7.2 to
7.4(5H,m).

Example 5

Synthesis of 6-O-acetyl-6-epifumagillol (5)

The compound 2 (0.20 g) was dissolved in
dichloromethane (2 ml), to which were added acetic
anhydride (0.13 ml) and dimethyl aminopyridine (10 mg).
The mixture was stirred for 15 minutes, which was
diluted with ethyl acetate (30 ml), followed by washing
with a 1M aqueous solution of citric acid, a saturated
aqueous solution of sodium chloride, a saturated
aqueous solution of sodium hydrogencarbonate and
further with a saturated aqueous solution of sodium
chloride. The solution was dried over anhydrous
magnesium sulfate. The solvent was distilled off under
reduced pressure. The residue was purified by means of
a silica gel chromatography (developing solvent : ethyl
acetate - hexane = 1:2) to afford 6-O-acetyl-6-
epifumagillol (0.23 g).

NMR spectrum (δ value; CDCl_3) : 1.19(1H,m),
1.26(3H,s), 1.60(1H,d,11Hz), 1.66(3H,s), 1.75(3H,s),
1.70 to 2.25(4H,m), 2.38(1H,m), 2.56(1H,t,7Hz),
2.57(1H,d,4Hz), 2.97(1H,d,4Hz), 3.55(3H,s),
3.80(1H,dd,9Hz,1Hz), 4.92(1H,m), 5.20(1H,m).

Example 6

Synthesis of 6-O-benzoyl-6-epifumagillol (6)

In dichloromethane (2 ml) were dissolved the
compound 2 (0.20 g) and dimethyl aminopyridine (0.13
g), to which was added dropwise at 0 C benzoyl chloride
(0.1 ml). The mixture was stirred for 15 minutes at
the same temperature, which was diluted with ethyl
acetate (30 ml).

The solution was washed with a saturated aqueous solution of sodium chloride, a 1M aqueous solution of citric acid and further with a saturated aqueous solution of sodium chloride. The resultant was dried over anhydrous magnesium sulfate, then the solvent was distilled off under reduced pressure. The residue was purified by means of a silica gel chromatography (developing solvent : ethyl acetate - hexane = 1:4) to afford 6-O-benzoyl-6-epifumagillol (0.26 g)

10 NMR spectrum (δ value ; CDCl_3) : 1.24(1H,m),
1.30(3H,s), 1.68(1H,d,1Hz), 1.68(3H,s), 1.75(3H,s),
1.70 to 2.25(4H,m), 2.38(1H,m), 2.59(1H,t,7Hz),
2.60(1H,d,4Hz), 3.01(1H,d,4Hz), 3.59(3H,s),
3.97(1H,dd,9Hz,11Hz), 5.10 to 5.28(2H,m), 7.42 to
15 7.64(3H,m), 8.10(2H,m).

Example 7

Synthesis of 6-O-(3-carboxypropionyl)-6-epifumagillol (7)

20 In dichloromethane (1 ml) were dissolved the compound 2 (0.30 g) and dimethyl aminopyridine (0.39 g), to which was added succinic anhydride (0.21 g). The mixture was stirred for 15 minutes and diluted with ethyl acetate (30 ml). The solution was washed with a
25 1M aqueous solution of citric acid, followed by washing three times with a saturated aqueous solution of sodium chloride. The resultant was dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to afford 6-O-(3-carboxypropionyl)-6-
30 epifumagillol (0.38 g).

NMR spectrum (δ value; CDCl_3) : 1.19(1H,m),
1.26(3H,s), 1.61(1H,d,11Hz), 1.66(3H,s), 1.74(3H,s),
1.70 to 2.25(4H,m), 2.38(1H,m), 2.56(1H,t,8Hz),
2.57(1H,d,4Hz), 2.70(4H,m), 2.97(1H,d,4Hz), 3.54(3H,s),
35 3.81(1H,dd,9Hz,11Hz), 4.96(1H,m), 5.20(1H,m)

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Example 8

Synthesis of 6-O-phenoxy carbonyl-6-epifumagillol
(8)

5 In dichloromethane (8 ml) were dissolved the
compound 2 (0.53 g) and dimethyl aminopyridine (0.46
g). To the solution was added phenyl chloroformate
(0.45 g), and the mixture was stirred for 30 minutes.
To the resultant was added water, and the mixture was
10 diluted with dichloromethane (40 ml), followed by
washing with water and a saturated aqueous solution of
sodium chloride. The resultant was dried over
magnesium sulfate, then the solvent was distilled off
under reduced pressure. The residue was purified by
15 means of a silica gel chromatography (developing
solvent : ethyl acetate - hexane = 1:5), which was then
crystallized from ethanol to afford 6-O-
phenoxy carbonyl-6-epifumagillol (0.55 g), m.p. 118 to
119°C.

NMR spectrum (δ value; CDCl_3) : 1.22(1H,m),
20 1.28(3H,s), 1.63(1H,d,11Hz), 1.66(3H,s), 1.75(3H,s),
1.6 to 2.5(5H,m), 2.57(1H,t,7Hz), 2.59(1H,d,4Hz),
2.98(1H,d,4Hz), 3.65(3H,s), 3.83(1H,d,4Hz),
2.98(1H,d,4Hz), 3.65(3H,s), 3.84(1H,dd,9Hz,11Hz),
4.88(1H,m), 5.20(1H,m), 7.1 to 7.5(5H,m)

25

Example 9

Synthesis of 6-O-carbamoyl-6-epifumagillol (9)

The compound 8 (0.16 g) was dissolved in ethanol
(3 ml). To the solution was added a conc. ammoni water
30 (1 ml), and the mixture was stirred for 3 hours at room
temperature. The solvent was distilled off under
reduced pressure. The residue was purified by means of
a silica gel chromatography (developing solvent : ethyl
acetate - hexane = 1:1) to afford 6-O-carbamoyl-6-
35 epifumagillol (0.12 g), m.p. 52 to 53°C.

NMR spectrum (δ value ; CDCl_3) : 1.20(1H,m),

1.25(3H,s), 1.66(3H,s), 1.75(3H,s), 1.6 to 2.5(6H,m),
2.57(1H,t,7Hz), 2.58(1H,d,4Hz), 2.97(1H,d,4Hz),
3.54(3H,s), 3.79(1H,dd,11Hz), 4.83(1H,m), 5.21(1H,m),
5.26(2H,brs).

5

Example 10

Synthesis of 6-O-(N-chloroacetyl carbamoyl)-6-
epifumagillol (10)

10 The compound 2 (0.30 g) was dissolved in
dichloromethane (5 ml), to which was added dropwise at
0°C chloroacetyl isocyanate (0.11 ml). The mixture was
stirred for 15 minutes at the same temperature, which
was diluted with ethyl acetate (50 ml), followed by
15 washing with a saturated aqueous solution of sodium
hydrogen carbonate, then with a saturated aqueous
solution of sodium chloride. The resultant was dried
over anhydrous magnesium sulfate, then the solvent was
distilled off under reduced pressure. The residue was
purified by means of a silica gel chromatography
20 (developing solvent : ethyl acetate - hexane = 1:3),
followed by crystallization from isopropyl ether to
afford 6-O-(N-chloroacetylcarbamoyl)-6-epifumagillol
(0.25 g), m.p. 125 to 126°C.

NMR spectrum (δ value ; CDCl₃) : 1.24(1H,m),
25 1.26(3H,s), 1.66(3H,s), 1.75(3H,s), 1.70 to 2.25(5H,m),
2.38(1H,m), 2.56(1H,t,6Hz), 2.61(1H,d,4Hz),
2.99(1H,d,4Hz), 3.55(3H,s), 3.83(1H,dd,9Hz,11Hz),
4.52(2H,s), 4.92(1H,m), 5.20(1H,m), 8.06(1H,brs).

30 Example 11

Synthesis of 6-O-morpholinocarbonyl-6-
epifumagillol (11)

35 The compound 8 (0.17 g) was dissolved in
dichloromethane (4 ml), to which was added morpholine
(1 ml), and the mixture was stirred for one day at room
temperature. The reaction mixture was diluted with

ethyl acetate (30 ml), which was washed with a saturated aqueous solution of ammonium chloride and a saturated aqueous solution of sodium chloride. The resultant was dried over magnesium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified by means of a silica gel chromatography (developing solvent : ethyl acetate - hexane = 1:2) to afford 6-O-morpholinocarbonyl-6-epifumagillol (0.13 g), m.p. 139 to 140°C.

NMR spectrum (δ value ; CDCl_3) : 1.20(1H,m), 1.26(3H,s), 1.66(3H,s), 1.75(3H,s), 1.6 to 2.6(7H,m), 2.58(1H,d,4Hz), 2.97(1H,d,4Hz), 3.50(4H,m), 3.54(3H,s), 3.67(4H,m), 3.80(1H,dd,9Hz,11Hz), 4.87(1H,m), 5.21(1H,m).

Example 12

Synthesis of 6-O-methanesulfonyl-6-epifumagillol (12)

The compound 2 (0.18 g) and dimethyl aminopyridine (0.15 g) were dissolved in dichloromethane (3 ml). To the solution was added dropwise, under ice-cooling, a solution of methanesulfonyl chloride (0.11 g) in dichloromethane (1 ml). The mixture was stirred for 30 minutes at room temperature, then the reaction mixture was diluted with ethyl acetate (30 ml), followed by washing with a saturated aqueous solution of ammonium chloride, water, then a saturated aqueous solution of sodium chloride. The resultant was dried over magnesium sulfate, then the solvent was distilled off under reduced pressure. The residue was purified by means of a silica gel chromatography (developing solvent : ethyl acetate - hexane = 1:3) to afford 6-O-methanesulfonyl-6-epifumagillol (0.21 g).

NMR spectrum (δ value ; CDCl_3) : 1.29(3H,s), 1.56(1H,d,11Hz), 1.66(3H,s), 1.75(3H,s), 1.8 to 2.6(7H,m), 2.59(1H,d,4Hz), 2.94(1H,d,4Hz), 3.12(3H,s),

3.67(3H,s), 3.77(1H,dd,9Hz,11Hz), 4.50(1H,m),
5.20(1H,m).

Example 13

5 Synthesis of 6-O-(p-toluenesulfonyl)-6-
epifumagillol (13)

10 The compound 2 (0.17 g) and dimethyl aminopyridine
(0.15 g) were dissolved in dichloromethane (4 ml). To
the solution was added p-toluenesulfonyl chloride (0.18
g). The mixture was stirred for one day, which was
diluted with ethyl acetate (30 ml), followed by washing
with a saturated aqueous solution of ammonium
chloride, water and a saturated aqueous solution of
sodium chloride. The resultant was dried over
15 magnesium sulfate, then the solvent was distilled off
under reduced pressure. The residue was purified by
means of a silica gel chromatography(developing solvent
: ethyl acetate - hexane = 1:3) to afford 6-O-(p-
toluenesulfonyl)-6-epifumagillol (0.24 g).

20 NMR spectrum (δ value ; CDCl₃) : 1.20(1H,m),
1.21(3H,s), 1.47(1H,d,11Hz), 1.64(3H,s), 1.73((3H,s),
2.44(3H,s), 1.6 to 2.6(6H,m), 2.56(1H,d,4Hz),
2.92(1H,d,4Hz), 3.30(3H,s), 3.67(1H,dd,9Hz),11Hz),
4.53(1H,m), 5.17(1H,m), 7.33(2H,d,8Hz), 7.84(2H,d,8Hz).

25

Example 14

Synthesis of 6-epidihydrofumagillol (14)

30 A solution of the compound 2 (225 mg) in ethanol
(7 ml) was subjected to catalytic reduction at normal
pressure for 30 minutes at room temperature using 5%
palladium carbon (200 mg) as the catalyst. The
reaction mixture was subjected to filtration and the
solvent was distilled off from the filtrate under
reduced pressure. The residue was purified by means of
35 a silica gel chromatography (developing solvent : ethyl
acetate - hexane = 1:1) to afford 6-

g).

NMR spectrum (δ value : CDCl_3) : 0.91(6H,d,J=6Hz),
1.27(3H,s), 1.2 to 2.1(10H,m), 2.5 to 2.65(3H,m),
2.83(1H,d,J=4Hz), 3.62(3H,s), 3.5 to 3.8(2H,m).

5

Example 15

Synthesis of 6-O-(N-chloroacetylcarbamoyl)-6-epidihydrofumagillol (15)

10 In substantially the same manner as Example 10, 6-O-(N-chloroacetylcarbamoyl)-epidihydrofumagillol (0.18 g) was obtained from the compound 15 (0.16 g) and chloroacetyl isocyanate (0.11 g).

15 NMR spectrum (δ value ; CDCl_3) : 0.91(6H,d,J=7Hz),
1.23(3H,s), 1.2 to 2.2(10H,m), 2.53(1H,dd,J=7Hz,
J=5Hz), 2.90(1H,d,J=4Hz), 3.25(3H,s),
3.82(1H,dd,J=11Hz,9Hz), 4.52(2H,s), 4.95(1H,m),
8.14(1H,brs).

Example 16

20 Synthesis of 6-O-(2-dimethylaminoethylcarbamoyl)-6-epifumagillol (16)

To a solution of compound 8 (1.17 g) in dichloromethane (8 ml), was added dimethylaminoethylamine (1.1 g) and stirred over night
25 at room temperature. The reaction mixture was concentrated under reduced pressure, and the residue was purified by means of silica gel column chromatography (developing solvent: dichloromethane-methanol-concentrated ammonia water = 10:1:0.01) to
30 obtain 6-O-(2-dimethylaminoethylcarbamoyl)-6-epifumagillol (0.71 g).

35 NMR spectrum (δ value, CDCl_3) : 1.10(1H,m),
1.26(3H,s), 1.66(3H,s), 1.75(3H,s), 2.24 (6H,s), 1.5-
2.5(8H,m), 2.55(1H,t,6Hz), 2.56(1H,d,4Hz),
2.96(1H,d,4Hz), 3.29(2H,m), 3.52(3H,s),
3.78(1H,dd,11Hz,9Hz), 4.87(1H,m), 5.15-5.3(2H,m).

Example 17

Synthesis of 6-O-(2,4-difluorophenylcarbamoyl)-6-epifumagillol (17)

5 To a solution of compound 2 (1 g) and dimethylaminopyridine (0.2 g) in dichloromethane (7 ml), was added 2,4-difluorophenylisocyanate (1.37 g) and stirred at room temperature for 30 minutes. The reaction mixture was diluted by ethyl acetate (100 ml) and washed with an aqueous 1M citric acid, an aqueous saturated sodium hydrogencarbonate and an aqueous saturated sodium chloride. The resultant was dried over anhydrous magnesium sulfate, then the solvent was distilled off under reduced pressure.

15 The resulting residue was purified by means of silica gel column chromatography (developing solvent: ethyl acetate-hexane=4:1) to obtain 6-O-(2,4-difluorophenylcarbamoyl)-6-epifumagillol (1.15 g).

20 NMR spectrum (δ value, CDCl_3) : 1.21(1H,m), 1.28(3H,s), 1.64(1H,d,11Hz), 1.67(3H,s), 1.75(3H,s), 1.7-2.5(5H,m), 2.57(1H,t,7Hz), 2.59(1H,d,4Hz), 2.99(1H,d,4Hz), 3.55(3H,s), 3.85(1H,dd,11Hz,9Hz), 4.97(1H,m), 5.22(1H,m), 6.78(1H,brs), 6.8-6.95(2H,m), 8.03(1H,m).

25 Example 18

Synthesis of 6-O-[1-(4-ethyl-2,3-dioxopiperazinyl)carbonyl]-6-epifumagillol (18)

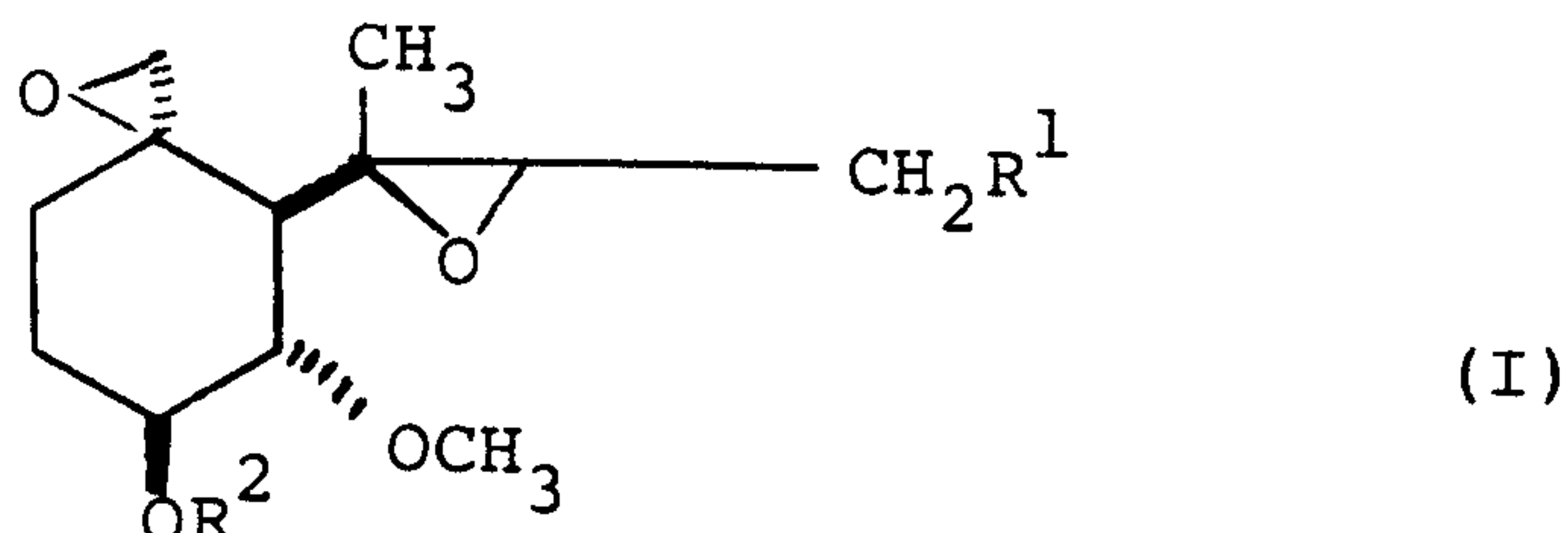
30 To a solution of compound 2 (1.28 g) and dimethylaminopyridine (1.11 g) in dichloromethane (8 ml), was added 1-(4-ethyl-2,3-dioxopiperazinyl)carbonyl chloride (1.20 g) and stirred at room temperature for 15 minutes. The reaction mixture was diluted with ethyl acetate (100 ml) and washed with an aqueous solution of saturated sodium hydrogencarbonate, water and a aqueous solution of

saturated sodium chloride. The resultant was dried over anhydrous magnesium sulfate, then the solvent was distilled off under reduced pressure. The resulting residue was purified by means of silica gel column chromatography (developing solvent: ethyl acetate-hesane=1:1) to obtain 6-O-[1-(4-ethyl-2,3-dioxopiperazinyl)carbamoyl]-6-epifumagillol (0.24 g).
m.p.: 161 - 162°C

NMR spectrum (δ value, CDCl_3) : 1.20(1H,m),
1.23(3H,t,7Hz), 1.27(3H,s), 1.60(1H,d,11Hz),
1.66(3H,s), 1.75(3H,s), 1.6-2.5(5H,m), 2.56(1H,t,7Hz),
2.58(1H,d,4Hz), 2.97(1H,d,4Hz), 3.5-3.7(4H,m),
3.63(3H,s), 3.91(1H,dd,11Hz,9Hz), 4.09(2H,m),
4.95(1H,m), 5.21(1H,m).

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A 6-epifumagillol compound of the formula:



(wherein:

R^1 is 2-methyl-1-propenyl or isobutyl; and

R^2 is (1) hydrogen;

(2) an aliphatic hydrocarbon residue selected from the class consisting of:

(a) C_{1-20} straight-chain or branched alkyl which may be epoxidated or may have one to three substituents each independently selected from the class consisting of amino, C_{1-6} alkylamino, di- C_{1-6} alkylamino, nitro, halogen, hydroxyl, lower alkoxy, cyano, carbamoyl, carboxyl, C_{1-6} alkoxy-carbonyl, carboxy- C_{1-6} alkoxy, phenyl [which may further have one to five substituents each independently selected from the class consisting of C_{1-6} alkyl, C_{1-6} alkoxy, halogen, halogenated C_{1-6} alkyl and nitro], 2-furyl, 2-thienyl, 4-thiazolyl, 4-imidazolyl and 4-pyridyl,

(b) C_{2-20} alkenyl,

(c) C_{2-20} alkynyl, and

(d) C_{3-8} cycloalkyl; or

(3) an acyl selected from the class consisting of:

(a) C_{1-20} alkanoyl which may have one to three substituents each independently selected from the class consisting of amino, C_{1-6} alkylamino, di- C_{1-6} alkylamino, nitro, halogen, hydroxyl, lower alkoxy, cyano, carbamoyl, carboxyl, C_{1-6} alkoxy-carbonyl, carboxy- C_{1-6} alkoxy, phenyl [which may further have one to five substituents each independently selected from the class consisting of C_{1-6} alkyl, C_{1-6} alkoxy, halogen, halogenated C_{1-6} alkyl and nitro], 2-furyl, 2-thienyl, 4-thiazolyl, 4-imidazolyl and 4-pyridyl,

(b) aroyl which is selected from the class consisting of benzoyl, 1-naphthoyl and 2-naphthoyl, and may be substituted by one to three substituents each independently selected from the class consisting of C_{2-6} alkyl, amino, halogen, hydroxyl, C_{1-6} alkoxy, cyano, and carbamoyl and carboxyl,

(c) aromatic heterocyclic carbonyl group which is selected from the class consisting of 2-furoyl, 2-thenoyl, nicotinoyl, isonicotinoyl and imidazole-1-carbonyl, and may be substituted by one to three substituents each independently selected from the class consisting of C_{2-6} alkyl, amino, halogen, hydroxyl, C_{1-6} alkoxy, cyano, and carbamoyl and carboxyl,

(d) carbamoyl group which may be mono- or disubstituted by substituents each independently selected from the class consisting of C_{1-6} alkyl [which may further be substituted by mono- or di- C_{1-6} alkylamino], C_{1-6} alkanoyl, chloroacetyl, dichloroacetyl, trichloroacetyl, C_{1-6} alkoxy-carbonylmethyl, carboxymethyl, phenyl [which may further have one to five substituents each independently selected from the class consisting of

C₁₋₆alkyl, C₁₋₆alkoxy, halogen, halogenated C₁₋₆alkyl and nitro], naphthyl and benzoyl, or which has two substituents that together with the nitrogen of the carbamoyl group form a cyclic amino group selected from the class consisting of pyrrolidino, piperidino, morpholino and piperazino, the said cyclic amino group being unsubstituted or further substituted by up to three substituents each independently selected from the class consisting of C₁₋₆alkyl, phenyl and oxo,

(e) C₁₋₆alkoxy-carbonyl group which may be substituted by one to three substituents each independently selected from the class consisting of amino, C₁₋₆alkylamino, di-C₁₋₆alkylamino, nitro, halogen, hydroxyl, lower alkoxy, cyano, carbamoyl, carboxyl, C₁₋₆alkoxy-carbonyl, carboxy-C₁₋₆alkoxy, phenyl [which may further have one to five substituents each independently selected from the class consisting of C₁₋₆alkyl, C₁₋₆alkoxy, halogen, halogenated C₁₋₆alkyl and nitro], 2-furyl, 2-thienyl, 4-thiazolyl, 4-imidazolyl and 4-pyridyl,

(f) phenoxycarbonyl group which may be substituted by one to three substituents each independently selected from the class consisting of C₁₋₆alkyl and halogen,

(g) benzenesulfonyl group which may be substituted by one to three substituents each independently selected from the class consisting of C₁₋₆alkyl and halogen,

(h) C₁₋₆alkylsulfonyl group which may have one to three substituents each independently selected from the class consisting of amino, C₁₋₆alkylamino, di-C₁₋₆alkylamino, nitro,

halogen, hydroxyl, lower alkoxy, cyano, carbamoyl, carboxyl, C_{1-6} alkoxy-carbonyl, carboxy- C_{1-6} alkoxy, phenyl [which may further have one to five substituents each independently selected from the class consisting of C_{1-6} alkyl, C_{1-6} alkoxy, halogen, halogenated C_{1-6} alkyl and nitro], 2-furyl, 2-thienyl, 4-thiazolyl, 4-imidazolyl and 4-pyridyl, or

(i) sulfamoyl group which may be mono- or disubstituted by substituents each independently selected from the class consisting of C_{1-6} alkyl and phenyl [which may further have one to five substituents each independently selected from the class consisting of C_{1-6} alkyl, C_{1-6} alkoxy, halogen, halogenated C_{1-6} alkyl and nitro]), or a pharmaceutically acceptable salt thereof.

2. The compound or salt as claimed in claim 1, wherein R^1 is 2-methyl-1-propenyl group.

3. The compound as claimed in claim 1, wherein R^2 is hydrogen atom.

4. The compound or salt as claimed in claim 1, wherein R^2 is the said aliphatic hydrocarbon residue.

5. The compound or salt as claimed in claim 1, wherein R^2 is the said C_{1-20} straight-chain or branched alkyl.

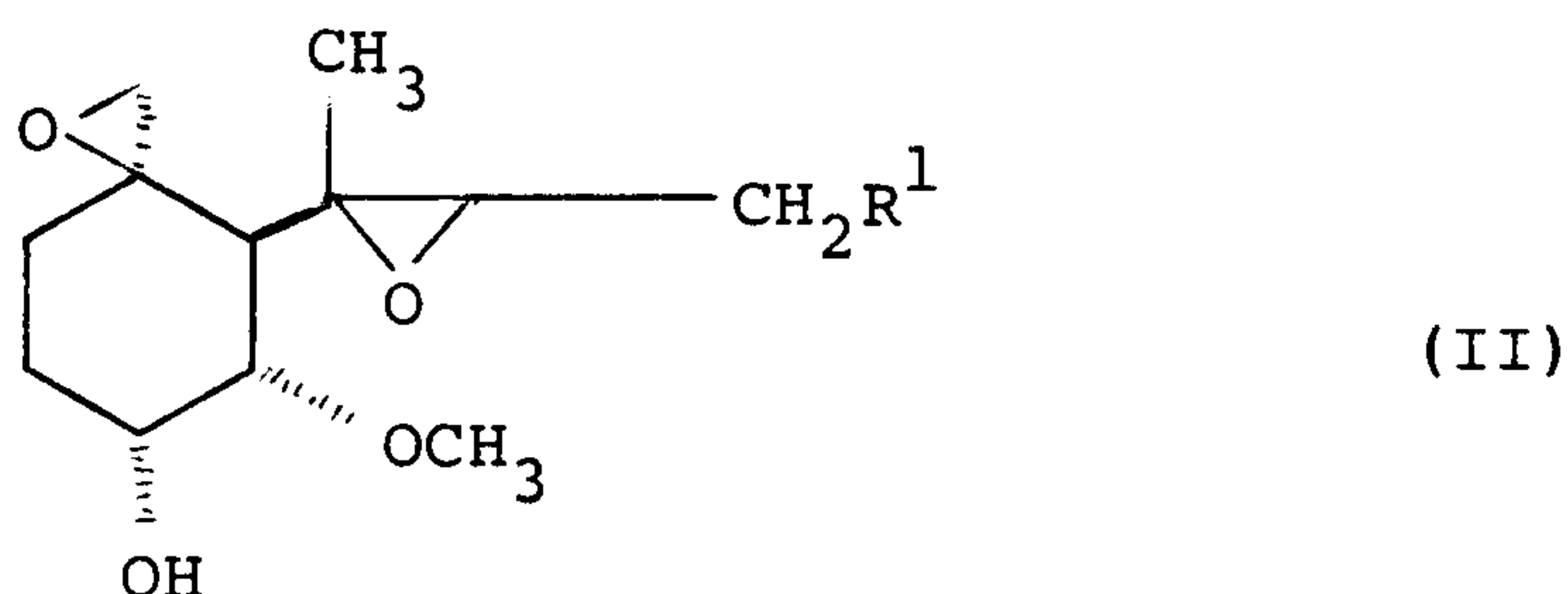
6. The compound as claimed in claim 1, wherein R^2 is C_{1-6} alkyl which may be substituted by phenyl.

A

7. The compound or salt as claimed in claim 1, wherein R^2 is the said acyl.
8. The compound or salt as claimed in claim 1, wherein R^2 is C_{1-20} alkanoyl which may be substituted with amino, hydroxy, halogen or carboxy.
9. The compound or salt as claimed in claim 1, wherein R^2 is the said aroyl.
10. The compound or salt as claimed in claim 1, wherein R^2 is an aromatic heterocyclic carbonyl selected from the class consisting of 2-furoyl, 2-thenoyl, nicotinoyl, isonicotinoyl and imidazole-1-carbonyl.
11. The compound or salt as claimed in claim 1, wherein R^2 is the said carbamoyl group.
12. The compound or salt as claimed in claim 1, wherein R^2 is C_{1-6} alkoxy-carbonyl which may be substituted with amino, hydroxy, halogen or carboxy.
13. The compound or salt as claimed in claim 1, wherein R^2 is the said phenoxycarbonyl group.
14. The compound or salt as claimed in claim 1, wherein R^2 is C_{1-6} alkylsulfonyl which may be substituted with amino, hydroxy, halogen or carboxy.
15. The compound as claimed in claim 1, wherein R^2 is

sulfamoyl which may be substituted with C₁₋₆ alkyl or phenyl.

16. The compound as claimed in claim 1, wherein R¹ is 2-methyl-1-propenyl group and R² is hydrogen, C₁₋₆ alkanoyl or phenoxycarbonyl.
17. The compound 6-epifumagillol.
18. The compound 6-O-acetyl-6-epifumagillol.
19. The compound 6-O-phenoxycarbonyl-6-epifumagillol.
20. The compound or salt as claimed in claim 1, wherein R² is formyl, methyl, benzyl, acetyl, benzoyl, 3-carboxy-propionyl, phenoxycarbonyl, carbamoyl, N-chloroacetylcarbamoyl, morpholino-carbonyl, methanesulfonyl, p-toluenesulfonyl, 2-dimethylamino-ethylcarbamoyl, 2,4-difluorophenylcarbamoyl, or 1-(4-ethyl-2,3-dioxopiperazinyl)carbonyl.
21. The compound or salt as claimed in claim 1, wherein R² is the said benzenesulfonyl group.
22. The compound or salt as claimed in any one of claims 6, 8, 10, 12, 14 and 15, wherein R¹ is 2-methyl-1-propenyl.
23. A process of producing the compound of the formula (I) defined in claim 1, which comprises:
epimerizing the hydroxyl group in a compound of the formula:



(wherein R^1 is as defined in claim 1), thereby obtaining a compound (I) in which R^2 is hydrogen and,

where required, treating the resulting compound with a reagent to introduce the aliphatic hydrocarbon residue or the acyl, thereby obtaining a compound (I) in which R^2 is other than hydrogen.

24. The process as claimed in claim 23, wherein the epimerization of the hydroxyl group is carried out by subjecting fumagillol or its catalytic reduction product to Matsunobu reaction using diethylazocarboxylate, triphenylphosphine and a carboxylic acid, followed by hydrolysis; and

the reagent is a member selected from the class consisting of:

1) an activated derivative of a carboxylic acid R^3OH in which R^3 is an acyl of the C_{1-20} alkanoyl (a), the aroyl (b), the aromatic heterocyclic carbonyl (c), the alkoxy carbonyl (e) or the phenoxycarbonyl (f), each defined in claim 1,

2) a compound of the formula R^4X in which R^4 is the aliphatic hydrocarbon residue (2) defined in claim 1 and X is a leaving group selected from the class consisting of halogen and lower alkylsulfate,

3) an isocyanate of the formula $R^5\text{-NCO}$ in which R^5 is the substituent defined in claim 1 for mono-substituted carbamoyl group (d),

4) a carbamoyl halide in which the carbamoyl group may be substituted as defined in claim 1,

5) an activated derivative of a sulfonic acid of the formula $R^6\text{OH}$ in which R^6 is the benzenesulfonyl (g), the C_{1-6} alkylsulfonyl (h) or the sulfamoyl (i) each defined in claim 1.

25. A pharmaceutical composition for inhibiting immune reactions which contains an effective amount of the compound or salt as claimed in any one of claims 1 to 21 and a pharmaceutically acceptable carrier, diluent or excipient therefor.

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PATENT AGENTS