

DESCRIPTION

MEDICAMENT CONTAINING
A SULFOPYRANOSYLACYLGLYCEROL DERIVATIVE

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Technical Field

The present invention relates to a medicament containing at least one compound selected from the group consisting of sulfopyranosylacylglycerol derivatives and pharmaceutically acceptable salts thereof, as an active ingredient.

Background Art

Sulfur-containing glycolipids contained in natural products derived from, e.g., algae and higher plants are known to have physiological activities.

For example, in a document of Ohta et al. (Chemical & Pharmaceutical Bulletin, 46(4), (1998)), it is described that a specific sulfoquinovosyldiacylglycerol derivative derived from red algae, Gigartina tenella, exhibits not only inhibitory activities against DNA polymerases α and β of higher organisms but also an HIV-derived reverse-transcriptase inhibitory activity. The sulfoquinovosyldiacylglycerol derivative disclosed in the Ohta document is the one whose fatty acid that bonded, through ester-bond, at the C1 carbon atom of the glycerol is an unsaturated fatty acid having 20 carbon atoms with 5 double bonds, and whose another

fatty acid that bonded at the C2 carbon atom of the glycerol is a saturated fatty acid having 16 carbon atoms.

Furthermore, in a document of Mizushima et al.
5 (Biochemical Pharmacology 55, 537-541 (1998)), it is described that a mixture of specific sulfoquinovosyldiacylglycerol derivatives derived from a pteridophyte exhibits inhibitory activities against a calf DNA polymerase α and a rat DNA polymerase β ,
10 however, the mixture has no effect upon an HIV-derived reverse-transcriptase activity.

On the other hand, in a document of Sahara et al. (British Journal of Cancer, 75(3), 324-332 (1997)), it is described that a fraction of
15 sulfoquinovosylmonoacylglycerols contained in an acetone extract from a sea urchin intestine exhibits anticancer activities in-vivo and in-vitro. However, the sulfoquinovosylmonoacylglycerol fraction for which Sahara found the anticancer activities
20 principally contains sulfoquinovosylmonoacylglycerol having, bonded thereto through an ester-bond, a saturated fatty acid with 16 carbon atoms. In the sulfoquinovosylmonoacylglycerol fraction, sulfoquinovosylmonoacylglycerols whose acyl moiety is
25 that of an unsaturated fatty acid, are contained only in an extremely small amount. In addition, Sahara et al. have not yet investigated on anticancer activities

with respect to individual components contained in the sulfoquinovosylmonoacylglycerol mixture.

Furthermore, National Patent Publication No. 5-501105 describes that a

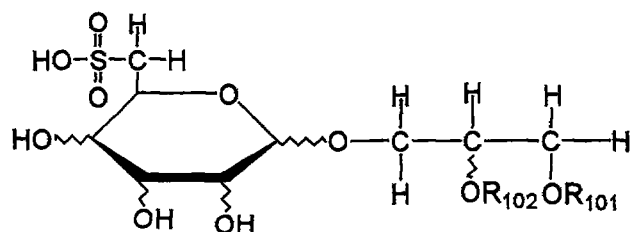
5 sulfoquinovosyldiacylglycerol derivative has an anti-virus activity. More specifically, it discloses that the derivative has an anti-HIV (human immunodeficiency virus) activity, however it does not disclose that the derivative has DNA polymerase
10 inhibitory activities and anticancer activities.

Disclosure of Invention

An object of the present invention is to provide a medicament containing a sulfopyranosylacylglycerol derivative as an active ingredient.

15 The present inventors found that specific sulfopyranosylacylglycerol derivatives have medicinal activities and achieved the present invention. The present invention provides a medicament containing, as an active ingredient, at least one compound selected
20 from the group consisting of:

compounds represented by the following General Formula (1):



(1)

wherein R₁₀₁ represents an acyl residue of an unsaturated higher fatty acid, and R₁₀₂ represents a hydrogen atom or an acyl residue of an unsaturated higher fatty acid; and

5 pharmaceutically acceptable salts thereof.

Brief Description of Drawings

FIG. 1 shows anticancer activities of medicaments of the present invention against tumor cells.

FIG. 2 shows an anticancer activity of
10 a medicament of the present invention obtained by an animal test.

FIG. 3 shows an anticancer activity of a medicament of the present invention obtained by an animal test.

15 FIG. 4 shows an anticancer activity of a medicament of the present invention obtained by an animal test.

FIG. 5 shows an anticancer activity of a medicament of the present invention obtained by
20 an animal test.

FIG. 6 shows an anticancer activity of a medicament of the present invention obtained by an animal test.

FIG. 7 shows an anticancer activity of
25 a medicament of the present invention obtained by an animal test.

Best Mode for Carrying Out of the Invention

In the specification, the term "carbon atoms" of a protecting group refers to the number of carbon atoms assuming that the protecting group is unsubstituted.

5 To be more specific, when the group represented by R^6 is a substituted alkyl group, its number of carbon atoms is that of the alkyl group itself, and the number of carbon atoms of the substituent on the alkyl group is not counted. The same conditions are applicable to
10 the case where the protecting group is other than the alkyl group.

First, the sulfopyranosylacylglycerol derivative represented by General Formula (1) and contained in the medicament of the present invention as an active
15 ingredient will be more specifically explained.

In the sulfopyranosylacylglycerol derivative represented by General Formula (1), the pyranose, which is a sugar skeleton constituting the pyranoside, may include α -D-quinovose (i.e., 6-deoxy- α -D-glucose),
20 β -D-quinovose (i.e., 6-deoxy- β -D-glucose), α -D-fucose (i.e., 6-deoxy- α -D-galactose), β -D-fucose (i.e., 6-deoxy- β -D-galactose), α -D-rhamnose (i.e., 6-deoxy- α -D-mannose) and β -D-rhamnose (i.e., 6-deoxy- β -D-mannose).

25 The absolute configuration of the carbon (asymmetric carbon) at the 2-position of the glycerol moiety may be either the S- or R-configuration.

The sugar skeleton of the pyranoside may be either a boat or chair configuration. However, the chair configuration is preferable in view of stability.

In the sulfopyranosylacylglycerol derivative represented by General Formula (1), R₁₀₁ represents an acyl residue of an unsaturated higher fatty acid.

The fatty acid giving the acyl residue represented by R₁₀₁ may be a straight-chain or branched-chain, unsaturated higher fatty acid. From the viewpoint of using the compound represented by General Formula (1) as a medicament, the straight-chain unsaturated higher fatty acid is preferably used.

The acyl residue of the straight-chain unsaturated higher fatty acid has 14-26 carbon atoms (preferably even number of 14-26) with 1-6 unsaturated bonds.

The acyl residue of the straight-chain unsaturated higher fatty acid is represented by Formula: R-C(=O)-, where R is a straight-chain aliphatic unsaturated hydrocarbon group of 13-25 carbon atoms (preferably, an odd number of 13-25), and 1-6 unsaturated bonds are included in the hydrocarbon group.

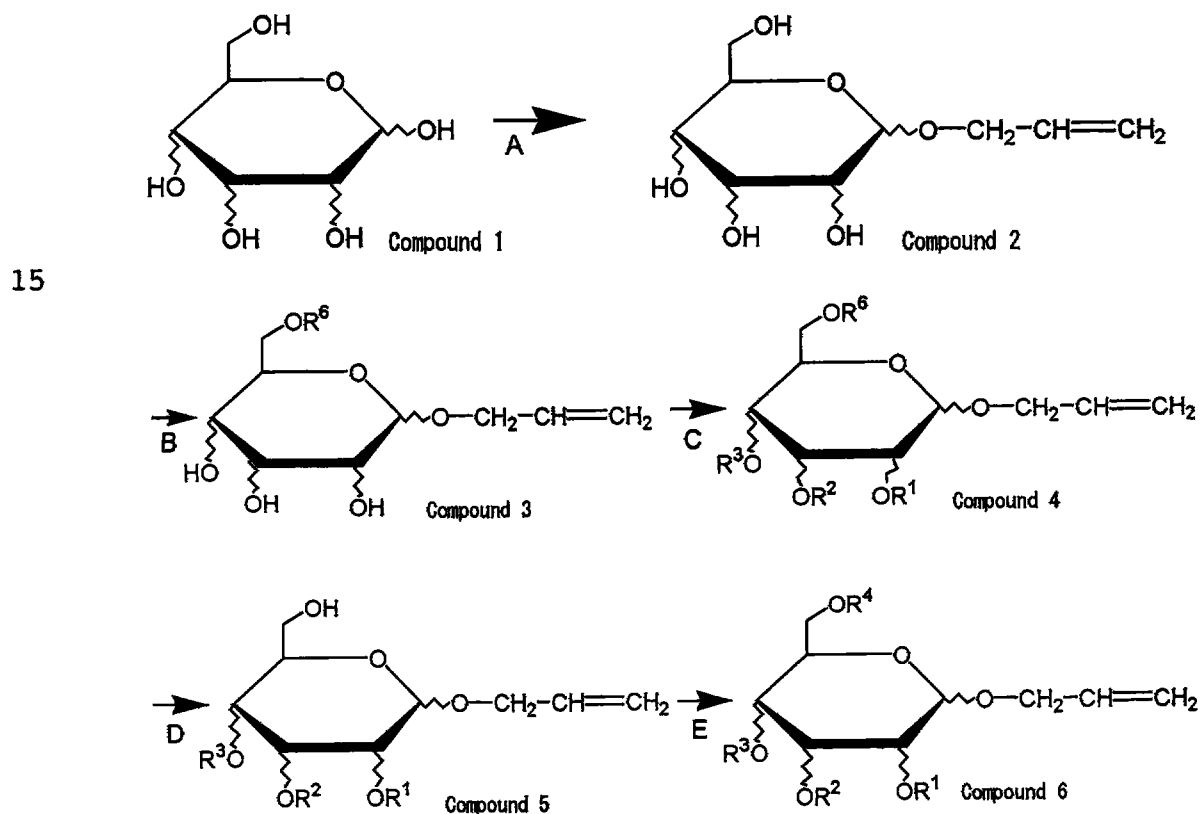
In the sulfopyranosylacylglycerol derivative represented by General Formula (1), R₁₀₂ represents a hydrogen atom or an acyl residue of an unsaturated higher fatty acid. In particular, R₁₀₂ preferably represents a hydrogen atom in consideration of an anti-cancer activity. When R₁₀₂ is an acyl residue

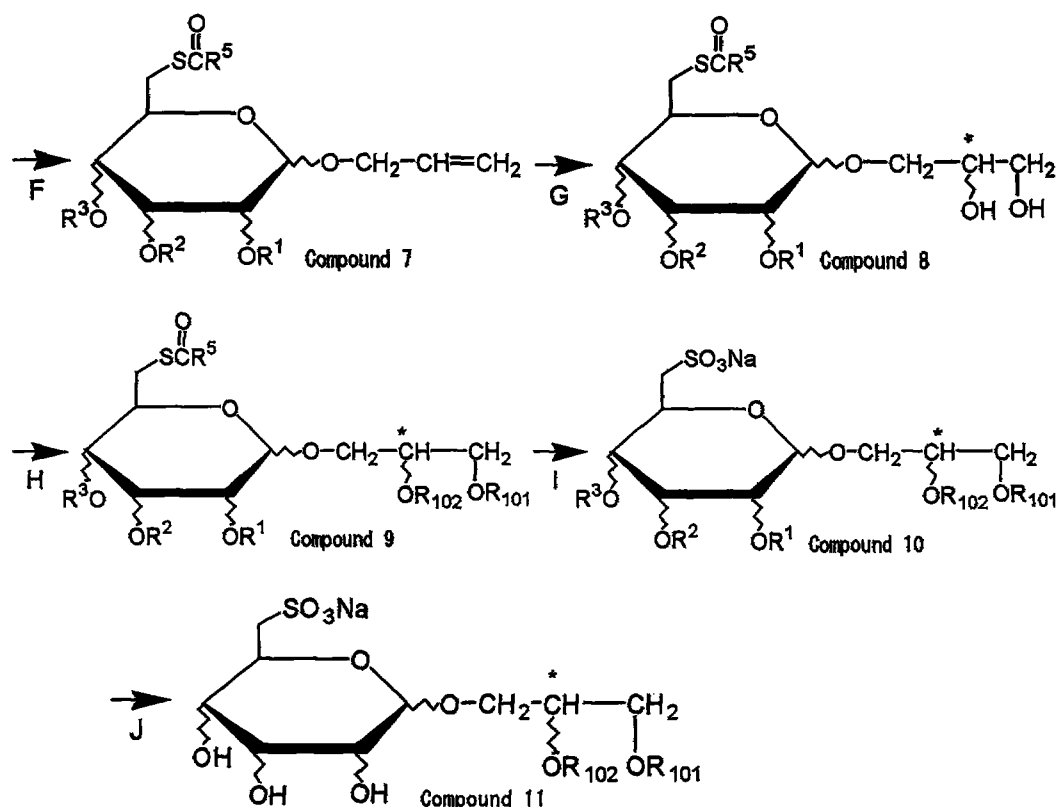
of the unsaturated higher fatty acid, the same fatty acid giving an acyl residue as defined in R_{101} may be selected. R_{101} and R_{102} may be the same or different acyl residue.

5 Now, a method of preparing the sulfoxyranosylacylglycerol derivatives of the present invention will be explained below.

The sulfoxyranosylacylglycerol derivatives of the present invention can be prepared via (Step A) to
 10 (Step J) in accordance with the reaction procedure shown in Scheme 1 below:

Scheme 1





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(Step A) The hydroxyl group bonded to the C1 carbon of the pyranose is converted into a 2-propenyl group. (Step B) The hydroxyl group of the C6 carbon of the pyranose is protected. (Step C) The hydroxyl groups bonded to the C2, C3 and C4 carbons of the pyranose are protected. (Step D) The protecting group of the C6 carbon previously protected is deprotected. (Step E) The hydroxyl group bonded to the C6 carbon is substituted with a group (for example, an alkylsulfonyloxy group or arylsulfonyloxy group) which can be converted to a carbonylthio group. (Step F) The C6 carbon is converted into a carbonylthio group. (Step G) The 2-propenyl group bonded to the C1 carbon is converted into a diol. (Step H) Both of the hydroxyl groups or only the hydroxyl group at the

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1-position of the diol thus obtained are/is esterified with a desired unsaturated higher fatty acid. (Step I) The carbonylthio group at the C6 carbon is converted into a sulfonate salt. (Step J) The protecting groups of C2, C3 and C4 carbons of the sulfonate salt obtained are deprotected. As a result, a salt of a sulfopyranosylacylglycerol derivative of the present invention can be produced. The salt thus obtained is subjected to titration with an acid such as hydrochloric acid to give the sulfopyranosylacylglycerol derivative of the present invention.

The aforementioned Steps A-J will be further explained in detail.

In Step A, the 2-propenylation is carried out by reacting the pyranose with allyl alcohol in the presence of a strong acid, such as trifluoromethanesulfonic acid, usually at room temperature to 100°C, preferably from 80 to 90°C, for a half day to two days. However, the reaction time varies depending upon the reaction conditions.

In Step B, the hydroxyl group bonded to the C6 carbon is protected to obtain the compound to which -OR⁶ is bonded at the C6 carbon (where R⁶ represents an alkyl or substituted silyl group).

As the compound capable of protecting the hydroxyl group, a compound can be used which can provide an

alkyl group or substituted silyl group as the R^6 group.

Examples of the alkyl group represented by R^6 preferably include bulky and substituted alkyl groups. The substituents of the bulky and substituted alkyl groups include methyl and phenyl groups. The specific examples of the substituted alkyl group include t-butyl and trityl groups.

When the group represented by R^6 represents a substituted silyl group, examples of substituents of the substituted silyl group include lower alkyl groups, preferably alkyl groups having 1-4 carbon atoms (for example, methyl, ethyl, isopropyl and t-butyl groups); and aryl groups, preferably aryl groups having 6 carbon atoms (for example, a phenyl group). The substituted silyl group represented by R^6 preferably includes tri-substituted silyl groups, more preferably, a t-butyldiphenylsilyl group.

When the compound 3, where R^6 represents an alkyl group, is to be obtained, the protection of the hydroxyl group in Step B can be carried out by adding a compound represented by R^6-X (where R^6 represents the alkyl group defined above, and X represents a halogen atom such as chlorine atom) to a solution of the compound 2 dissolved in an organic solvent, such as anhydrous pyridine, and reacting the solution mixture at room temperature in the presence of a catalyst such as p-dimethylaminopyridine (DMAP). As the

compound R^6-X , trityl chloride is preferably used in view of manufacturability and reactivity.

When the compound 3, where R^6 represents a substituted silyl group, is to be obtained, 5 t-butyldiphenylsilyl chloride, for example, is used as the compound R^6-X , and the reaction is carried out usually in the presence of a catalyst, such as imidazol, at room temperature for a half day to two days. Note that the reaction time varies depending 10 upon the reaction conditions.

In Step C, the hydroxyl groups bonded to the C2, C3 and C4 carbons are protected and converted into $-OR^1$, $-OR^2$ and $-OR^3$, respectively, where R^1 to R^3 15 independently represent an alkyl or substituted silyl group. The protection of these hydroxyl groups can be carried out by activating, with sodium hydride, the hydroxyl groups bonded to the C2, C3 and C4 carbons of the compound 3 dissolved in an organic solvent, such as N, N-dimethylformamide (DMF), and reacting with the 20 compound capable of protecting these hydroxyl groups at room temperature.

As the compound capable of protecting the hydroxyl groups, benzyl bromide, p-methoxybenzyl bromide, t-butyldimethylsilyl chloride or triethylsilyl chloride 25 may be used.

The reaction using the compound capable of protecting the hydroxyl groups can be carried out

under a suitable reaction condition for each of the protecting groups.

The deprotection of the protecting group bonded to the C6 carbon in Step D may be carried out by reacting
5 a solution of the compound 4 dissolved in an organic solvent, such as methanol, in the presence of a catalyst, such as p-toluenesulfonic acid, generally for 12 hours to one day at room temperature. The reaction time varies depending upon the reaction conditions.

10 In Step E, R^4 , that is, an alkylsulfonyl or arylsulfonyl group is bonded to the hydroxyl group at the C6 carbon of the compound 5, so that the hydroxyl group is converted into $-OR^4$ to give the compound 6.

The reaction to give the $-OR^4$ group is performed
15 by adding a compound having the alkylsulfonyl group or a compound having the arylsulfonyl group to a solution of the compound 5 dissolved in an organic solvent, and reacting them. The alkyl group of the compound having the alkylsulfonyl group preferably includes
20 unsubstituted alkyl groups, more preferably, lower alkyl groups, much more preferably, alkyl groups having 1-2 carbon atoms (methyl and ethyl groups). The compound having an alkylsulfonyl group can be represented by $R^{4'}-X$ (where $R^{4'}$ represents an
25 alkylsulfonyl group, and X represents a halogen atom). Specific examples include methanesulfonyl chloride and ethanesulfonyl chloride.

On the other hand, the aryl group of the compound having the arylsulfonyl group may include unsubstituted and substituted aryl groups, preferably aryl groups having 6 carbon atoms (e.g., a phenyl group). In the case of the substituted aryl group, examples of the substituent thereof include p-methyl and p-methoxy groups. Examples of the compound having an arylsulfonyl group include compounds represented by $R^{4''}-X$ (where $R^{4''}$ represents an arylsulfonyl group, and X represents a halogen atom). Specific examples include p-toluenesulfonyl chloride, p-methoxybenzenesulfonyl chloride and benzenesulfonyl chloride.

Of the compounds having an alkylsulfonyl or arylsulfonyl group, a compound having a tosyl group is preferably used from the viewpoint of reaction facility.

In the reaction of Step E, as an organic solvent, pyridine or dichloromethane may be used.

The reaction mentioned above may be performed, as the case may be, in the presence of a catalyst, such as DMAP, at room temperature for 2 hours to one day. The reaction time varies depending upon the reaction conditions.

In Step F, the sulfonyloxy group ($-OR^4$) of the compound 6 is replaced with a carbonylthio group represented by $-SC(=O)R^5$, where R^5 represents

a hydrogen atom, an alkyl or aryl group.

In the reaction, a compound capable of substituting the alkylsulfonyloxy or arylsulfonyloxy group of the compound 6 with the carbonylthio group, is allowed to react in an organic solvent to give a compound 7. Hereinafter, this compound will be referred to as "O-substituted $\rightarrow$ S-substituted compound".

Examples of the O-substituted $\rightarrow$ S-substituted compound include alkali metal salts and alkali earth metal salts of a thiocarboxylic acid. Examples of the thiocarboxylic acid include thioformic acid, lower thiocarboxylic acids, preferably aliphatic thiocarboxylic acids each having 1-5 carbon atoms in its aliphatic hydrocarbon moiety (for example, thioacetic acid or thiopropionic acid), and aromatic thiocarboxylic acids each having 6-10 carbon atoms in its aromatic hydrocarbon moiety (for example, thiobenzoic acid).

The alkali metal that forms a salt with the thiocarboxylic acid includes potassium and sodium. The alkali earth metal includes magnesium and calcium.

Of the above-mentioned O-substituted $\rightarrow$ S-substituted compounds, salts of thioacetic acid may be preferably used since a reaction can proceed stably and the sulfur atom can be easily oxidized in a later step.

Examples of an organic solvent used in the reaction include alcohols, preferably lower alcohols, (for example, methanol, ethanol and propanol), N,N-dimethylformamide and dimethylsulfoxide.

5 The aforementioned reaction may be performed usually at room temperature to the boiling point of a solvent to be used while stirring for one hour to one day. Note that the reaction time varies depending upon the reaction conditions.

10 The dihydroxylation of Step G may be performed by adding an oxidizing agent, such as osmium tetroxide, to a solution of the compound 7 dissolved in a solvent mixture, such as a mixture of t-butanol and water, and then reacting the resultant mixture in the presence of
15 a re-oxidizing agent, such as trimethylamine N-oxide, at room temperature for one hour to one day. Note that the reaction time varies depending upon the reaction conditions.

 By the esterification of Step H, a
20 sulfoxyranosylacylglycerol derivative having a desired unsaturated higher fatty acid bonded, through an ester-bond, to its glycerol moiety can be obtained.

 This reaction can be carried out by adding an unsaturated higher fatty acid corresponding to a final
25 product to a solution of the compound 8 dissolved in a suitable organic solvent, such as dichloromethane, and then reacting the resultant mixture, if necessary,

in the presence of a suitable catalyst, such as ethyldimethylaminopropylcarbodiimide (EDCI)-DMAP.

In the reaction of Step H, as the fatty acid to be added, use may be made of an unsaturated higher fatty acid whose acyl group is that represented by R_{101} of General Formula (1).

In the reaction of Step H, the compound 9 is obtained in the form of a mixture of a diacylester and a monoacylester. The diacylester herein is represented by Formula (1) of the present invention where each of R_{101} and R_{102} is an acyl residue of the unsaturated higher fatty acid added. The monoacylester herein has the acyl residue of the unsaturated higher fatty acid added, as the R_{101} only. Two or more unsaturated higher fatty acids may be added, if desired, in the reaction of Step H. In this case, the resultant mixture contains diacylesters represented by General Formula (1) where R_{101} and R_{102} are the same or different acyl residues, and monoesters having different acyl residues as R_{101} .

If necessary, the mixture of the monoesters and diesters can be isolated from each other by, for example, chromatography, and subjected to the next reaction Step I.

Furthermore, if desired, by reacting a monoester obtained in Step H with a fatty acid having a different acyl residue from the acyl residue (R_{101}) of the

monoester, it is possible to obtain a diester where R_{102} and R_{101} are different acyl residues. This additional esterification step may be performed under the same conditions as those of Step H except that
5 a different fatty acid is used.

In Step I, the conversion into a sulfonate salt can be carried out by adding an oxidizing agent, such as OXONE ($2\text{KHSO}_5 + \text{KHSO}_4 + \text{K}_2\text{SO}_4$) or molybdenum oxidizing agent (for example, hexaammonium
10 heptamolybdate), into a solution of the compound 9 dissolved in an organic solvent, which is buffered with acetic acid and potassium acetate, and then allowing the resultant mixture to react at room temperature.

The deprotection of the protecting groups bonded
15 to carbons at the C2 to C4 carbons in Step J can be carried out by a method suitable for a protecting group to be used and capable of maintaining a double bond of the unsaturated fatty acid. For example, when the protecting group is a silyl group, deprotection can be
20 made by using acid catalyst (e.g., trifluoroacetic acid).

Note that the pyranosyl moiety of a starting material usually takes α - and β -anomer configurations in a solution. Therefore, the product in each step
25 results in a mixture of α - and β -anomers. The mixture is separated into α - and β -anomers by chromatography. Furthermore, depending upon a type of the sugar, it is

helpful to carry out a benzilydenation after Step A, thereby to separate an anomer by crystallization.

Now, we will explain the medicament of the present invention containing at least one compound selected
5 from the group consisting of sulfopyranosylacylglycerol derivatives of the present invention and pharmaceutically acceptable salts thereof, as an active ingredient.

The sulfopyranosylacylglycerol derivative serving
10 as an active ingredient for the medicament of the present invention may be an isomer having quinovose, rhamnose or fucose as the pyranose constituting the pyranosyl moiety. The derivative may be an isomer in which the pyranosyl moiety is bonded to glyceridyl
15 moiety with an α - or β -configuration. The derivative may be an isomer regarding the asymmetric carbon at the C2 carbon of the glyceridyl moiety. The medicament of the present invention may include one of these isomers alone or in combination of two or more isomers as long
20 as they do not adversely affect the activity.

In the present invention, the medicinal use includes a DNA polymerase inhibitor and an anticancer agent.

Examples of the pharmaceutically acceptable salts
25 employed in the medicament of the present invention include, but not limited to, a salt of a monovalent cation such as a sodium or potassium ion. Hereinafter,

the compounds of the group consisting of
sulfopyranosylacylglycerol derivatives and
pharmaceutically acceptable salts thereof are sometimes
referred to as "medicinally active substance of the
5 present invention".

The medicinally active substance of the present
invention can be orally or parenterally administered.
Medicinally active substance of the present invention
can be combined with, for example, a pharmaceutically
10 acceptable excipient or diluent depending on an
administration route thereby to form a medicinal
formulation.

The forms of the agent suitable for oral
administration include, solid-, semi-solid, liquid- and
15 gas-states. Specific examples include, but not limited
to, tablet, capsule, powder, granule, solution,
suspension, syrup and elixir agents.

In order to formulate the medicinally active
substance of the present invention into tablets,
20 capsules, powders, granules, solutions or suspensions,
the substance is mixed with a binder, a disintegrating
agent and/or a lubricant, and, if necessary, the
resultant is mixed with a diluent, a buffer, a wetting
agent, a preservative and/or a flavor, by a known
25 method. Examples of the binder include crystalline
cellulose, cellulose derivatives, cornstarch and
gelatin. Examples of the disintegrating agent

include cornstarch, potato starch and sodium carboxymethylcellulose. Examples of the lubricant include talc and magnesium stearate. Furthermore, additives such as lactose and mannitol may also be used
5 as long as they are used conventionally.

Moreover, the medicinally active substance of the present invention may be administered in the form of aerosol or inhalant, which is prepared by charging the active substance of liquid- or fine powder-form,
10 together with a gaseous or liquid spraying agent, and, if necessary, a known auxiliary agent such as a wetting agent, into a non-pressurized container such as an aerosol container or a nebulizer. As the spraying agent, a pressurized gas, for example,
15 dichlorofluoromethane, propane or nitrogen may be used.

For parenteral administration, the medicinally active agent of the present invention can be injected by, for example, rectal administration or injection.

For rectal administration, a suppository may be
20 used. The suppository may be prepared by mixing the medicinally active substance of the present invention with an excipient that can be melted at body temperature but is solid at room temperature, such as cacao butter, carbon wax or polyethylene glycol, and
25 molding the resultant material, by a known method.

For the administration by injection, the medicinally active agent of the present invention

can be injected hypodermically, intracutaneously,
intravenously or intramuscularly. An injection
preparation may be formulated by dissolving, suspending
or emulsifying the medicinally active substance of the
5 invention into an aqueous or non-aqueous solvent such
as a vegetable oil, a synthetic glyceride with a fatty
acid, an ester of a higher fatty acid or propylene
glycol by a known method. If desired, a conventional
additive such as a solubilizing agent, an
10 osmoregulating agent, an emulsifier, a stabilizer or
a preservative, may be added to the preparation.

For formulating the medicinally active substance
of the invention into solutions, suspensions, syrups
or elixirs, a pharmaceutically acceptable solvent such
15 as sterilized water for injection or normalized
physiological saline solution may be used.

The medicinally active substance of the invention
may be used together with a pharmaceutically acceptable
compound having another activity, to prepare a
20 medicinal preparation.

The dose of the medicinally active substance of
the present invention may be appropriately set or
adjusted in accordance with an administration form,
an administration route, a degree or stage of a target
25 disease, and the like. For example, in the case of
oral administration, a dose of the medicinally active
substance may be set at 1 - 10 mg/kg body weight/day.

In the case of administration by injection, a dose of the medicinally active substance may be set at 1 - 5 mg/kg body weight/day. In the case of rectal administration, a dose of the medicinally active substance may be set at 1 - 5 mg/kg body weight/day. However, the dose is not limited to these.

When the medicinally active substance of the present invention is used as an anticancer agent, examples of cancers to be treated include those having features of malignant tumors such as solid tumors including adenocarcinoma, epithelioma, sarcoma, glioma, melanoma and lymphoma, and a fluid cancer such as leukemia.

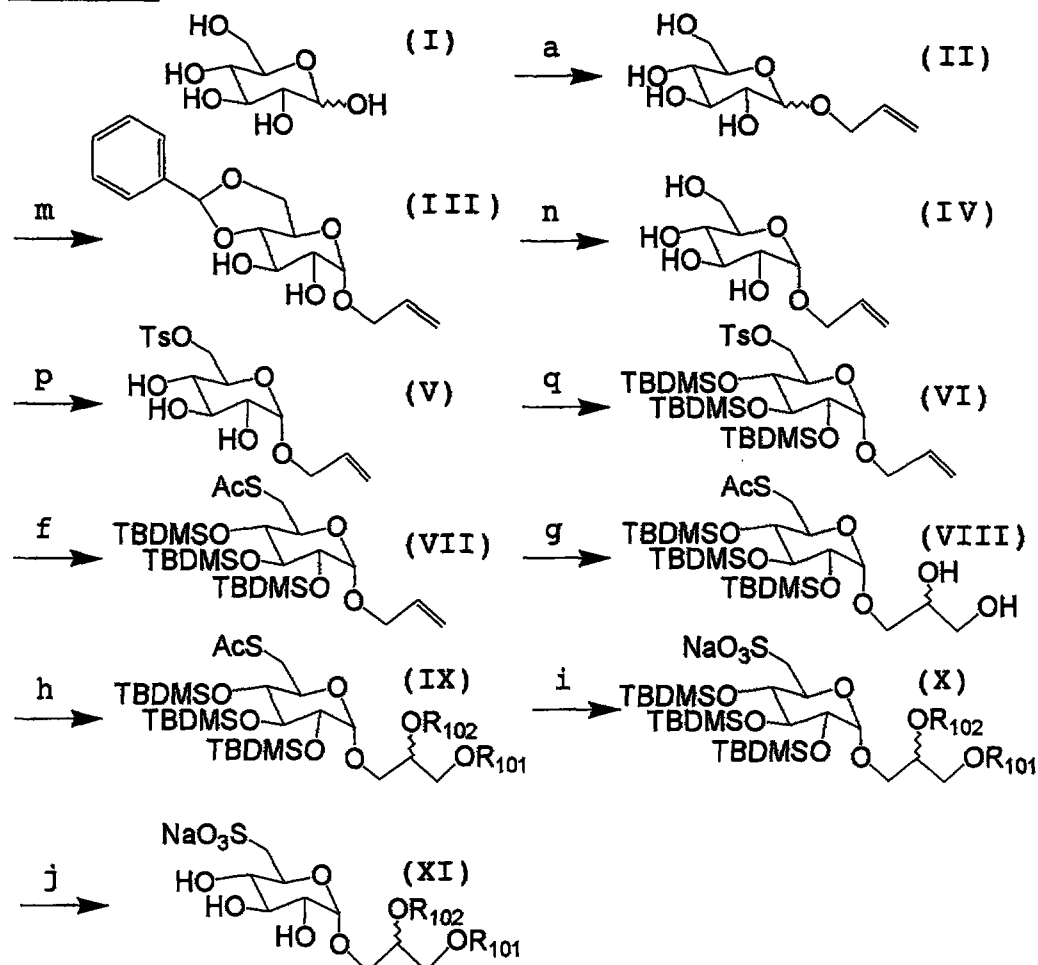
Examples

The present invention will now be described by way of its Examples. However, the present invention is not limited to these Examples.

<Synthesis Example>

Preparation steps of a sulfopyranosylacylglycerol derivative will be shown in Scheme 2 by way of a sulfoquinovosylacylglycerol α derivative.

Scheme 2



5 Ts=tosyl, TBDMS=t-butyldimethylsilyl, AcS=acetylthio,
 R₁₀₁=an acyl residue of an unsaturated higher fatty
 acid, and
 R₁₀₂=a hydrogen atom or an acyl residue of
 an unsaturated higher fatty acid.

10 Reaction conditions:

a; allyl alcohol, trifluoromethanesulfonic acid,
 at 80°C

m; benzaldehyde, zinc chloride, at room temperature

n; acetic acid, water, at 100°C

15 p; toluenesulfonyl chloride, dimethylaminopyridine,
 pyridine, at room temperature

q; t-butyldimethylsilyl trifluoromethanesulfonate,
 2,6-lutidine, dichloromethane, at room temperature

f; potassium thioacetate, ethanol, under reflux

20 g; osmium tetroxide, trimethylamine N-oxide dihydrate,
 t-butanol, water

h; fatty acid, 1-ethyl-3-(3-dimethylaminopropyl)-
 carbodiimide hydrochloride(EDCI), at room
 temperature

- i; OXONE, glacial acetic acid, potassium acetate,
at room temperature
j; acetic acid, tetrahydrofuran, trifluoroacetic acid,
5 water, at room temperature

Scheme 2 is the same as those of Scheme 1 except
for Steps B to E of Scheme 1. More specifically, in
Scheme 2, Step m is employed instead of Step B in
Scheme 1. In Step m, compound (II) is reacted with
10 benzaldehyde to prepare a benzylidene derivative.
By virtue of this reaction, α -anomer is crystallized
and separated.

In the reaction of Scheme 2, p-toluenesulfonyl
chloride is reacted with compound (IV) thereby to
15 bond a tosyl group at C6 carbon thereof in Step p,
and then, the C2, C3 and C4 carbons are protected with
t-butyldimethylsilyl groups (Step q). In this case,
Step B of protecting the C6 carbon by an alkyl or
substituted silyl group, and Step D of deprotecting the
20 C6 carbon in the process of Scheme 1 may be omitted,
because of the stable nature of the tosyl group.

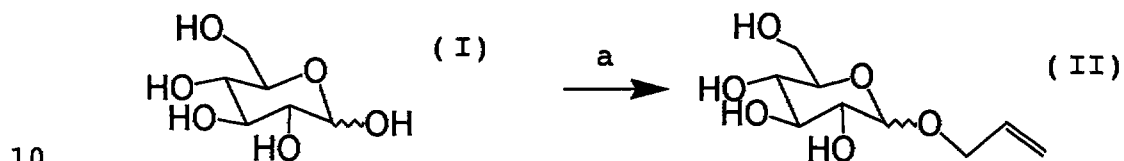
Furthermore, in Step h, a mixture of a monoester
and diester is obtained. The monoester and the diester
are separated from each other by chromatography and
25 subjected to Step i, respectively.

<Example 1>

Route a: 1-O-(2-propenyl)-D-glucose (II)

One hundred grams of D-glucose (I) was added
into 250 mL of allyl alcohol and sufficiently
30 dissolved therein. To the solution, 0.8 mL of

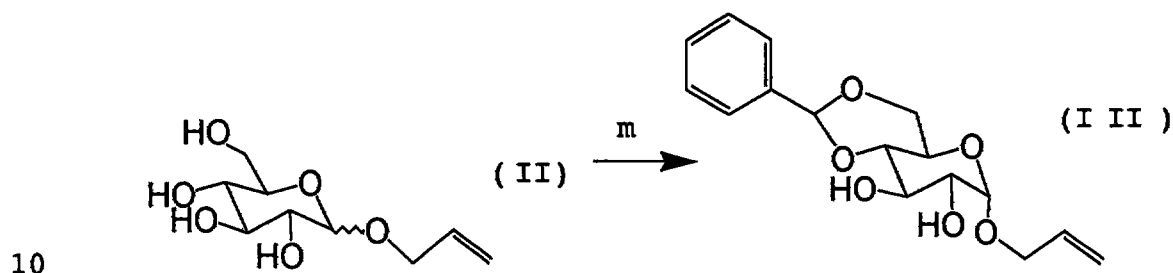
trifluoromethanesulfonic acid was gradually added under an ice-cooled condition. Then, the solution was reacted in an oil bath at 80°C for 30 hours while stirring. At the stage where the reaction sufficiently proceeded, the reaction mixture was neutralized with 1 mL of trimethylamine and concentrated in vacuo. The thin layer chromatography demonstrated a yield of about 60 - 70%.



Route m: 1-O-(2-propenyl)-4,6-O-benzylidene- α -D-glucose (III)

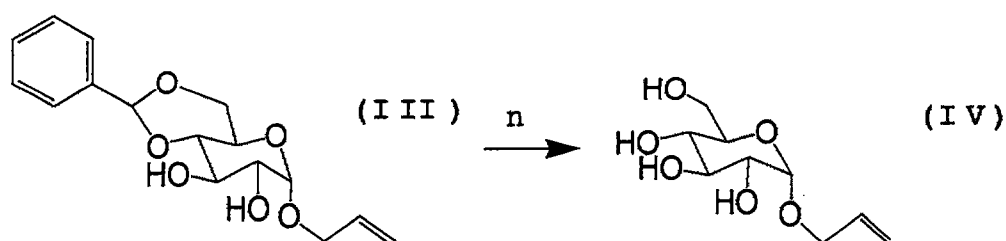
37.5 grams of the compound (II) was added to 210 mL of benzaldehyde and dissolved well. To the solution, 98 g of zinc chloride was added. The reaction mixture was reacted at room temperature for 4 hours. Thereafter, the reaction mixture was added to 500 mL of hexane, and then 100 mL of diluted sodium hydrogencarbonate was added. The reaction mixture was allowed to stand at 0°C for 30 minutes to crystallize. The crystal was filtered with suction, and dissolved in 50 mL of ethanol. The solution was allowed to stand at 0°C for 30 minutes for recrystallization (yield: 21g (68.1 mmol), recovery: 40.0%).

^1H NMR(300MHz, CDCl_3+TMS); 7.51-7.49(2H, m, Ar),
 7.38-7.33(3H, m, Ar), 5.98-5.85(1H, m, $-\text{CH}=\text{CH}_2$),
 5.51(1H, s, Ar- CH), 5.31(1H, dd, $J=1.5\&15.9$, $-\text{CH}=\text{CH}_2$),
 5.23(1H, dd, $J=1.2\&10.4$, $-\text{CH}=\text{CH}_2$), 4.90(1H, d, $J=3.9$,
 5 H-1), 4.28-4.19(2H, m, $-\text{CH}_2-\text{CH}=\text{CH}_2$), 4.06-4.00(1H, m,
 H-5), 3.93(1H, t, $J=9.3$, H-3), 3.87-3.78(1H, m, H-6a),
 3.70(1H, t, $J=10.2$, H-2), 3.60(1H, dd, $J=3.8\&9.2$, H-6b),
 3.47(1H, t, $J=9.3$, H-4)



Route n: 1-O-(2-propenyl)- α -D-glucose (IV)

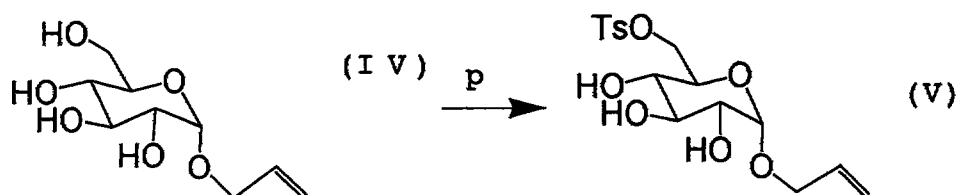
Into 260 mL of a solution of acetic acid and water
 (8 : 5), 10.7g (34.7 mmol) of the compound (III) was
 15 dissolved. The solution was reacted at 100°C for 1
 hour, concentrated in vacuo, and purified by silica
 gel flash chromatography (dichloromethane : methanol =
 6 : 1) (yield: 6.3g (28.6 mmol), recovery: 82.4%).
 ^1H NMR(300MHz, $\text{CD}_3\text{OD}+\text{TMS}$); 5.92-5.79(1H, m, $-\text{CH}=\text{CH}_2$),
 20 5.26-5.18(1H, m, $-\text{CH}=\text{CH}_2$), 5.07-5.03(1H, m, $-\text{CH}=\text{CH}_2$),
 4.23-3.23(7H, m)



Route p: 1-O-(2-propenyl)-6-O-(4-tolylsulfonyl)- α -D-glucose (V)

Into 200 mL of anhydrous pyridine, 6.3g (28.6 mmol) of the compound (IV) was dissolved, and 195 mg of p-dimethylaminopyridine (DMAP) and 7.0g of p-toluenesulfonyl chloride were added. The solution was reacted for 16 hours at room temperature while stirring. Thereafter, the reaction was quenched by adding 20 mL of cold distilled water, and the reaction mixture was extracted with ethyl acetate (3 $\times$ 200 mL). The organic layers were combined, neutralized to pH 4 with 1.0 M and 0.1 M hydrochloric acids, washed with brine (2 $\times$ 200 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo, and purified by silica gel flash chromatography (dichloromethane : methanol = 20 : 1) (yield: 8.6 mg (23.0 mmol), recovery: 83.8%).

^1H NMR(300MHz, CDCl_3 +TMS) ; 7.77(2H,d,J=8.3,Ar at TsCH₃), 7.30(2H, d, J=8.1 Ar at TsSO₂), 5.90-5.77(1H, m, -CH=CH₂), 5.24(1H, dd, J=1.4&17.2, -CH=CH₂), 5.11(1H, dd, J=1.2&12.4, -CH=CH₂), 4.79(1H, d, J=3.3, H-1), 4.38-3.38(8H, m), 2.40(3H, s, TSCH₃)



Route q: 2,3,4-tri-O-(t-butyltrimethylsilyl)-1-O-(2-propenyl)-6-O-(4-tolylsulfonyl)- α -D-glucose (VI)

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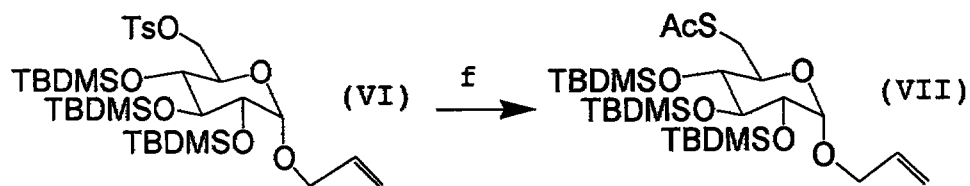


Route f: 2,3,4-tri-O-(t-butyldimethylsilyl)-1-O-(2-propenyl)-6-deoxy-6-acetylthio- α -D-glucose (VII)

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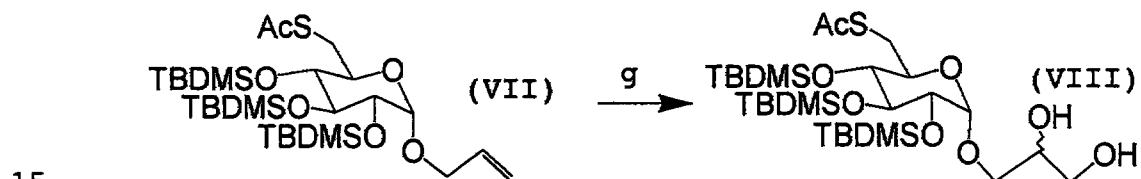
Route q: 3-O-[2,3,4-tri-O-(t-butylidimethylsilyl)-6-

20

25

1.5 hours to adsorb the osmium tetroxide. After filtration with suction, the reaction was quenched by adding 200 mL of cold distilled water, and extracted with ethyl acetate (3×200 mL). The organic layers were combined, washed with brine (2×300 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo, and purified by silica gel flash chromatography (hexane : ethyl acetate = 3 : 1 → 2 : 1) (yield: 5.2 g (7.94 mmol), recovery: 88.0%).

¹H NMR (300 MHz, CDCl₃ + TMS); 4.73 (1H, m, H-1(R and S)), 4.12–3.40 (10H, m), 2.86 (1H, dd, J=9.2&13.6, H-6b), 2.32 (3H, s, SC(=O)CH₃), 0.88–0.79 (27H, m, CH₃ at t-Bu), 0.08 – -0.03 (18H, m, Si-CH₃)



Route h: 3-O-[2,3,4-tri-O-(t-butyldimethylsilyl)-6-deoxy-6-acetylthio- α -D-glucopyranosyl]-1-O-oleoyl-glycerol (IX) and 3-O-[2,3,4-tri-O-(t-butyldimethylsilyl)-6-deoxy-6-acetylthio- α -D-glucopyranosyl]-1,2-di-O-oleoyl-glycerol (IX')

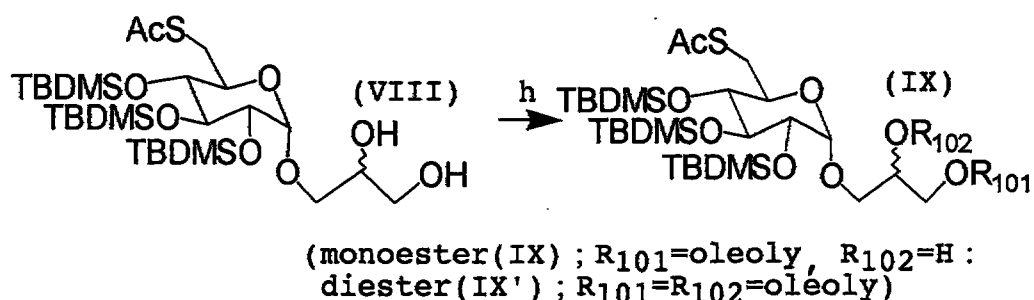
20

Into 20 mL of anhydrous dichloromethane, 1.37g (2.09 mmol) of the compound (VIII) was dissolved and then 600 mg of EDCI, 26 mg of DMAP and 660 mg of oleic acid were added. The solution was reacted at room temperature for 16 hours while stirring. Thereafter, the reaction was quenched by adding 200 mL of

25

dichloromethane, and washed with brine (2×100 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo, and purified by silica gel flash chromatography (hexane : ethyl acetate = 20 : 1 → 10 : 1 → 7 : 1) (yield of the diester: 772 mg (652 μmol) and yield of the monoester: 895 mg (974 μmol); recovery (both esters in total) of 78.0%).

¹H NMR(300MHz, CDCl₃+TMS); 5.32-5.28(2H, m, -CH=CH-), 4.68(1H, m, H-1(R and S)), 3.98-3.36(10H, m), 2.81(1H, dd, J=9.5&13.4, H-6b), 2.32-2.27(5H, m, OCOCH₂&SCOCH₃), 1.98-1.93(4H, m, CH₂-CH=CH-CH₂), 1.61-1.56(2H, m, OCOCH₂CH₂), 1.28-1.23(20H, br, -CH₂-), 0.88-0.79(30H, m, CH₃ at t-Bu & CH₃ at Acyl), 0.09 - -0.04(18H, m, Si-CH₃)(NMR of the monoester)

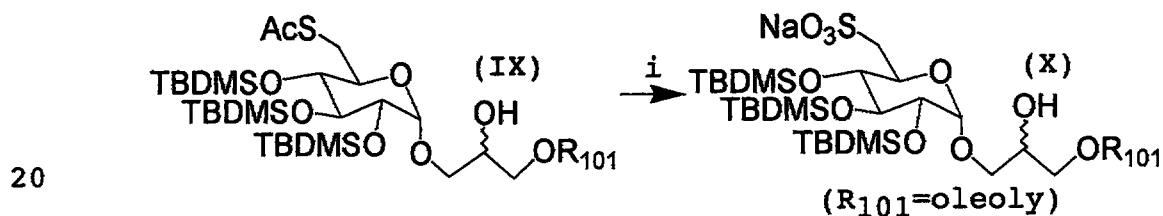


20 Route i: 3-O-[2,3,4-tri-O-(t-butyldimethylsilyl)-6-deoxy-6-sulfo-α-D-glucopyranosyl]-1-O-oleoyl-glycerol sodium salt (X)

Into 3.5 mL of glacial acetic acid, 21.4 mg (23.2 μmol) of the compound (IX: monoester) was dissolved and then 500 mg of potassium acetate and 35.4 mg of OXONE were added. The mixture was reacted at room temperature for 6 hours while stirring.

Thereafter, the reaction was quenched by adding 15 mL of cold distilled water, extracted with ethyl acetate (5×20 mL). The organic layers were combined, neutralized with saturated sodium hydrogencarbonate solution (5×70 mL), washed with brine (2×60 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo, and purified by silica gel flash chromatography (dichloromethane : methanol = 50 : 1 → 20 : 1). Thereafter, the reaction product was further purified by high performance liquid chromatography (ODS column, methanol : water = 80 : 20) (yield: 3.3 mg (3.49 μ mol), recovery: 15.0%).

^1H NMR (300MHz, $\text{CDCl}_3 + \text{TMS}$); 5.16-5.14 (2H, br, $-\text{CH}=\text{CH}-$), 4.60 (1H, br, H-1(R and S)), 4.31-2.88 (11H, m), 2.17-2.13 (2H, br, OCOCH_2), 1.82-1.80 (4H, br, $\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2$), 1.42 (2H, br, $\text{OCOCH}_2\text{CH}_2$), 1.11 (20H, br, $-\text{CH}_2-$), 0.72 (30H, m, CH_3 at t-Bu & CH_3 at Acyl), -0.08 (18H, br, Si- CH_3)

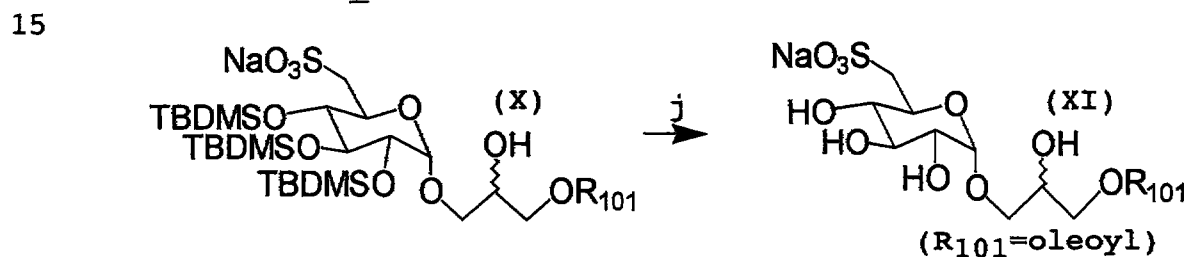


Route j: 3-O-(6-deoxy-6-sulfo- α -D-glucopyranosyl)-1-O-oleoyl-glycerol sodium salt (XI)

Into 7 mL of a solution of acetic acid, tetrahydrofuran, trifluoroacetic acid and water (3 : 1: 0.4 : 1), 358.4 mg (378 μ mol) of the compound (X) was dissolved. The solution was reacted at room

temperature for 16 hours while stirring, and the reaction mixture was extracted with ethyl acetate (3×10 mL). The organic layers were combined, washed with brine (2×20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo, and purified by silica gel flash chromatography (dichloromethane : methanol = 10 : 1 → dichloromethane : methanol : water = 65 : 25 : 4)(yield: 138.1 mg (229 μmol), recovery: 62.7%).

¹H NMR(300MHz, CD₃OD+TMS); 5.24-5.17(2H, m, -CH=CH-), 4.69(1H, m, H-1(R and S)), 4.18-2.75(11H, m), 2.29-2.21(2H, m, OCOCH₂), 1.94-1.90(4H, m, CH₂-CH=CH-CH₂), 1.49(2H, br, OCOCH₂CH₂), 1.20(20H, br, -CH₂-), 0.78(3H, t, J=6.3, CH₃)



<Example 2>

The Steps h-j were carried out in the same manner as in Example 1 except that myristoleic acid was used in place of oleic acid to synthesize 3-O-(6-deoxy-6-sulfo-α-D-glucopyranosyl)-1-O-myristoleoyl-glycerol sodium salt (yield: 118.7 mg (217 μmol), recovery: 59.8 %).

25 <Example 3>

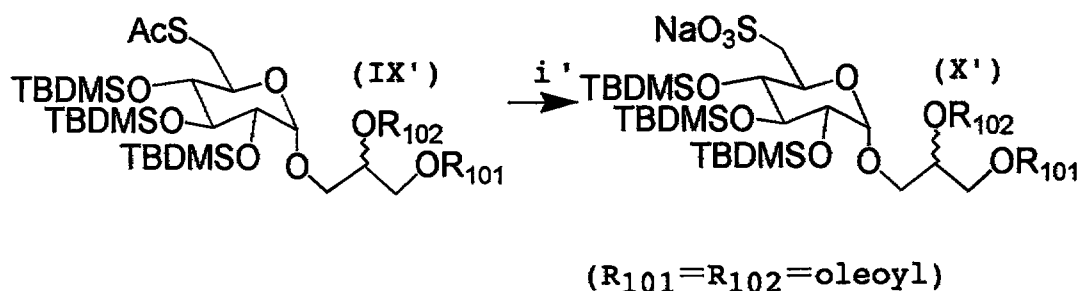
The same procedure as in Example 2 was repeated

except that palmitoleic acid was used in place of oleic acid to synthesize 3-O-(6-deoxy-6-sulfo- α -D-glucopyranosyl)-1-O-palmitoleoyl-glycerol sodium salt (yield: 142 mg (247 μ mol), recovery: 67.7 %).

5 <Example 4>

The same synthesis example as in Example 1, except that the compound (IX': diester) was used in place of the compound (IX: monoester) in the route i of preparing the compound (X) from the compound (IX), and
10 a molybdenum oxidizer was used in place of OXONE, will be described.

13.1 mg (11.0 μ mol) of the compound (IX': diester) was dissolved in 0.5 mL of dichloromethane and 0.5 mL of methanol. 50 μ L of 0.06M solution
15 of hexaammonium heptamolybdate tetrahydrate ((NH₄)₆Mo₇O₂₄ · 4H₂O) in 30% hydrogen peroxide was further added thereto and stirred at room temperature for 50 hours. Thereafter, 10 mL of ethyl acetate was added to the reaction solution, and the resultant
20 solution was washed with saturated sodium hydrogencarbonate solution (2×5 mL) and brine (2×5 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo, and purified by silica gel flash chromatography (dichloromethane : methanol =
25 50 : 1 → 10 : 1). As a result, a colorless oily substance was obtained (yield: 7.8 mg (6.4 μ mol), recovery: 58.2 %).



The compound represented by General Formula (1) of the present invention was subjected to a physiological assay.

<Assay 1>

An assay on inhibitory effect against a DNA polymerase α was carried out in the following manner.

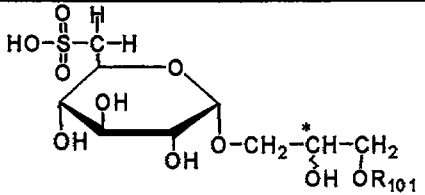
0.05 U of a DNA polymerase α purified and isolated from a bovine thymus by an immunoaffinity column was mixed with each of test compounds, sulfopyranosylacylglycerol (hereinafter, simply referred to as "SQAG") derivatives, namely, SQAG 1, SQAG 2 and SQAG 3 (listed in table 1) dissolved in DMSO. Each mixture was added with a buffer containing inorganic salts for the enzymatic reaction, [³H]-labeled dTTP and compounds for reaction containing a template DNA strand, and incubated at 37°C for 60 minutes.

After the enzymatic reaction was quenched, the resultant reaction product was fixed on a dedicated filter and subjected to measurement by a liquid scintillation counter. The amount of enzymatically incorporated dTTP was calculated as a radiation dose (cpm) of [³H]. Note that, each of the

sulfonylpyranosylglycerol derivatives is a mixture of the S- and R-configurations with respect to an absolute configuration of the carbon of the 2-position of the glycerol moiety.

5 The results are shown as IC₅₀ in Table 1 below.

Table 1: Inhibitory activity on DNA polymerase α

		DNA polymerase inhibitory activity
Compound	R ₁₀₁	IC ₅₀ (μg/mL)
SQAG1 (14:1)	CH ₃ -(CH ₂) ₃ -(CH=CH-CH ₂) ₁ -(CH ₂) ₆ -CO-	9.0
SQAG2 (16:1)	CH ₃ -(CH ₂) ₅ -(CH=CH-CH ₂) ₁ -(CH ₂) ₆ -CO-	5.5
SQAG3 (18:1)	CH ₃ -(CH ₂) ₇ -(CH=CH-CH ₂) ₁ -(CH ₂) ₆ -CO-	2.0

As is clear from Table 1, the compounds subjected to the assay exhibit significant inhibitory activity against the DNA polymerase α .

10 Colon cancer cells and gastric cancer cells used in the following two assays are only for the purpose of illustration of cancer cells for which the medicinally active agent of the present invention effectively works. Thus, in these assays, cancer cells for which
15 the medicament of the invention is effective are not limited.

<Assay 2>

An assay on anticancer activity against cultured colon cancer cells was carried out in the following

manner.

Colon cancer cells DLD-1 were maintained and subcultured in RPMI 1640 medium (containing 10% calf serum). Each of the test compounds (SQAG 2, SQAG 3 shown in Table 1) was suspended and diluted in the medium, and then the cancer cells were cultivated together with the medium in a 96-well plate at 3×10^3 cells/well. After 48 hour cultivation, the MTT assay (Mosmann, T: Journal of Immunological Method, 65, 55-63 (1983)) was carried out to compare survival rates.

The results are shown in FIG. 1.

In FIG. 1, open squares connected by a solid line indicate SQAG2 and open circles connected by a solid line indicate SQAG3.

As is clear from FIG. 1, all of the sulfopyranosylacylglycerol derivatives have significant anticancer activities against the colon cancer cells used.

20 <Assay 3>

An assay on anticancer activity against cultured gastric cancer cells was carried out in the same manner as in the assay 2 except that gastric cancer cells NUGC-3 were used instead of the colon cancer cells DLD-1.

The results are shown also in FIG. 1.

In FIG. 1, solid squares connected by a solid line

indicate SQAG2 and solid circles connected by a solid line indicate SQAG3.

As is clear from FIG. 1, the
sulfopyranosylacylglycerol derivatives have anticancer
5 activities against the gastric cancer cells used.

<Assay 4>

Tests for human cancer-cell implanted mice were
conducted in the following manner.

5×10^5 of human lung cancer cells A-549
10 cultured in an MEM medium containing 5% calf serum were
implanted in nude mice BALB/cAcl-nu. The size of the
tumor formation site was periodically measured.
When the size of the tumor reached 30-50 mm³ (42 days
after the implantation), the mice were subjected to
15 an administration test.

Five mice were assigned at random to test groups
and a control group. A test compound (SQAG1, SQAG2
and SQAG3 listed in Table 1) suspended in PBS in
a concentration of 100 μ g/100 μ L was administered to
20 the test groups, and PBS was administered to the
control group at a dose of 100 μ L, every 3 days.
This administration operation was repeated 8 times.
The size of the tumor formation site was measured at
all the administration times. The volume of the tumor
25 was calculated in accordance with the following
formula.

Volume of tumor = tumor-site length $\times$ (tumor site

width)² × 0.5

The results obtained for the test compounds are respectively shown in FIG. 2 (SQAG1), FIG. 3 (SQAG2) and FIG. 4 (SQAG3).

5 In each Figure, the horizontal axis represents days after implantation of the cancer-cell and the vertical axis represents the volume of a tumor.

 It was demonstrated that each of the test compounds significantly suppresses the formation of
10 the tumor, compared to the control group.

 No particular change was observed in the state of mice in the test groups at the aforementioned dose. The mice were alive in the same state as in the control group.

15 <Assay 5>

 5 × 10⁵ of cultured lung cancer cells A-549 were subcutaneously injected into each of 7 week-old female nude mice BALB/cAcl-nu having 20-22 g weight, and the size of tumor was measured every 3 days from 37 days
20 after the implantation. At 43 days after the implantation when the sizes of the tumor in all of the tumor-bearing mice reach 25-35 mm³, the mice were randomly divided into 7 groups of 4 mice for each. Of the 7 groups, one is used as a control group.
25 100 μL of PBS was subcutaneously injected into the mice of the control group. To the remaining 6 groups, SQAG1 (14:1), SQAG2(16:1) and SQAG3(18:1) were

subcutaneously injected by dissolving each of the test compounds in 100 μ L of PBS so as to give doses of 4 mg and 20 mg per 1 kg weight. The injection was performed every 3 days. This administration operation was
5 repeated 8 times from 43 days to 64 days after the cancer-cell implantation. The size of the tumor was measured every 3 days until 70 days after the implantation. The volume of the tumor was calculated in the same manner as in Assay 4.

10 The results obtained for the test compounds are respectively shown in FIG. 5 (SQAG1), FIG. 6(SQAG2) and FIG. 7 (SQAG3).

In each of the test groups, it was demonstrated that the formation of the tumors is significantly
15 suppressed compared to the control group.

After completion of the test, major organs, such as lung, heart, stomach, liver, pancreas, kidney, intestine and brain, of all the mice of each administration group were subjected to pathological
20 evaluation, and no pathologically abnormality was observed in any organ.

Industrial Applicability

As explained in the foregoing, according to the present invention, there is provided a medicament
25 containing at least one compound selected from the group consisting of sulfonyranosylacylglycerol derivatives represented by General Formula (1) and

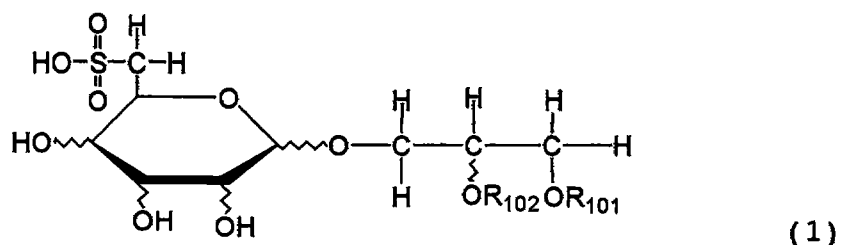
pharmaceutically acceptable salts thereof, as an active ingredient.



C L A I M S

1. A medicament containing, as an active ingredient, at least one compound selected from the group consisting of:

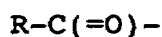
5 compounds represented by General formula (1):



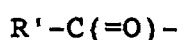
wherein R_{101} represents an acyl residue of an unsaturated higher fatty acid, and R_{102} represents a hydrogen atom or an acyl residue of an unsaturated higher fatty acid; and

pharmaceutically acceptable salts thereof.

2. The medicament according to claim 1, wherein said R_{101} of General formula (1) is represented by Formula:



wherein R represents a straight-chain aliphatic unsaturated hydrocarbon group having 13-25 carbon atoms and including 1-6 unsaturated bonds; and R_{102} represents a hydrogen atom or a group represented by Formula:



wherein R' represents a straight-chain aliphatic unsaturated hydrocarbon group having 13-25 carbon atoms and including 1-6 unsaturated bonds.



3. The medicament according to claim 2, wherein
R₁₀₂ of General formula (1) represents a hydrogen atom.

4. The medicament according to any one of
claims 1 to 3 wherein the pyranose in the compound
5 represented by General formula (1) is a quinovose.

5. The medicament according to claim 4, wherein
the quinovose is α -quinovose.

6. The medicament according to claim 4 being
a DNA polymerase inhibitor.

10 7. The medicament according to claim 4 being
an anticancer agent.