



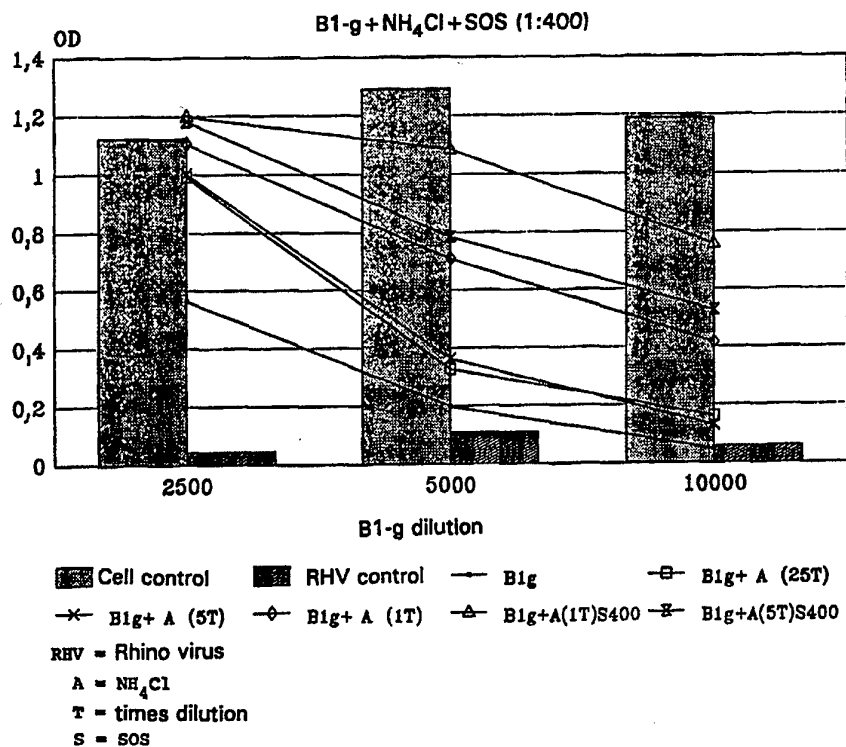
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/045, 9/68		A1	(11) International Publication Number: WO 95/35103
			(43) International Publication Date: 28 December 1995 (28.12.95)
(21) International Application Number: PCT/DK95/00256 (22) International Filing Date: 20 June 1995 (20.06.95) (30) Priority Data: 0722/94 20 June 1994 (20.06.94) DK 0926/94 9 August 1994 (09.08.94) DK (71)(72) Applicant and Inventor: BERG, Kurt [DK/DK]; Paul-Petersens Vej 5, DK-2820 Gentofte (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): CHRISTENSEN, Søren, Brøgger [DK/DK]; Åtoften 18-7, DK-02990 Nivå (DK). BOYE-KNUDSEN, Carsten [DK/DK]; Ægirsgade 19, 2. tv., DK-2200 Copenhagen N (DK). MING, Chen [CN/DK]; Henrik Harpestrangs Vej 5, 1. tv., DK-2100 Copenhagen Ø (DK). SIMONSEN, Beth [DK/DK]; Frihedsvej 6, DK-2960 Rungsted (DK). (74) Agent: CHAS.HUDE; H.C. Andersens Boulevard 33, DK-1553 Copenhagen V (DK).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published With international search report. In English translation (filed in Danish).	

(54) Title: A PHARMACEUTICAL COMPOSITION FOR THE PREVENTION AND/OR TREATMENT OF VIRAL INFECTIONS AND OPTIONALLY INFLAMMATIONS AS WELL AS A METHOD FOR THE TREATMENT THEREOF

(57) Abstract

A pharmaceutical composition for the prevention and/or treatment of viral infections and optionally inflammations comprises one or more β -lupeol derivatives, optionally in combination with an ammonium ion releasing compound, and/or in combination with one or more mono or polysulphated mono, oligo or polysaccharides or analogues and/or derivatives thereof. The pharmaceutical composition may be in the form of chewing gums, lozenges, chewing tablets, resorbibles, drops, troches, gels, mouth ointments, solutions, mucoadhesive formulations or depot preparations. Furthermore, a method of preventing and treating viral infections and optionally inflammations by oral administration of the pharmaceutical composition.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Title: A pharmaceutical composition for the prevention and/or treatment of viral infections and optionally inflammations as well as a method for the treatment thereof

Field of the Invention

5 The present invention relates to a pharmaceutical composition for the prevention and treatment of viral infections and optionally inflammations accompanying viral infections. The invention relates more specifically to pharmaceutical compositions comprising β -lupeol as the antivirally active substance. The invention relates furthermore to a method of preventing
10 and treating viral infections and optionally inflammations by oral administration of the pharmaceutical composition to a person with a need thereof.

Background Art

Until now, it has been impossible to provide an efficient composition for
15 preventing and/or treating viral infections caused by cold virus etc, such as influenza virus, Rhino virus, Corona virus etc. or other viruses in the upper respiratory passages. Practically all humans suffer from infections in the upper respiratory passages from time to time, such as cold and flu. The symptoms of these infections include a sore throat and earache (otitis), a
20 runny nose, itchy eyes, and a pain in the muscles and the joints. The infections are caused by a variety of different viruses which together are referred to as "cold virus". Although vaccines are available for a restricted number of influenza strains, no efficient methods are known for preventing or treating most of the infections in the upper respiratory passages. Such
25 viral infections, e.g. caused by Rhino virus, which is responsible for approximately 50% of all viral infections in the upper respiratory passages, are wide-spread and can cause ill health or be directly potentially lethal for susceptible groups, such as children, elderly people, and persons suffering

from a deficient immunity, such as AIDS-patients, cancer patients etc. A method of treating these symptoms and the underlying infections would be of immense importance.

GB Patent Application No. 2,198,041 A discloses compositions which i.a. contain lupeol. The compositions are stated to have an effect on alcohol addiction, but it does not appear that this effect can be ascribed to lupeol.

EP-A-O 287 000 discloses a process for the preparation of plant extracts, which i.a. may contain lupeol. These extracts are stated to be applicable by the treatment of prostatic hypertrophy, but it does not appear whether the effect can be ascribed to lupeol.

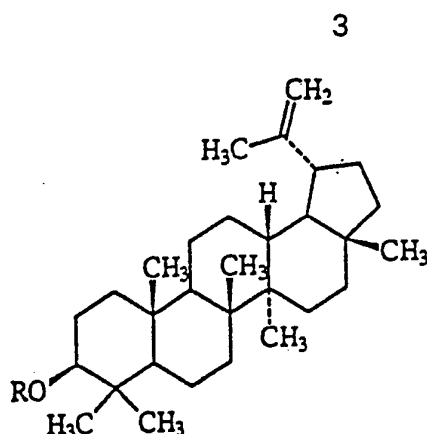
WO 90/14764 discloses a number of terpenozonides having an antiviral effect. These compounds differ, however, essentially from β -lupeol, as they contain three oxygen atoms to form a trioxycyclopentane ring. The antiviral effect is ascribed to this trioxycyclopentane ring system.

Aqueous, unpurified extracts of bitter ginseng orally administered have been used for many years in China against chronic hepatitis. The chemical compound or compounds active by the above treatment are, however, not known. Thus it could not be foreseen that a specific fraction can be extracted from bitter ginseng, viz. β -lupeol, which has the unexpected useful effect described in the present specification.

Brief Description of the Invention.

The invention relates according to a first aspect to a pharmaceutical composition for the prevention and/or treatment of viral infections, said composition being characterised by comprising

one or more β -lupeol derivatives of the formula



where R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group, which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, as well as conventional pharmaceutically acceptable adjuvants, additives, and carriers.

- 5 10 The aliphatic C₁₋₆-hydrocarbyl group includes methyl, ethyl, branched and unbranched propyl, butyl, pentyl and hexyl, ethenyl, branched and unbranched propenyl, butenyl, pentenyl and hexenyl, ethynyl, branched and unbranched propynyl, butynyl and hexynyl and corresponding compounds containing two or more double or triple bonds.
- 15 The C₁₋₆-acyl group includes methanoyl, ethanoyl, branched and unbranched propanoyl, butanoyl, pentanoyl and hexanoyl, ethenoyl, branched and unbranched propenoyl, butenoyl, pentenoyl and hexenoyl, butynoyl, branched and unbranched propynoyl, butynoyl, pentynoyl and hexynoyl and corresponding compounds containing two or more double or
- 20 triple bonds.

It should be understood that a group which is easily decomposed under

the conditions prevailing in the human or animal body includes any group that can be transformed into the β -lupeol derivative under physiological conditions.

According to a particularly preferred embodiment of the invention, R is
5 hydrogen.

In addition, it has been found that the presence of ammonium ions provides an antiviral effect against a number of laboratory viruses, such as VSV (= Vesicular Stomatitis Virus) and Semliki virus, as well as against for instance Rhino virus. The most probable mechanism of the antiviral
10 effect mediated through ammonium ions is considered to be related to the fact that ammonium ions interfere with the binding of ammonium-sensitive viruses to virus receptors on the target cell and therefore improve the capacity of the host or the environment of eliminating the virus via non-specific cell processes, or via neutralization by means of suitable
15 antibodies. Such viruses include for instance HIV-virus, hepatitis virus, usual cold viruses (such as Rhino virus, influenza virus etc.) or other infectious ammoniumion-sensitive viruses.

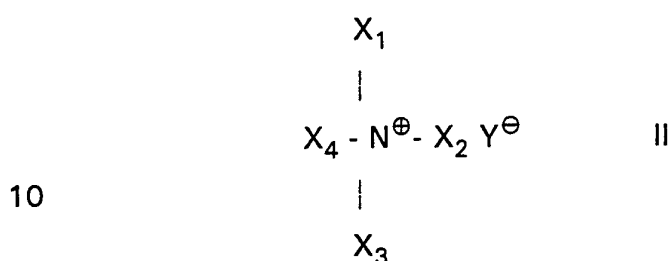
Based on preliminary experiments it appears that ammonium ions have an effect exclusively on the receptor level through membrane-like interactions,
20 as said ammonium ions must be constantly present at the time when the virus is introduced in the cell cultures in order to provide an optimum antiviral effect.

Therefore another aspect of the invention is to provide a pharmaceutical composition comprising a β -lupeol derivative of the above formula I as well
25 as an ammonium ion releasing compound.

The ammonium ions are preferably derived from a salt of a pharmaceutically acceptable inorganic or organic acid. Any pharmaceutically

acceptable acid can be used, and examples thereof are hydrochloric acid, sulphuric acid, phosphoric acid, carbonic acid, acetic acid, and tartaric acid. Ammonium chloride, ammonium sulphate, ammonium hydrogen carbonate or monoammonium dihydrogen phosphate are preferably used.

- 5 The ammonium ions may furthermore be derived from a compound of the general formula II



where X_1 - X_4 , which may be identical or different, are selected from hydrogen; C_{1-6} alkyl, which may be straight-chained or branched, saturated or unsaturated and may optionally contain one or more substituents
 15 selected from halogen, hydroxy, C_{1-4} -alkoxy or amino; aryl, which is optionally substituted with C_{1-4} alkyl, halogen, hydroxy, C_{1-4} -alkoxy or amino, and

Y is a physiologically acceptable salt-forming anion, preferably selected from F^- , Cl^- , Br^- and I^- .

20

It has been found that the combination of β -lupeol and ammonium ions provides a synergistic antiviral effect against a number of viruses, such as VSV, Rhino virus and probably also influenza virus.

A third aspect of the invention relates to a pharmaceutical composition as
 25 defined above and further comprising one or more mono or polysulphated mono, oligo or polysaccharides or analogues and/or derivatives thereof, including compounds with heparin or heparan structure, which do not possess essential anti-coagulant properties.

Viral infections are known to produce inflammations which are probably mediated via neutrophilic granulocytes accumulated in the affected area and causing further inflammation through the release of various substances, such as cytokines and other mediators. Furthermore, it is
5 thought that cationic protein complexes adjacent to or situated in the neutrophilic granulocytes play an important role as they promote the inflammatory reactions causing the known cold symptoms (sore throat, pain in the joints, fever, etc.). Preliminary experiments indicate that the mere presence of a highly anionic substance related to the heparin
10 structure, but without the anti-coagulant effect of heparin, such as the sodium salt of sucrose octasulphate (SOS), or another SOS-like component, can counteract this process because the latter may optionally "neutralize" the charge of the cationic proteins present in the accumulated neutrophilic granulocytes. The latter granulocytes are bound to the
15 virus-infected cells through ICAM-1-markers with the result that the usual inflammatory reactions are considerably reduced or completely suppressed.

It is known to use sulphated sugars including the aluminum complex of sucrose octasulphate, sucralphate, in the treatment of inflammations in the gastrointestinal region or for topical application on the skin for prophylaxis
20 or treatment of inflammation, cf. for instance DK printed accepted application No. 165,357 and DK-PS No. 169,018. Furthermore, EP Patent Application No. 0 230 023 A2 discloses pharmaceutical compositions comprising sulphated oligosaccharides including sucrose octasulphate, for promoting ulcer healing. Thus it is assumed that SOS together with local
25 growth factors form a biologically active complex which initiates and stabilizes, respectively, the growth factors resulting in accelerated ulcer healing processes.

The presence of sulphated saccharides in or around the upper respiratory passages is thought to be advantageous in that these substances can
30 accelerate the ulcer healing/curing in the throat or the oral cavity during

minor microbial infections, especially during virus infections causing inflammations, e.g. by the presence of cationic substances. The sulphated saccharides will be retained in the inflammatory areas and thereby reduce the inflammatory processes in the affected area.

- 5 A particular aspect of the present invention is therefore sulphated saccharides for use as an anti-inflammatory substance in the oral cavity and the lymphatic ring, respectively, around the lower respiratory passages (below the nasopharynx), as well as a method of treating inflammations in this area.
- 10 According to an embodiment of the present invention, the saccharide is a mono or polysulphated mono, di, tri or tetrasaccharide. According to a particular embodiment, the saccharide is a monosaccharide selected from xylose, fructose and glucose or a disaccharide selected from sucrose, lactose, maltose and cellobiose.
- 15 In a preferred embodiment the saccharide forms a complex or a salt with ammonium ions or with a metal selected from Al, Na, K, Ca, Mg, Ba, Zn, Cu, Zr, Ti, Bi, Mn, and Os, or with an amino acid.

- According to a preferred embodiment of the present invention the sulphated disaccharide is sucrose octasulphate or a complex or a salt of
- 20 sucrose octasulphate with ammonium ions or with a metal selected from Al, Na, K, Ca, Mg, Ba, Zn, Cu, Zr, Ti, Bi, Mn and Os, or a salt of sucrose octasulphate with an amino acid.

- Among these sucrose octasulphate or the sodium, potassium or NH_4^+ salt thereof or the aluminum complex of sucrose octasulphate, sucralphate, are
- 25 preferred.

Interferons usually present under ordinary virus infections, especially in

connection with colds, have been shown to intensify the antiviral effect of β -lupeol and ammonium ions. Thus it has been found that interferons in relatively low concentrations of 0.1-2 units/ml intensify the antiviral effect.

Furthermore, it can be advantageous as a further ingredient of the pharmaceutical composition to use human or non-human immunoglobulines directed towards the substances contributing to intensify colds, such as microorganisms (virus) etc.

According to a particular embodiment of the invention the pharmaceutical compositions comprise therefore as a further ingredient human or non-human immunoglobulines.

The pharmaceutical composition is preferably in the form of chewing gums, lozenges, chewing tablets, resorbibles, drops, troches, gels, mouth ointments, solutions or in form of mucoadhesive formulations, preferably in the form of depot preparations. By depot preparations is in this connection to be understood preparations and formulations with a controlled, sustained release of active ingredients.

The pharmaceutical composition is preferably in the form of a chewing gum, which per piece of chewing gum having a weight of 500 to 3000 mg, preferably of approximately 1000 mg, comprises:

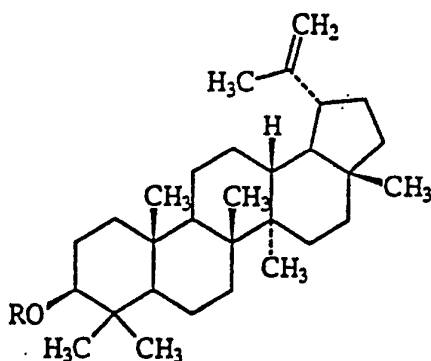
- 20 a) 0.01 to 2000, preferably 0.15-1000, particularly preferred 1-800, such as 20-600 μg of a β -lupeol derivative/piece, calculated as β -lupeol,
- b) 0 to 100, preferably 1-50, particularly preferred 2 to 40, such as 5-30 mg of NH_4^+ -ions/piece, calculated as ammonium chloride,

- c) 0 to 1000, preferably 10-500, particularly preferred 25-250 mg of a sulphated saccharide/piece, calculated as SOS,

as well as conventional chewing gum ingredients.

The chewing gum is prepared by means of conventional chewing gum bases and conventional chewing gum additives, such as sweeteners, flavours, colorants, softeners, and texturizing substances. It may furthermore be necessary to use solubilizers or other release-controlling measures in order to release the pharmacologically active substances disclosed herein from the chewing gum. A further illustration of solubilizers can for instance be found in EP-0 486 563 B1, in which a general mention of the preparation of chewing gum is found together with examples of applicable chewing gum ingredients.

The invention relates furthermore to the use of one or more β -lupeol derivatives of the general formula I

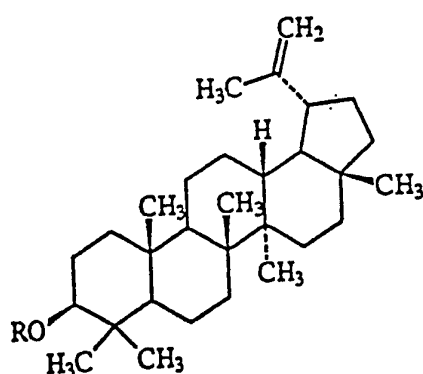


I

- 15 in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple

bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative for the preparation of a medicament for the prevention and/or treatment of viral infections.

- 5 Furthermore, the invention relates to the use of one or more β -lupeol derivatives of the general formula I

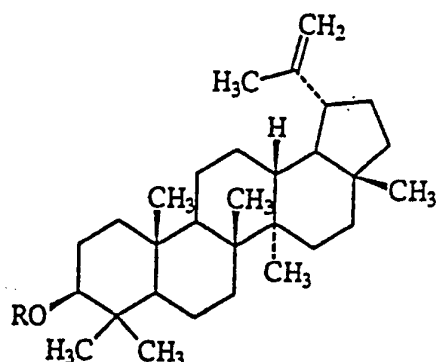


I

- in which R represents a hydrogen atom, a straight-chained or branched aliphatic C_{1-6} -hydrocarbonyl group, which may be saturated or may contain
- 10 one or more unsaturated bonds selected from double and triple bonds, a C_{1-6} -acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative,
- 15 as well as one or more ammonium ion releasing compounds for the preparation of a medicament for the prevention and/or treatment of viral infections.

Furthermore the invention relates to the use of one or more β -lupeol derivatives of the formula I

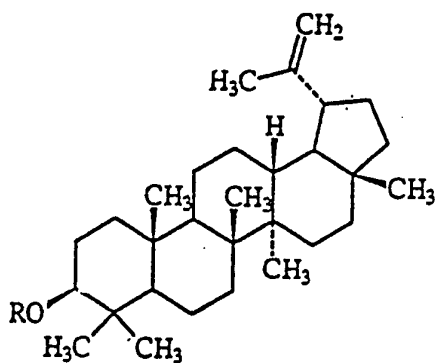
11



I

in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, as well as one or more mono or polysulphated mono, oligo, or polysaccharides or analogues or derivatives thereof, for the preparation of a medicament for the prevention and/or treatment of viral infections and associated inflammations.

Finally, the invention relates to the use of one or more β -lupeol derivatives of the formula I



I

in which R represents a hydrogen atom, a straight-chained or branched

aliphatic C₁₋₆-hydrocarbonyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple
5 bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, one or more ammonium ion releasing compounds, as well as one or more mono or polysulphated mono, oligo, or polysaccharides for the preparation of a medicament for the prevention and/or treatment of viral infections and
10 associated inflammations.

Thus the invention is particularly useful in treating infections in the upper respiratory passages, especially cold viruses, such as Rhino virus, influenza virus, enterovirus, Coxsackie virus and other cold viruses.

In addition, the invention allows the use of one or more of the above
15 mentioned active ingredients for treating HIV, hepatitis virus, cytomegalo virus, herpes virus and other viral infections as well as for treating atherosclerosis as well as for suppressing tumour cell growth.

Antivirally active substances may function in various ways:

(i) either as a substance capable of protecting the target cells provided it
20 is present simultaneously with the virus. If the latter is a condition for producing the antiviral activity, it is very likely that the antiviral effects involve a direct binding of the antiviral substance either to the virus or the receptor thereof or a combination thereof. Many plant extracts will show this type of "non-specific", receptor-dependent antiviral activity. Most
25 frequently, it is only possible to produce this type of antiviral activity provided the substance is present at all times, especially from the time the virus is added,

(ii) or as a substance which is capable of showing an effect without being present during the actual virus infection, such as in connection with a previous contact with the target cell, or by being present after the virus infection, but before a substantial production of viruses has taken place.

5 It is very likely that through this type of antiviral substances more fundamental changes inside the cells are produced via the synthesis of intracellular proteins/enzymes, which secondarily cause a relatively specific inhibition of the transcription and/or the translation of the virus in such a manner that the new intracellular proteins result in a so-called "antiviral
10 state" of the cell. When the antiviral state has been produced in the cell, the substance need no longer be present in principle as the cells are protected for a certain period of time, although the protection must be expected to decrease gradually over time.

Ammonium ions are thought to belong to type (i) in the effect mechanism,
15 although a certain, but weaker antiviral activity can be measured in cell cultures by the addition of NH_4^+ 2 to 4 hours after the infection.

β -lupeol is thought to belong to type (ii) in the effect mechanism.

β -lupeol is present in many plants, such as in the shell of lupin seeds, in chiccle rubber, in latex from figs and rubber plants, and in various
20 medicinal plants, such as in extracts from bitter ginseng. β -lupeol is commercially available and may be obtained e.g. from the company Sigma.

The scope of applicability of the invention will appear from the following with reference to the drawings and the examples. It should, however, be understood that the detailed description and the specific examples are
25 merely included to illustrate preferred embodiments, and that various alterations and modifications within the scope of protection will be obvious to persons skilled in the art on the basis of the detailed description.

Brief Description of the Drawings

The invention is explained in greater detail with reference to the drawings, in which

Fig. 1 illustrates the antiviral activity of β -lupeol (also called B1-g) against
5 Rhino virus,

Fig. 2 the antiviral activity of interferon- α (HuIFN- α) against Rhino virus,

Fig. 3 the antiviral activity of B1-g against EMC virus,

Fig. 4A the antiviral activity of B1-g against Rhino virus at various dilutions,

10 Fig. 4B the antiviral activity of B1-g + interferon- α against Rhino virus,

Fig. 5 the antiviral activity of NH_4^+ ions against VSV, Semliki virus and EMCIII virus,

Fig. 6 the antiviral activity of NH_4Cl against Rhino virus,

Fig. 7A the antiviral activity of B1-g, B1-g + NH_4Cl as well as B1-g,
15 NH_4Cl + SOS against Rhino virus at an SOS dilution of 1:100 relative to a 20% SOS stock solution in water,

Fig. 7B the same at an SOS dilution of 1:200,

Fig. 7C the same at an SOS dilution of 1:400,

Fig. 8 the antiviral activity of B1-g, NH_4Cl , SOS and interferon- α against
20 Rhino virus, and

Fig. 9 the kinetics for the induction of an antiviral state.

Detailed Description of the Invention

The method used for determining antiviral activity is described below.

The cell cultures employed are VERO cells, WISH cells, MDBK cells and
5 HEP cells which are common laboratory cell cultures and which are
described in greater detail in Berg, K.: Purification and characterisation of
murine and human interferons. A review of the literature of the 1970s
(thesis). Acta Pathol. Microbiol. Scand., Sec. C., Suppl. 279.: page 1-136,
1982. The viruses employed are VSV, EMC, Semliki virus, influenza virus
10 and Rhino virus.

Briefly a single-layer cell culture is established in microtrays. A certain
amount of the antivirally active substance in a suitable dilution is added to
the cell culture together with or followed by a suitable amount of virus
("challenge virus"). A control culture receives nothing but challenge virus.
15 The virus infected cultures are incubated until the production of virus is
distinct in the virus control culture (4 to 5 days as far as Rhino virus is
concerned). An MTS/PMS solution comprising 1.0 ml MTS stock solution
(110 μ g MTS + 39.2 ml PBS, pH-value 5.6 kept at +4°C in the dark), 2.3
ml medium and 30 μ l PMS stock solution (13 mg PMS (Sigma, H5004, Lot
20 13, P. 9625) + 6.5 ml distilled water, kept at 4°C in the dark with a layer
of paraffin oil on the top) is added, and based on OD(optical density)-
readings in an OD-scanner the relative protection of the cells against viral
attacks can be calculated. A high OD-reading indicates that the cells are
protected against virus, and a low OD-value indicates that the cells have
25 been killed by virus. Thus, the virus control cultures will typically have an
OD-value of < 0.100, while non-infected control cell cultures will have an
OD-value > 1.000. An antivirally active substance is thus a substance
being capable in the presence of medium and challenge virus to provide

protection against the test virus in a cell culture.

As far as the MTS-methods are concerned, reference is furthermore made to Berg, K., B. H. Simonsen, M. B. Hansen, and S. Nielsen: A method for analysing a sample for the presence of a biological substance, especially
5 a virus, use of the method for quantitative determination of biological substances and agents for use as novel substances detected by the method. PCT/DK/89/00010. 1 to 32, 1989.

Hansen M. B., S. E. Nielsen, and K. Berg: Re-examination and further development of a precise and rapid dye method for measuring cell
10 growth/cell kill. J. Immunol. Methods. 119: 203 to 210, 1989.

Berg, K., M. B. Hansen and S. E. Nielsen: A sensitive bioassay for precise quantification of interferon activity as measured via the mitochondrial dehydrogenase function in cells (MTT-method). AMPIS 98: 156 to 162, 1990.

15

EXAMPLES

Example 1

Antiviral activity of β -lupeol measured by means of the MTS-system

500 to 1000 WISH-cells in 100 μ l medium were seeded in wells in a microtray and incubated for 24 hours at 34°C in an atmosphere containing
20 5% CO₂. The medium was replaced by fresh medium containing dilutions of β -lupeol (25 to 1.6 μ g/ml, cf. Fig. 1) and incubated for further 24 hours at 34°C in an atmosphere containing 5% CO₂. The following day challenge Rhino virus was added and after 4 to 5 days at 34°C in an atmosphere containing 5% CO₂ MTS was added over 2 hours, whereafter the
25 microtray was measured in an OD-scanner. A total protection against

Rhino virus was obtained at 3 μ g/ml β -lupeol (= B1-g). However, at high concentrations of β -lupeol a decreasing cell number appears which must be ascribed to some toxicity of β -lupeol at such concentrations.

Example 2

5 Antiviral activity of interferon- α (rHuIFN- α -2b, "intron A") against Rhino virus

10,000 WISH cells were seeded in a microtray, and the following morning the medium was replaced by two-fold dilutions of HuIFN- α -2b ("intron A") in fresh medium containing 2% serum (cf. Fig. 2). On the following
10 morning the medium was replaced by fresh medium containing Rhino virus. The results in Fig. 2 clearly show that Rhino virus is relatively sensitive to HuIFN- α -2b, and that a protection of approximately 90% is achieved at approximately 8 units IFN/ml. Furthermore, the toxicity of intron A appears to be negligible.

15 Example 3

Antiviral activity of B1-g

10,000 WISH cells were seeded and incubated at 37°C for 24 hours as described in Example 2, and dilutions of B1-g were added to the cultures in dilutions corresponding to the concentration range indicated in Example
20 1. After 24 hours the medium was replaced by challenge virus in fresh medium while simultaneously growing challenge virus control cultures and non-infected control cultures. 24 hours later, the cultures were incubated with MTS for 2 hours at 37°C, and the tray was scanned as described above.

The results (Fig. 3) show that B1-g has a moderate antiviral activity against EMC virus. Similar results were obtained against VSV and Semliki virus. The addition of small amounts of interferon- α intensified the antiviral activity considerably. Thus, as little as 0.5 units of interferon resulted in
5 almost 80% protection compared to 30% protection without interferon. It should be noted, that very often interferon is present in these amounts (0.2 to 0.6 units/ml) in patients suffering from moderate viral infections, such as ordinary cold and the like.

Example 4

10 Anti-Rhino virus activity of B1-g

A corresponding experiment as described above was performed with Rhino virus. As illustrated in Fig. 4A, Rhino virus appears to be much more sensitive to B1-g at a dilution of 1:100 - 1:200 than for instance VSV and EMC (from a 1 mg/ml stock solution of B1-g), as it is able to suppress the
15 viral infection by more than 80 to 90%. Corresponding results must be expected with influenza virus. Thus it appears that B1-g has a very strong activity towards Rhino virus compared to the effect towards VSV and EMC. This difference could not be foreseen.

The addition of 0.5 units of interferon- α /ml intensified the antiviral activity
20 to a significant extent, cf. Fig. 4B.

Example 5

Antiviral activity of NH_4^+

10,000 WISH cells were seeded in wells in a microtray for 24 hours and incubated for 24 hours at 37°C in an atmosphere containing 5% CO₂.
25 Subsequently, the medium was replaced by fresh medium containing

dilutions of NH_4^+ and virus, and after incubation for 24 hours at 37°C in an atmosphere containing 5% CO_2 , MTS was added over 2 hours at 37°C and 5% CO_2 , whereafter the microtray was measured in a OD-scanner.

As illustrated in Fig. 5, NH_4^+ ions are capable of inhibiting VSV, and to a
5 minor extent Semliki virus, whereas no protection appears against EMC.

Example 6

Anti-Rhino virus activity of NH_4^+

As described in Example 5, the antiviral activity of NH_4^+ towards Rhino
virus was tested, and after incubation for 24 hours at 37°C in an
10 atmosphere containing 5% CO_2 , MTS was added over 2 hours at 37°C
and 5% CO_2 , whereafter the microtray was measured in an OD-scanner.

As it appears from the results in Fig. 6, a strong antiviral effect is obtained
by a dilution of a saturated NH_4Cl -solution of 1:900. In contrast a toxic
effect appears at higher concentrations of NH_4^+ for the laboratory culture
15 employed. The toxicity in vivo for humans is, however, as it is well-
known, negligible ammonium chloride being an ingredient of inter alia
liquorice.

As it appears from Figs. 5 and 6, NH_4^+ possesses a varying antiviral
strength towards different viruses, and furthermore the NH_4^+
20 concentration varies which in each particular case provides the optimum
antiviral effect.

Example 7

Antiviral activity of B1-g, B1-g + NH_4Cl as well as B1-g + NH_4Cl + SOS towards Rhino virus

Tests were carried out as described in Example 5, whereby, however, the temperature was 34°C and the incubation was carried out for 4 to 5 days. The results appear from Figs. 7A, 7B and 7C.

Neither the use of SOS alone in the dilutions of 1:100, 1:200 or 1:400, 5 NH₄Cl alone at the dilutions of 1:1000 or 1:2000 nor NH₄Cl in combination with SOS have any significant antiviral effect.

The use of B1-g alone reveals a good effect being intensified by the simultaneous use of NH₄Cl, which alone at 34°C only provides a very low protection. Nevertheless, an increasing effect is obtained with an 10 increasing NH₄⁺ concentration. The additional use of SOS in the dilution of 1:100 provides only a moderate increase of the effect.

When comparing Figs. 7A, 7B and 7C it appears that the favourable effect of the combination of B1-g, NH₄Cl and SOS is most significant at an SOS-dilution of 1:400 (Fig. 7C), where a protection of almost 95% is found 15 corresponding to a B1-g concentration of significantly less than 1 µg/ml. The fact that the most favourable effect is obtained at the lowest concentration of SOS tested is probably due to some toxicity of SOS towards the laboratory cells used. SOS is, however, known to be completely non-toxic to humans in all concentrations relevant in practice.

20 Example 8

Anti-Rhino virus activity of B1-g, NH₄Cl, SOS and interferon-α

Tests were carried out as described in Example 5, whereby all the substances were added simultaneously with the virus. The results appear from Fig. 8. As it appears, interferon-α in an amount of 0.5 units/ml 25 intensifies further the favourable effect obtained by a combination of

NH_4Cl , B1-g and SOS, whereby an almost total protection is obtained by the use of B1-g, NH_4^+ ions and interferon- α .

Accordingly, the natural presence of interferon in a human during an infection must be expected to have an intensifying effect on B1-g and
5 NH_4^+ . Analogous results appear with SOS in the dilutions of 1:200 and 1:400 (not shown). Similar results are obtained with 0.25 and 0.125 units of interferon/ml.

Example 9

Antiviral activity of B1-g, NH_4^+ ions, SOS, interferons and combinations
10 thereof

The antiviral activity was measured according to the above method. Four different viruses (EMC, VSV, Semliki Forest virus as well as Rhino virus) and three different cell lines (A-549, WISH, VERO) were used for the tests. The results appear from the Table below.

TABLE 1

Antiviral activity against different viruses on different cell lines.

Target cell	WISH cell			A-549 cell			VERO cell			WISH cell
Virus	VSV	Semliki	EMC	VSV	Semliki	EMC	Influenza A	Influenza B	Rhino	
Antiviral component										
SOS	-	-	+	-	-	+	-	-	-	-
(NH ₄) ₂ SO ₄	+	+	-	+	+	-	+	+	+	+
NH ₄ Cl	+	-	-	+	-	-	+	+	+	+
SOS+ (NH ₄) ₂ SO ₄	+	+	+	+	+	+	ND	ND	ND	+
HuIFN- α	++	++	+++	++	++	++	ND	ND	ND	++
B1-g	+	-/+	+	+	+	+	ND	ND	ND	++
B1-g+HuIFN- α	+	+	+	+	+	+	ND	ND	ND	+++
B1-g+NH ₄ Cl	ND	ND	ND	ND	ND	ND	ND	ND	ND	+++
B1-g+SOS	ND	ND	ND	ND	ND	ND	ND	ND	ND	++
B1-g+NH ₄ Cl+SOS	ND	ND	ND		ND	ND	ND	ND	ND	++++
B1-g+NH ₄ Cl+HuIFN- α	ND	ND	ND		ND	ND	ND	ND	ND	++++

VSV virus, Semliki Forest virus and influenza virus belong to the enveloped viruses; EMC and Rhino virus belong to the non-enveloped viruses.

ND = not determined.

As it appears from the above, Rhino virus is inhibited by ammonium ions and by B1-g as well as by interferon- α . Influenza virus is also assumed to be inhibited by ammonium ions. SOS appears to have some antiviral effect towards EMC, but no detectable antiviral activity towards Rhino virus. It
5 appears clearly that Rhino virus (which exemplifies a cold virus) is inhibited by the combination of B1-g, NH_4^+ , interferon $\pm$ SOS.

Example 10

Kinetic tests

A test was performed to examine possible differences in the antiviral
10 effect as a function of the time for the initiation of the antiviral treatment relative to the establishment of the viral infection.

500 to 1000 WISH cells were seeded on day -1 in wells in microtrays and divided into 3 groups. To one group of cells (group -24h) was added B1-g in various concentrations in the range of 25 to 1.6 $\mu\text{g/ml}$, whereafter all of
15 the cells were incubated for 24 hours at 37°C in an atmosphere containing 5% CO_2 . On day 0 Rhino-challenge virus was added to all of the wells, and simultaneously B1-g was added to another group of cells (group 0h). The incubation was continued for 24 hours at 34°C and 5% CO_2 . Subsequently, the third group of cells received B1-g (group +24h), and all
20 of the cells were further incubated for 4 to 5 days at 34°C and 5% CO_2 followed by an MTS treatment and measuring in an OD-scanner as described above. The results appear from Fig. 9.

As it appears, the antiviral effect is almost the same regardless whether the B1-g addition is carried out 24 hours before the viral infection or
25 simultaneously with said viral infection.

Furthermore it is seen that even if the B1-g treatment is not initiated until

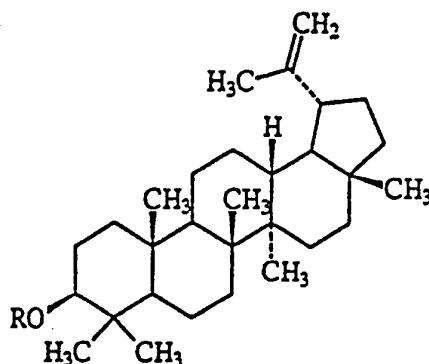
24 hours after the viral infection, i.e. at the time where the viral infection has manifested itself, a distinct antiviral effect is obtained.

While the invention has been described with reference to specific embodiments thereof, it is obvious that it can be varied in many ways.

- 5 Such variations are not to be considered a deviation from the scope of the invention, and all such modifications which are obvious to persons skilled in the art are also to be considered comprised by the scope of the accompanying claims.

Claims

1. A pharmaceutical composition for the prevention and/or treatment of viral infections, characterised in that it comprises one or more β -lupeol derivatives of the formula



I

5 in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple
 10 bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, as well as

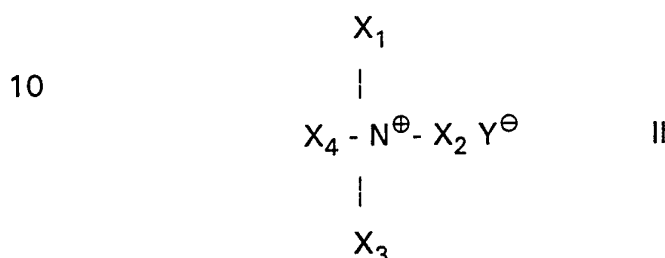
conventional pharmaceutically acceptable adjuvants, additives, and carriers.

15 2. A pharmaceutical composition as claimed in claim 1, characterised by R representing a hydrogen atom.

3. A pharmaceutical composition as claimed in claim 1 or 2, characterised in that it furthermore comprises an ammonium ion releasing compound.

4. A pharmaceutical composition as claimed in claim 3, c h a r a c t e r -
i s e d in that the ammonium ions are derived from a salt of a
pharmaceutically acceptable inorganic or organic acid preferably selected
from hydrochloric acid, sulphuric acid, phosphoric acid, carbonic acid,
5 acetic acid, and tartaric acid.

5. A pharmaceutical composition as claimed in claim 3, c h a r a c t e r -
i s e d in that the ammonium ions are derived from a compound of the
general formula II



- where X_1 - X_4 , which may be identical or different, are selected from
15 hydrogen; C_{1-6} alkyl, which may be straight-chained or branched, saturated
or unsaturated and optionally contain one or more substituents selected
from halogen, hydroxy, C_{1-4} -alkoxy or amino; aryl, which is optionally
substituted with C_{1-4} -alkyl, halogen, hydroxy, C_{1-4} -alkoxy or amino, and

- Y is a physiologically acceptable salt-forming anion, preferably selected
20 from F^- , Cl^- , Br^- and I^- .

6. A pharmaceutical composition as claimed in any of the preceding
claims, c h a r a c t e r i s e d in that furthermore it comprises one or
more mono or polysulphated mono, oligo or polysaccharides or analogues
and/or derivatives thereof.

- 25 7. A pharmaceutical composition as claimed in claim 6, c h a r a c t e r -
i s e d in that the saccharide is a mono or polysulphated mono, di, tri or

tetrasaccharide.

8. A pharmaceutical composition as claimed in claim 6, c h a r a c t e r -
i s e d in that the saccharide is a monosaccharide selected from xylose,
fructose and glucose.
- 5 9. A pharmaceutical composition as claimed in claim 6, c h a r a c t e r -
i s e d in that the saccharide is a disaccharide selected from sucrose,
lactose, maltose and cellobiose.
10. A pharmaceutical composition as claimed in any of the preceding
claims, c h a r a c t e r i s e d in that the saccharide forms a complex or
10 a salt with ammonium ions or with a metal selected from Al, Na, K, Ca,
Mg, Ba, Zn, Cu, Zr, Ti, Bi, Mn, and Os, or with an amino acid.
11. A pharmaceutical composition as claimed in claim 9, c h a r a c -
t e r i s e d in that the sulphated disaccharide is sucrose octasulphate, a
complex or a salt of sucrose octasulphate with ammonium ions or with a
15 metal selected from Al, Na, K, Ca, Mg, Ba, Zn, Cu, Zr, Ti, Bi, Mn, and Os,
or a salt of sucrose octasulphate with an amino acid.
12. A pharmaceutical composition as claimed in claim 11, c h a r a c -
t e r i s e d in that the sulphated disaccharide is sucrose octasulphate or
a sodium, potassium or NH_4^+ salt thereof or the aluminum complex of
20 sucrose octasulphate, sucralphate.
13. A pharmaceutical composition as claimed in any of the preceding
claims, c h a r a c t e r i s e d in that furthermore it comprises one or
more human or non-human immunoglobulines.
14. A pharmaceutical composition as claimed in any of the preceding
25 claims, c h a r a c t e r i s e d in that it is in the form of chewing gums,

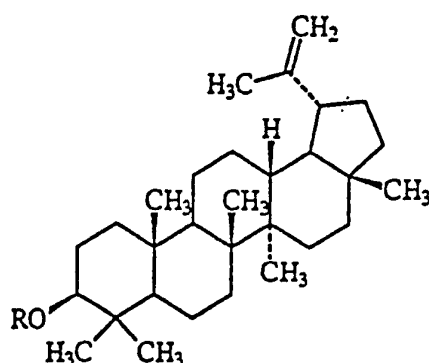
lozenges, chewing tables, resoriblets, drops, troches, gels, mouth ointments, solutions, mucoadhesive formulations and depot preparations.

15. A pharmaceutical composition as claimed in claim 14 in the form of a chewing gum, c h a r a c t e r i s e d in that per piece of chewing gum
5 it comprises:

- a) 0.01 to 2000, preferably 0.15 to 1000, particularly preferred 1 to 800, such as 20 to 600 μg of a β -lupeol derivative/piece, calculated as β -lupeol,
- b) 0 to 100, preferably 1 to 50, particularly preferred 2 to 40, such as
10 5 to 30 mg of NH_4^+ ions/piece, calculated as ammonium chloride,
- c) 0 to 1000, preferably 10 to 500, particularly preferred 25 to 250 mg of a sulphated saccharide/piece, calculated as SOS,

as well as conventional chewing gum ingredients.

16. The use of one or more β -lupeol derivatives of the general formula I

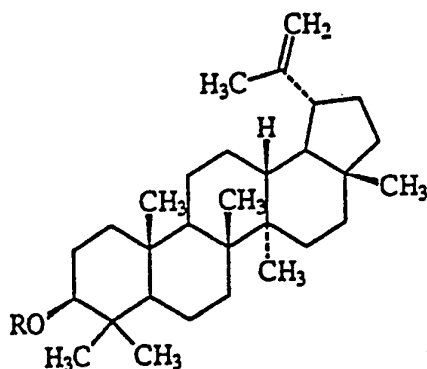


I

15 in which R represents a hydrogen atom, a straight-chained or branched aliphatic C_{1-6} -hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a

C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative for the preparation of a medicament for the prevention and/or treatment of viral infections.

17. The use of one or more β -lupeol derivatives of the general formula I

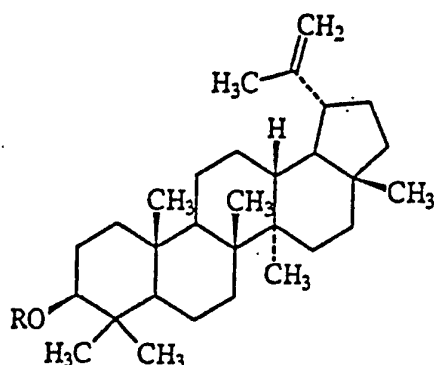


I

in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, as well as one or more ammonium ion releasing compounds, for the preparation of a medicament for the prevention and/or treatment of viral infections.

18. The use of one or more β -lupeol derivatives of the general formula I

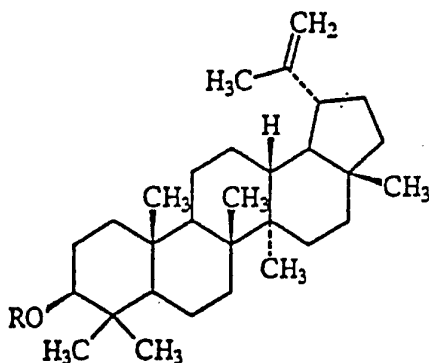
30



I

in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbonyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, as well as one or more mono or polysulphated mono, oligo or polysaccharides or analogues and/or derivatives thereof, for the preparation of a medicament for the prevention and/or treatment of viral infections and associated inflammations.

19. The use of one or more β -lupeol derivatives of the general formula I

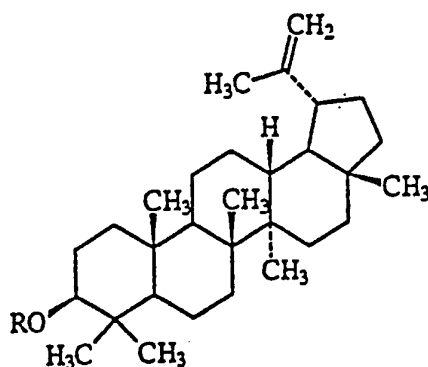


I

in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbonyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may
 5 contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, one or more ammonium ion releasing compounds, as well as one or more mono or polysulphated mono, oligo or polysaccharides or analogues and/or
 10 derivatives thereof, for the preparation of a medicament for the prevention and/or treatment of viral infections and associated inflammations.

20. The use as claimed in any of the preceding claims 16 to 19 for the treatment of infections in the upper respiratory passages, especially cold virus, such as Rhino virus and influenza virus.

15 21. A method for the prevention and treatment of viral infections, characterised in that it includes oral administration of a pharmacologically antiviral amount of one or more β -lupeol derivatives of the formula I

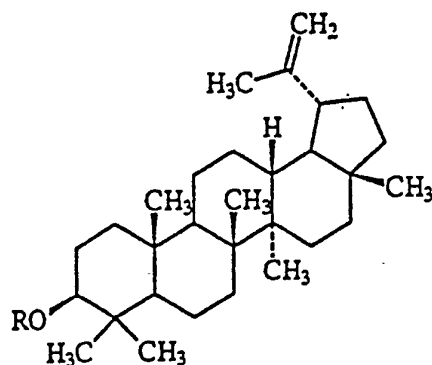


I

in which R represents a hydrogen atom, a straight-chained or branched
 20 aliphatic C₁₋₆-hydrocarbonyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a

C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative,
 5 in a pharmaceutically acceptable carrier.

22. A method for the prevention and treatment of viral infections, characterised in that it includes oral administration of a pharmacologically antiviral amount of one or more β -lupeol derivatives of the formula I

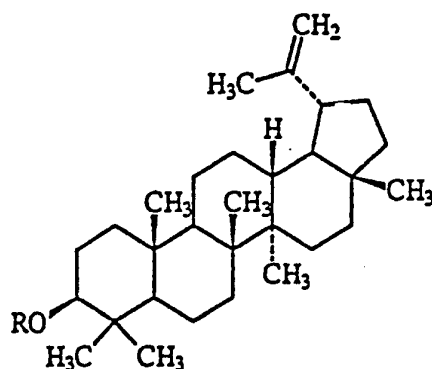


I

10 in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple
 15 bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative, as well as one or more ammonium ion releasing compounds, in a pharmaceutically acceptable carrier.

23. A method for the prevention and treatment of viral infections and
 20 associated inflammations, characterised in that it includes oral

administration of a pharmacologically antiviral amount of one or more β -lupeol derivatives of the formula I



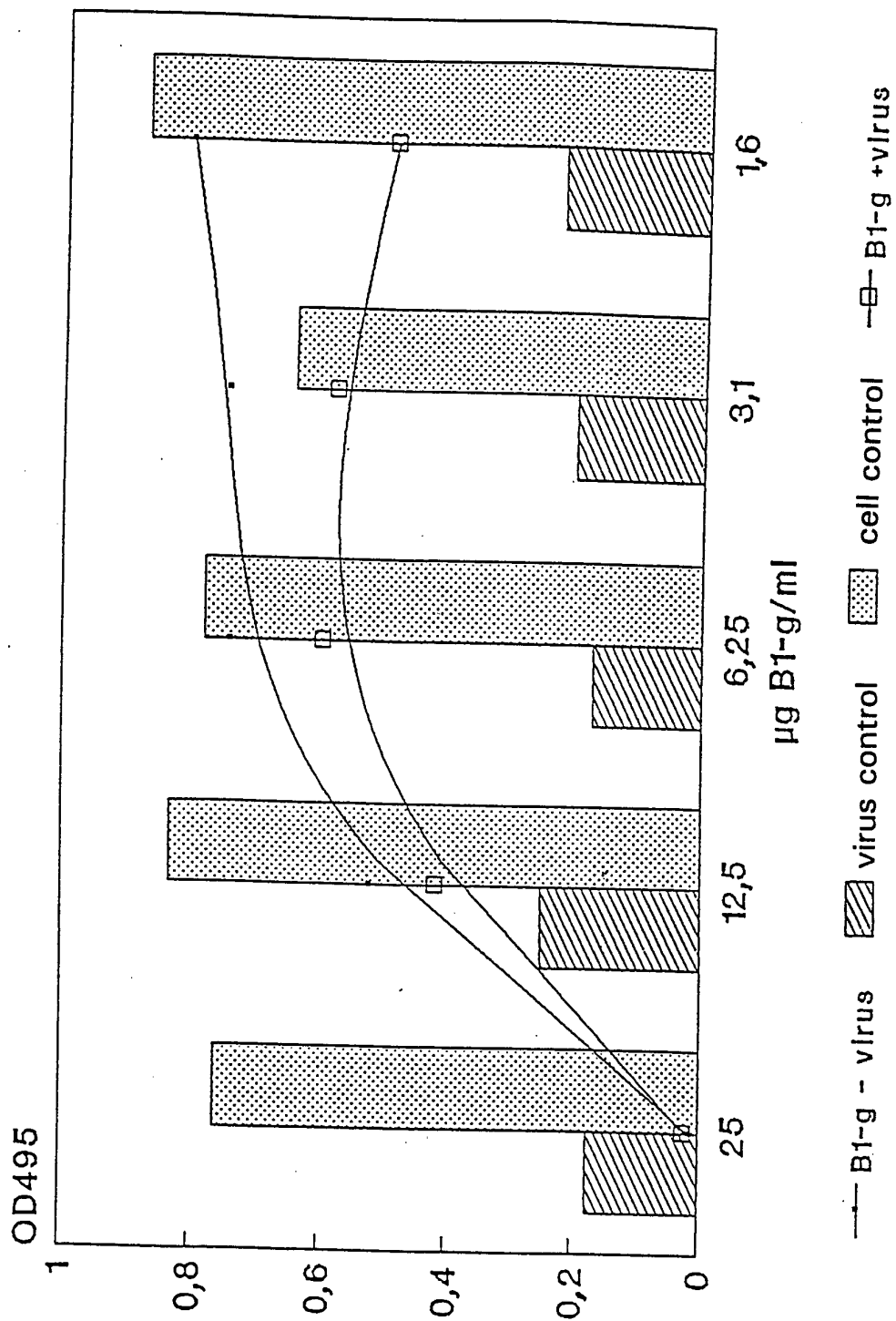
I

in which R represents a hydrogen atom, a straight-chained or branched aliphatic C₁₋₆-hydrocarbyl group, which may be saturated or may contain
 5 one or more unsaturated bonds selected from double and triple bonds, a C₁₋₆-acyl group, which may be straight-chained or branched and may contain one or more unsaturated bonds selected from double and triple bonds, or a group which is easily decomposed under the conditions prevailing in the human or animal body to release the β -lupeol derivative,
 10 as well as one or more mono or polysulphated mono, oligo or polysaccharides or analogues and/or derivatives thereof, in a pharmaceutically acceptable carrier.

24. A method for the prevention and treatment of viral infections and associated inflammations, characterised in that it includes oral
 15 administration of a pharmacologically antiviral amount of one or more β -lupeol derivatives of the formula I

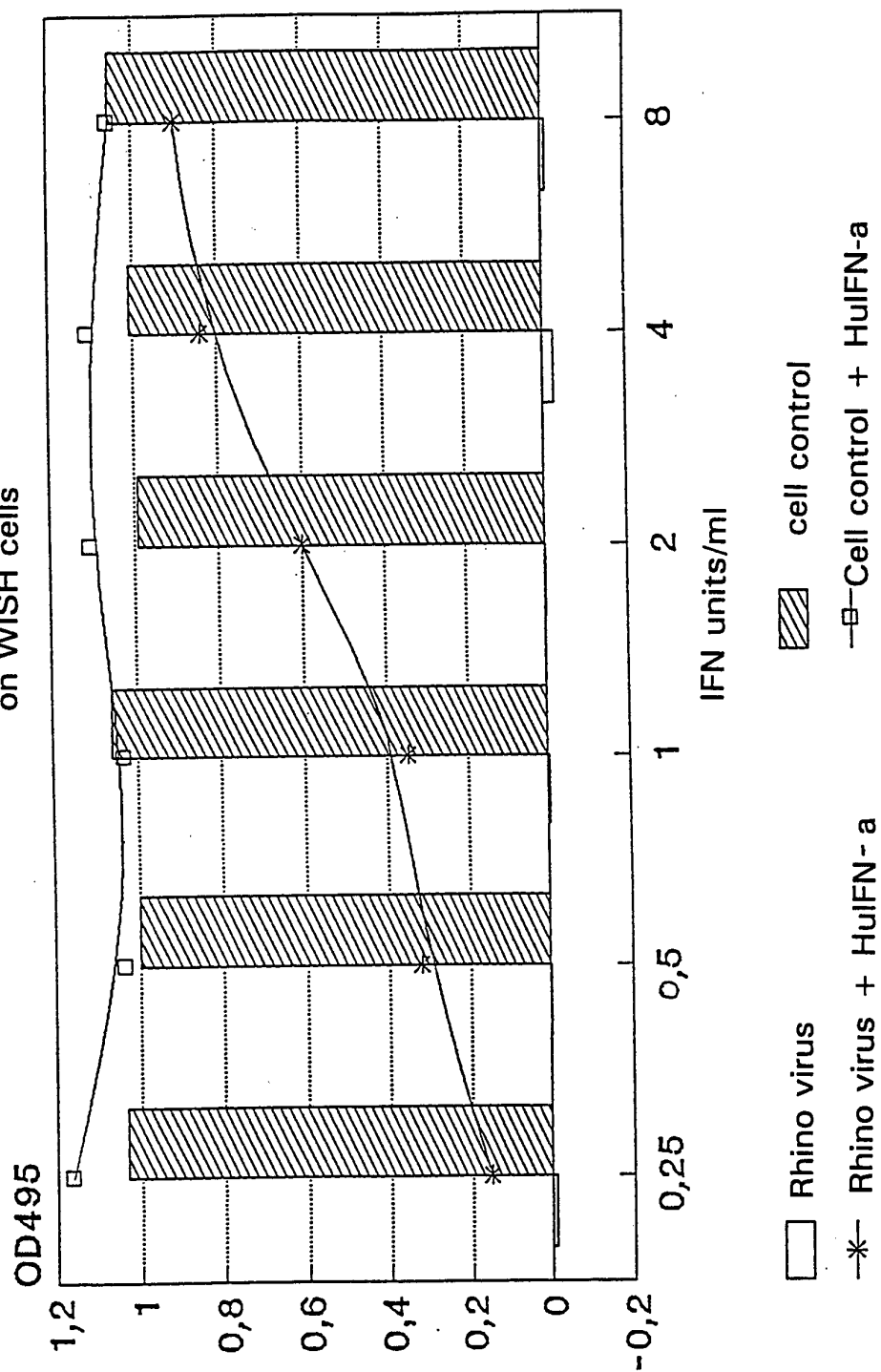
1/12

Fig. 1 Titration of B1-g against Rhino virus



2/12

Fig. 2 Anti-Rhino virus activity of HuIFN- α on WISH cells



3/12

Fig. 3 Antiviral activity of B1-g
EMC virus

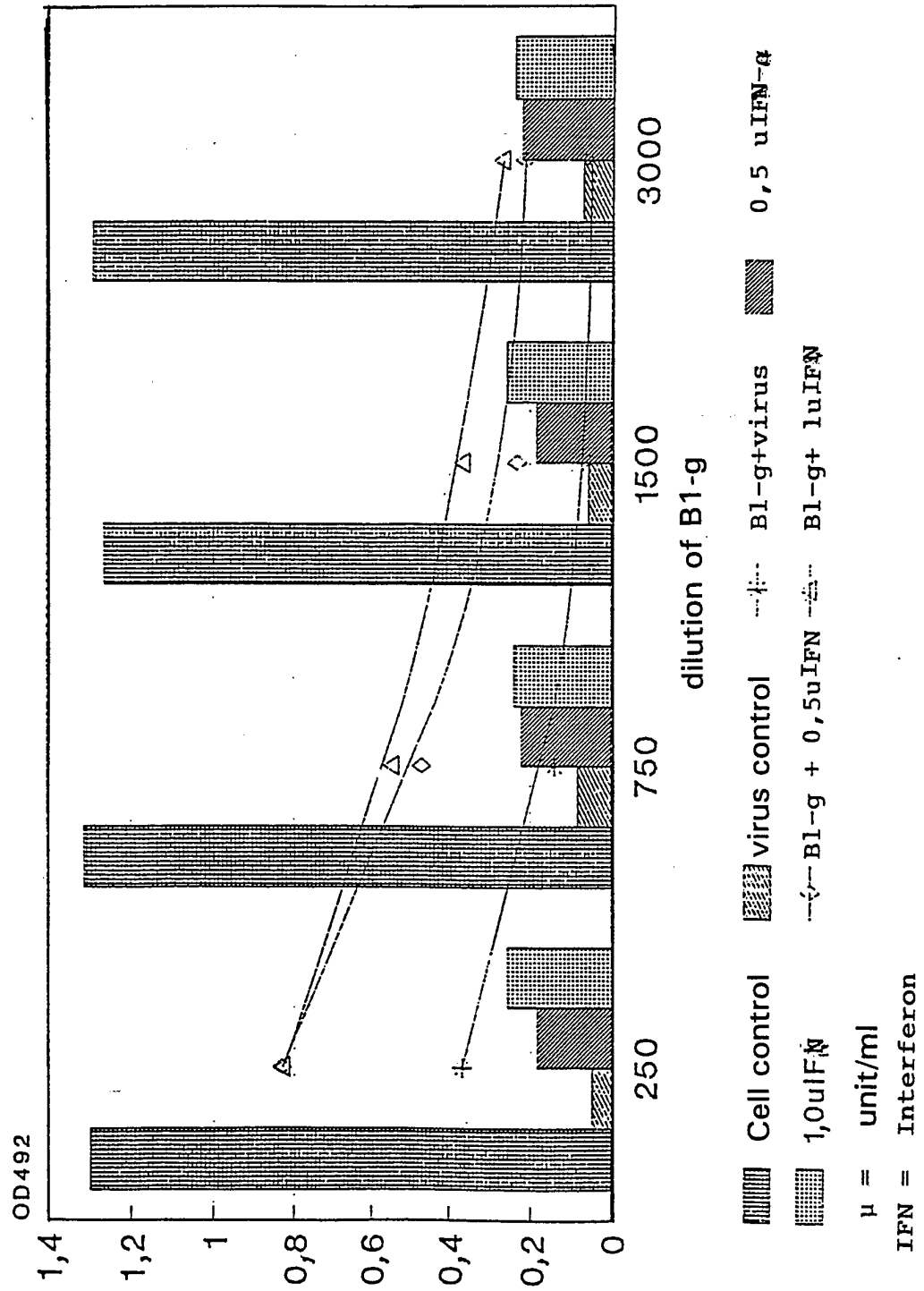
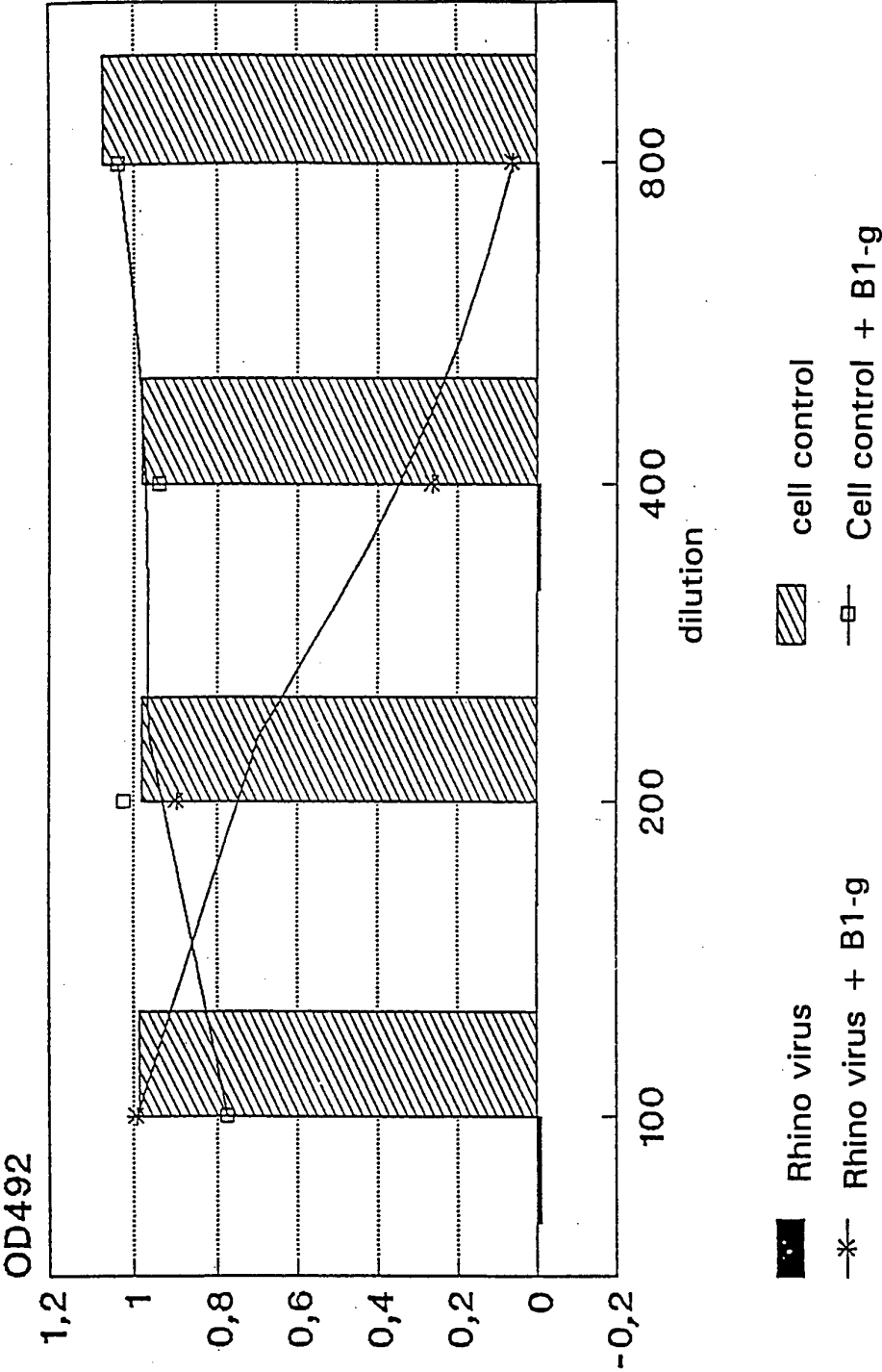
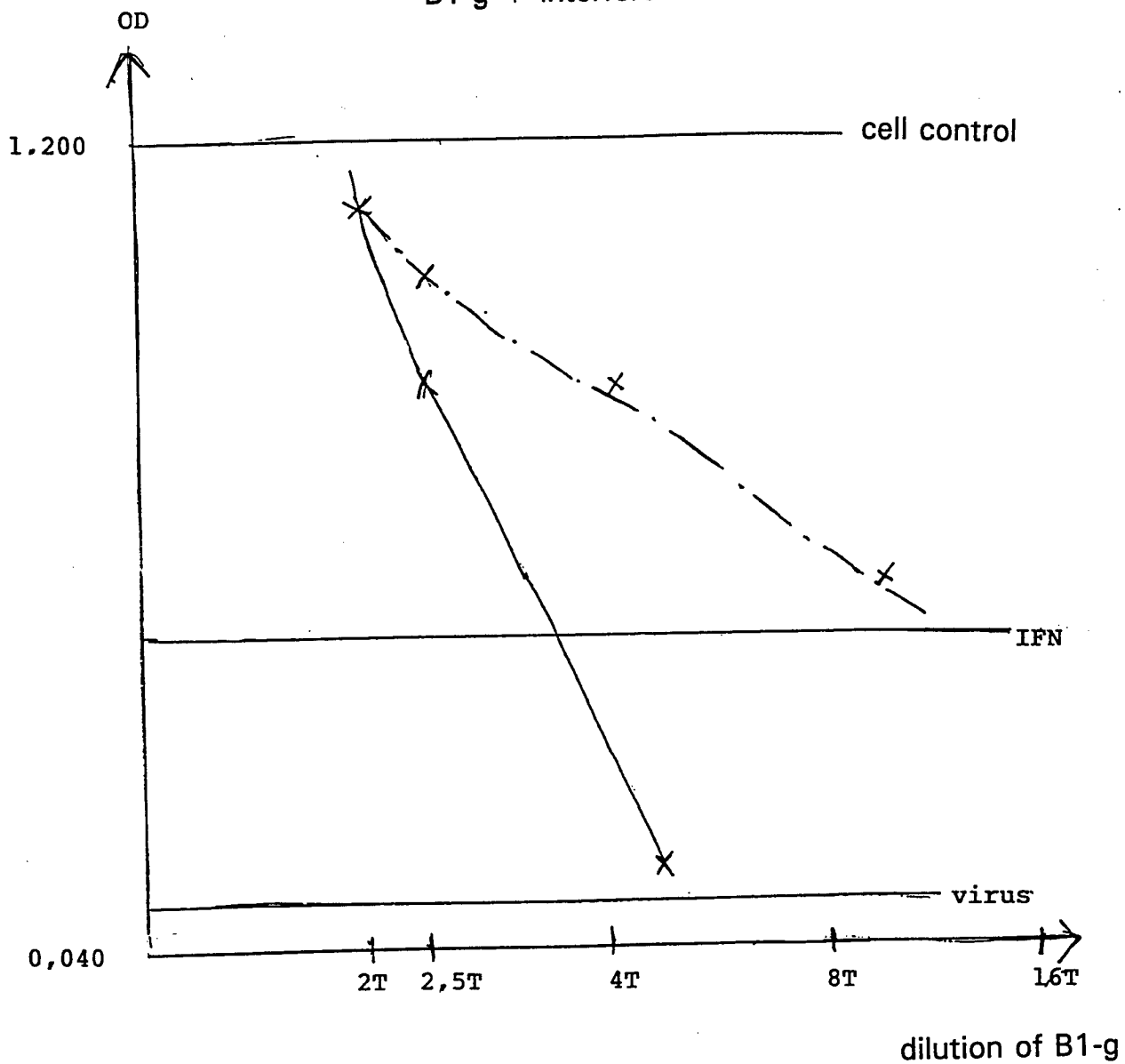


Fig. 4A Anti-Rhino virus activity of B1-g
on WISH cells



5/12

Fig. 4B
B1-g + interferon

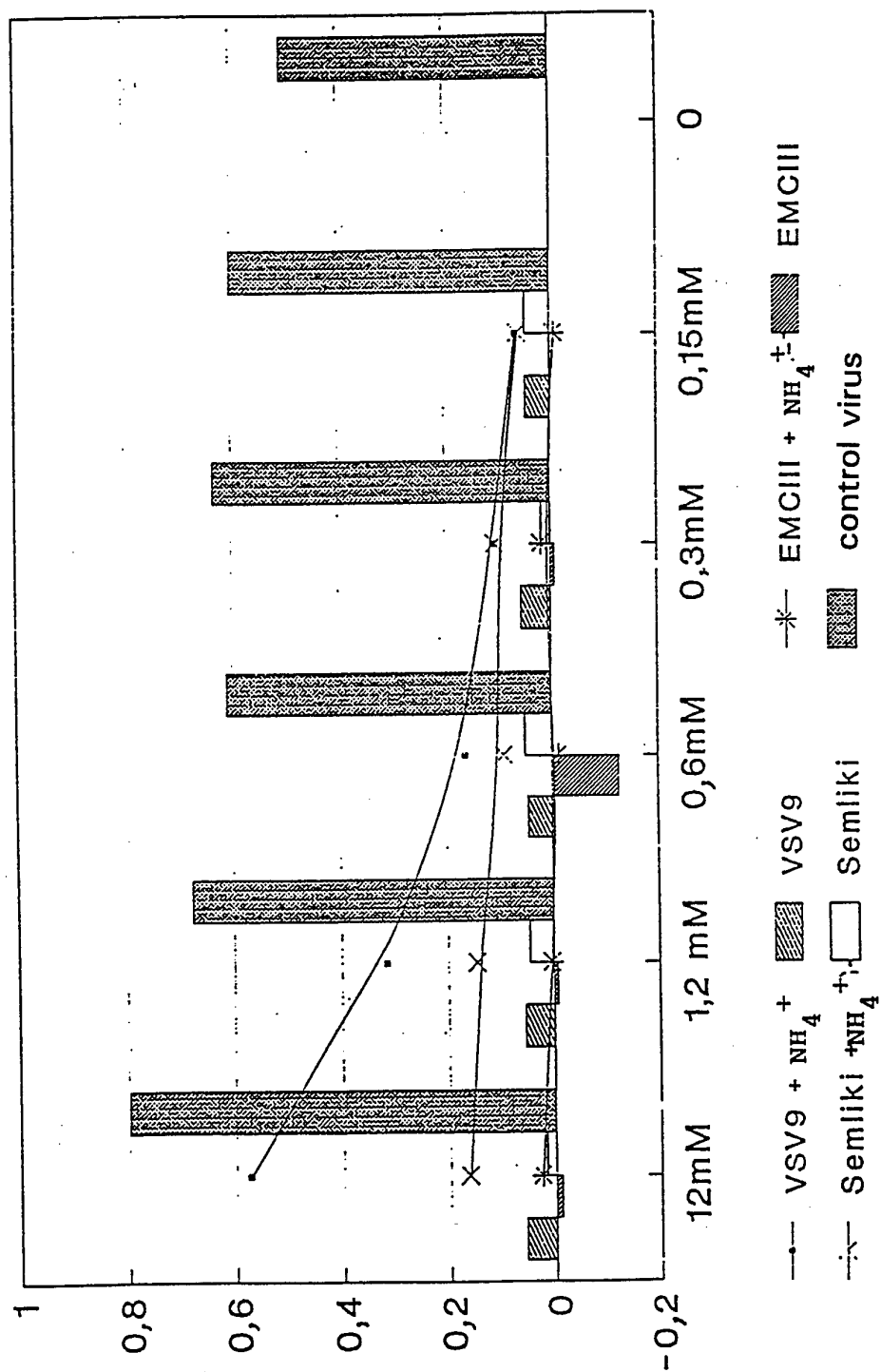


x—x B1-g alone

x-.-.-x B1-g + 0.5 units of interferon-α/ml

6/12

Fig. 5 Antiviral activity of NH_4^+ ions on A549 cells



7/12

Fig. 6 Anti-Rhino virus activity of NH_4Cl

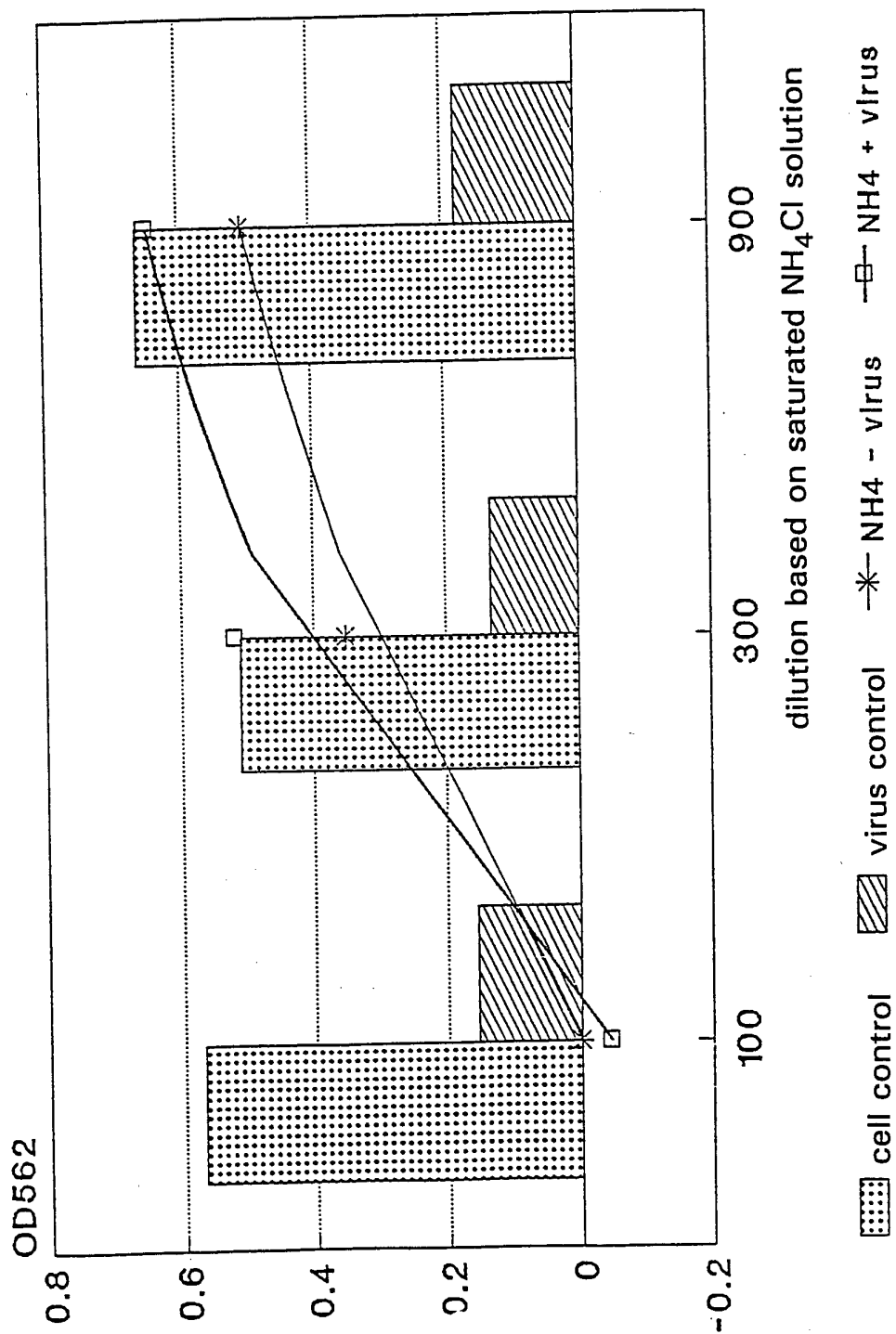
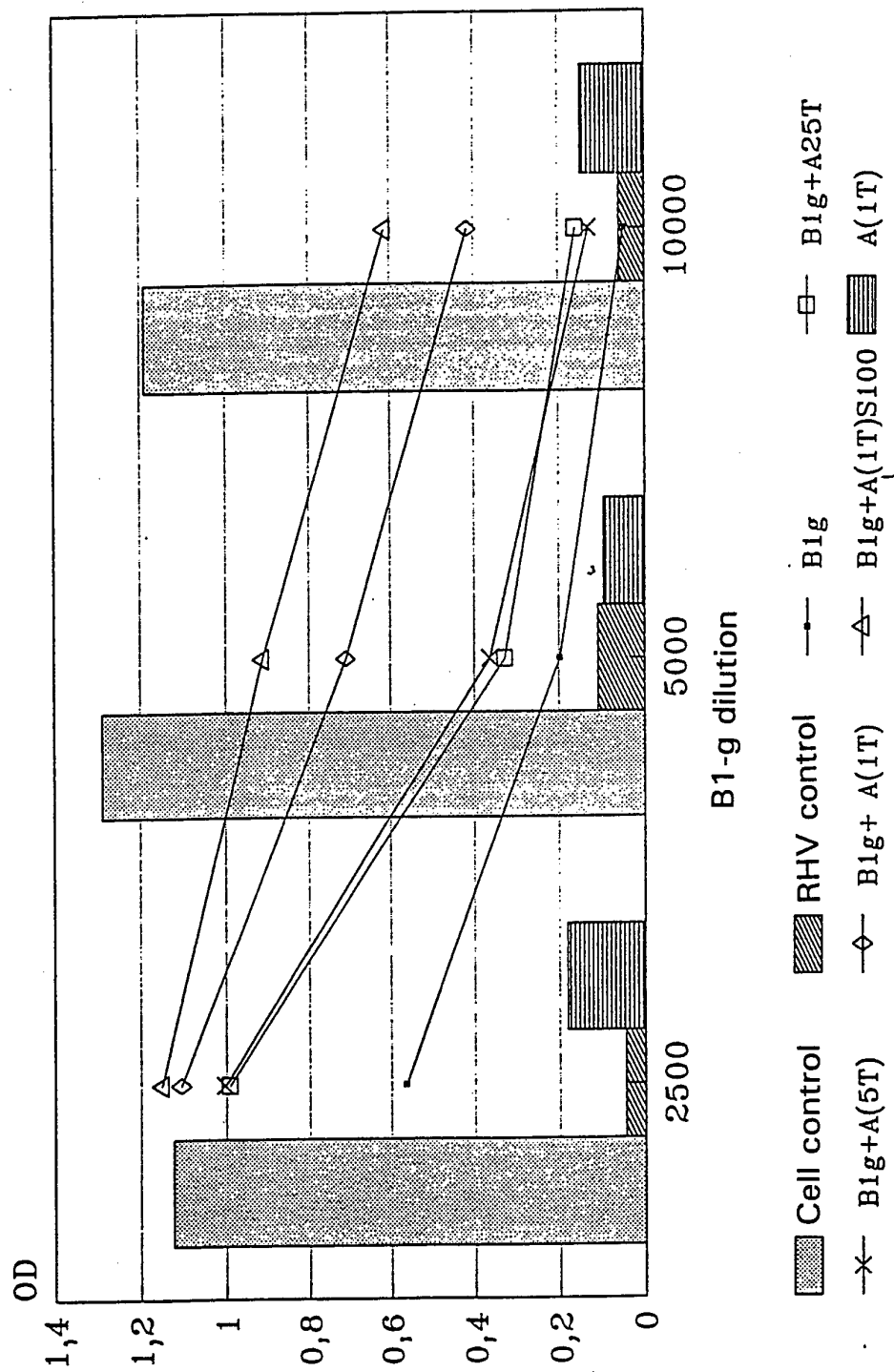


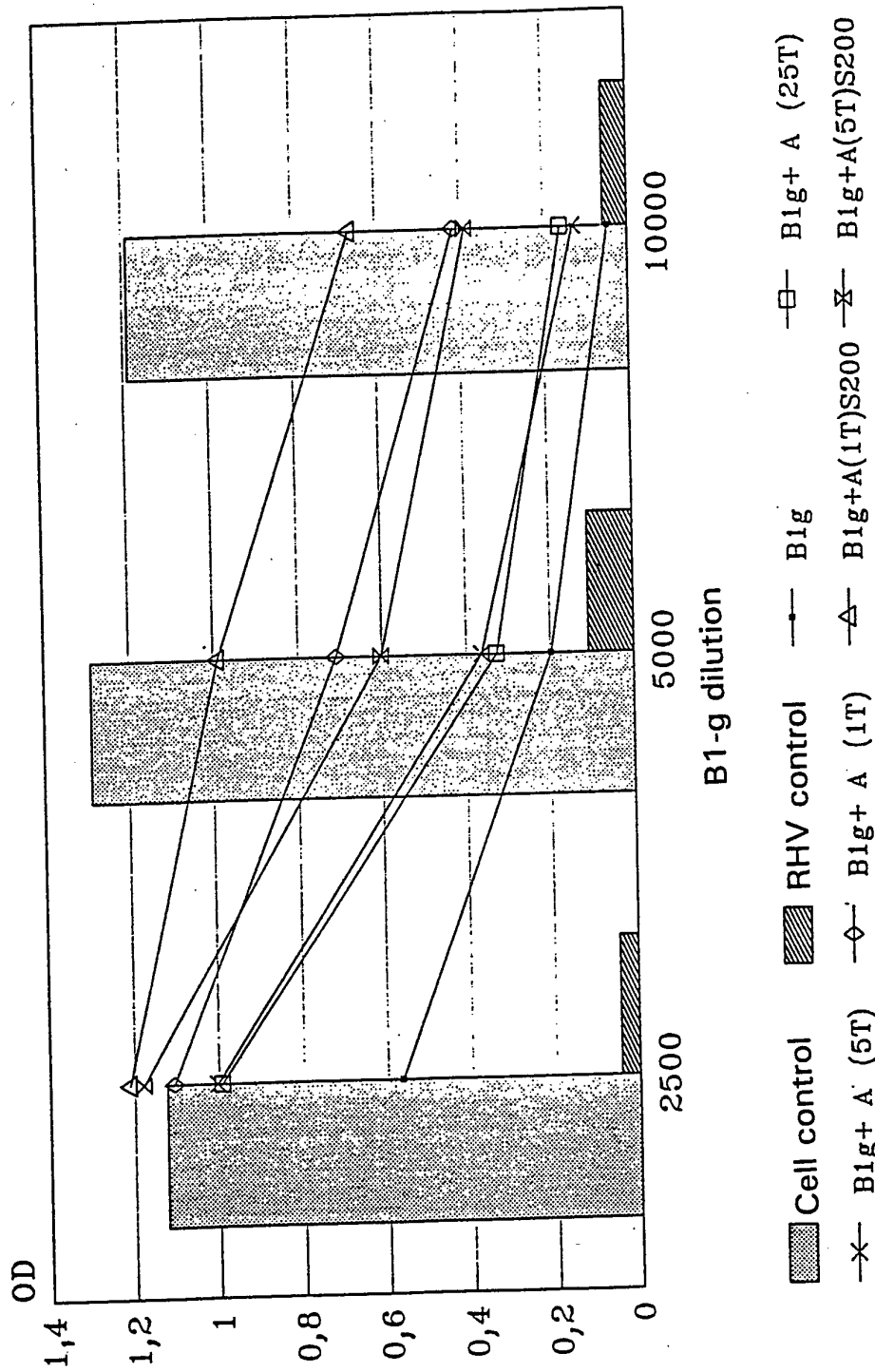
Fig. 7A B1-g + NH₄Cl + SOS (1:100)



RHV = Rhino virus
A = NH₄Cl
T = times dilution
S = SOS

9/12

Fig. 7B B1-g + NH₄Cl + SOS (1:200)



RHV = Rhino virus
A = NH₄Cl
T = times dilution
S = SOS

Fig. 7C B1-g + NH₄Cl + SOS (1:400)

Fig. 8 B1-g + NH₄Cl + SOS + IFN

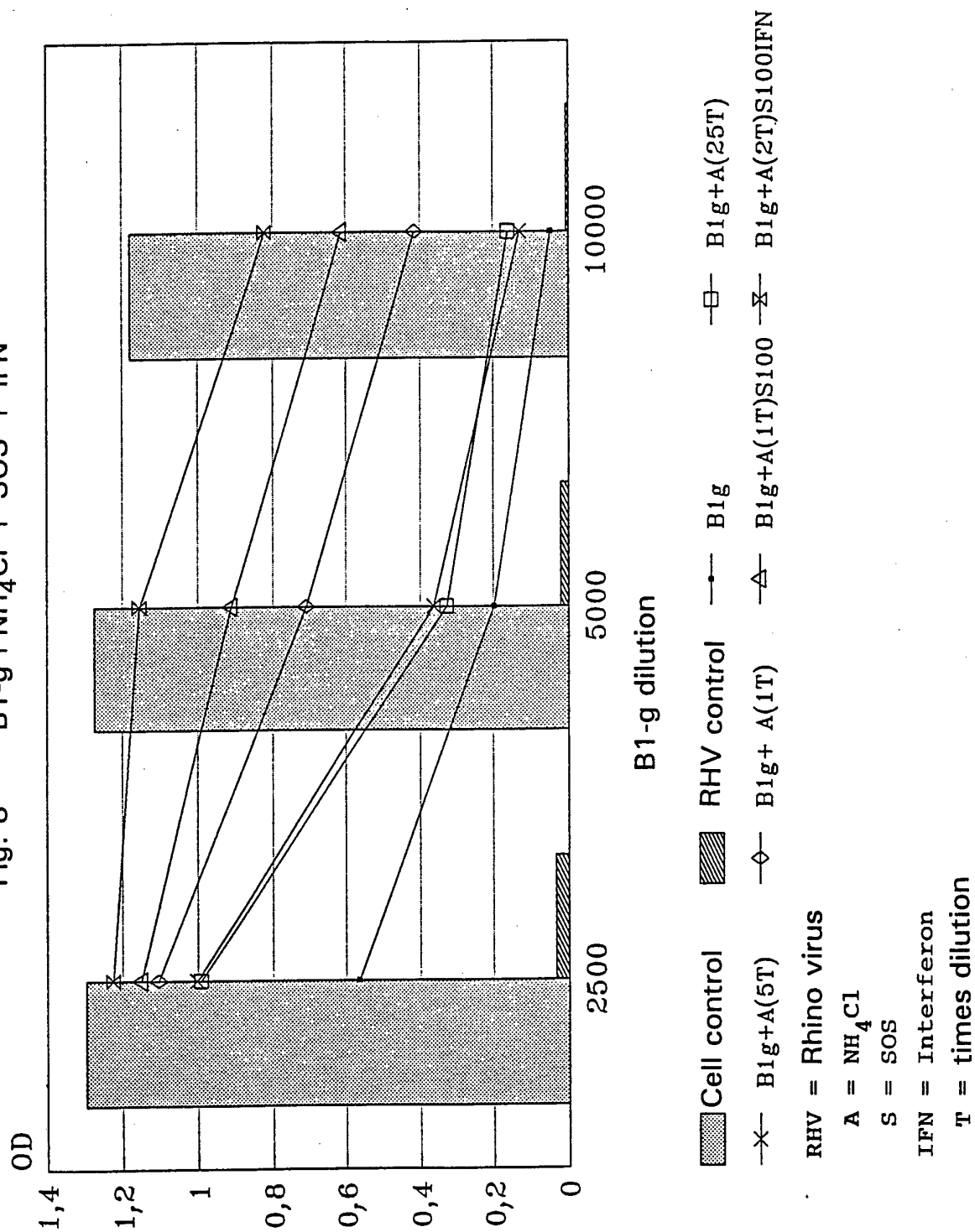
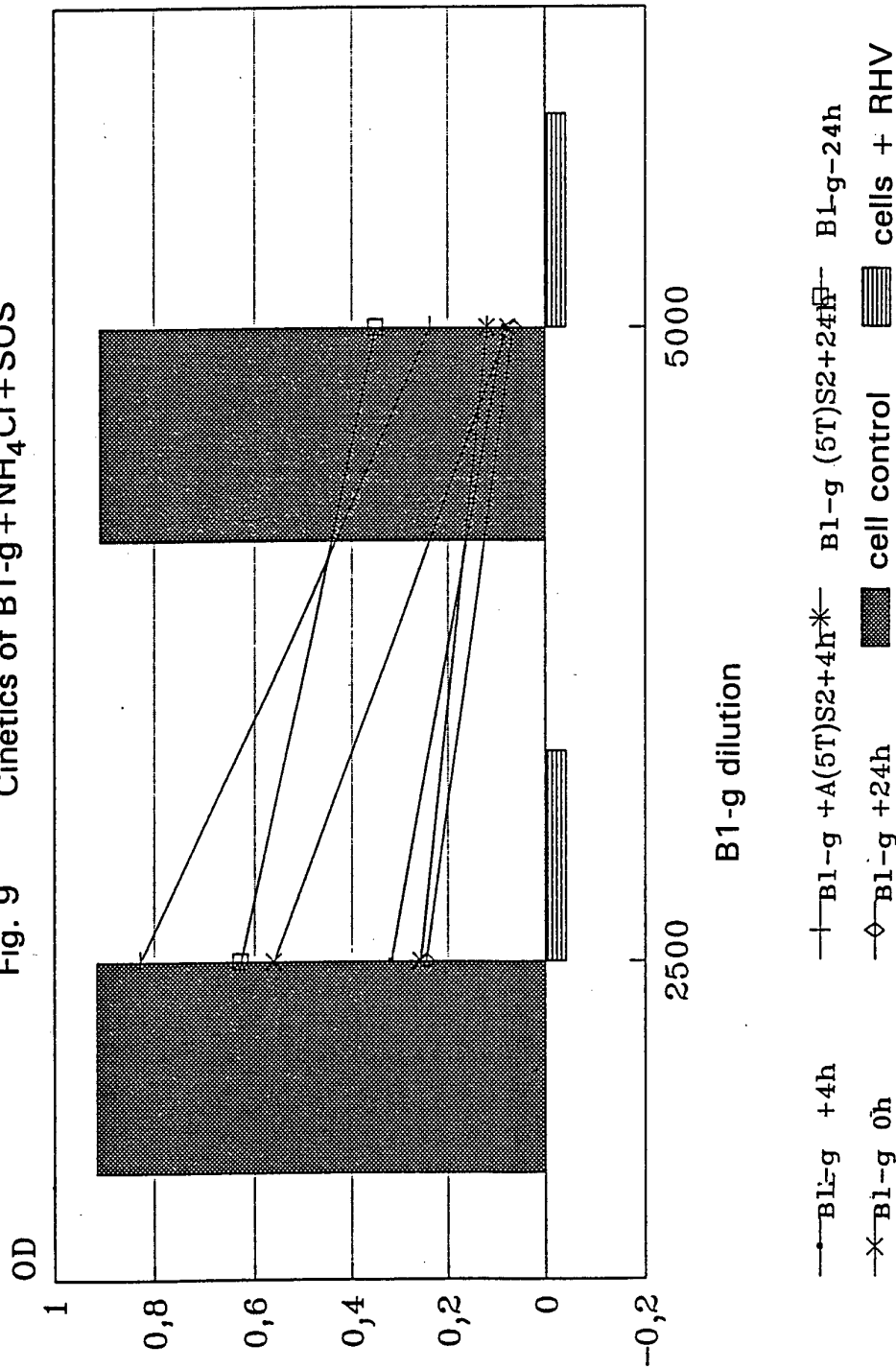


Fig. 9 Cinetics of B1-g + NH₄Cl + SOS



RHV = Rhino virus

A = NH₄Cl

S = SOS

T = times dilution

rhv45ki kb950619

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 95/00256

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/045, A61K 9/68

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

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

SE,DK,FI,NO classes as above

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

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INDIAN J MED RES, Volume 73, April 1981, M.B. Gupta, et al, "Antiulcer activity of some plant triterpenoids" page 649 - page 652	1-15,25
A	--	16-20
X	PHYTOTHERAPY RESEARCH, Volume 7, 1993, G. Kweifio-Okai et al, "Short communication, Antiarthritic effect of lupeol acetate" page 213 - page 215	1-15,25
A	--	16-20

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 Sept 1995

Date of mailing of the international search report

04-10-1995

Name and mailing address of the ISA/

Swedish Patent Office

Box 5055, S-102 42 STOCKHOLM

Facsimile No. +46 8 666 02 86

Authorized officer

Göran Karlsson

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 95/00256

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF NATURAL PRODUCTS, Volume 50, No 6, 1987, Takao Konoshima et al, "Studies on inhibitors of skin-tumor promotion, I. Inhibitory effects of triterpenes from euptelea polyandra on epstein-barr virus activation", page 1167 - page 1170, see compound 17 --	16-20
A	WO 9014764 A1 (HERMAN, STEPHEN), 13 December 1990 (13.12.90) --	16-20
A	Chemical Abstracts, Volume 94, No 16, 20 April 1981 (20.04.81), (Columbus, Ohio, USA), Tezuka, Shichigoro et al, "Natural gum resins for chewing gum", page 589, THE ABSTRACT No 82497v, Nippon Shokuhin Kogyo Gakkaishi 1980, 27 (9), 419-425 -- -----	15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK95/00256

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

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

1. ☒ Claims Nos.: 21-24, 26
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy,
see rule 39.1.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

Information on patent family members

28/08/95

International application No.

PCT/DK 95/00256

Form PCT/ISA/210 (patent family annex) (July 1992)