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ORGANIZATION

WIPO/GRTKF/IC/30/9
ORIGINAL: ENGLISH
DATE: JUNE 1, 2016

Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore

Thirtieth Session
Geneva, May 30 to June 3, 2016

SEEKING A BETTER UNDERSTANDING OF SWITZERLAND'S "FEDERAL ACT ON THE PROTECTION OF NATURE AND CULTURAL HERITAGE" AND "FEDERAL ACT ON PATENTS FOR INVENTIONS" BY HYPOTHETICALLY APPLYING THEM TO U.S. PATENT NUMBER 5,137,870

Document submitted by the Delegation of the United States of America

1. On May 31, 2016, the International Bureau of the World Intellectual Property Organization (WIPO) received a request from the Delegation of the United States of America to submit the Annexed document for discussion by the Thirtieth Session of the Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC), as a working document under the Agenda Item "Genetic Resources".

2. Pursuant to the request above, the Annex to this document contains the submission referred to.

3. The Committee is invited to take note of this document and the Annex to it.

[Annex follows]

SEEKING A BETTER UNDERSTANDING OF SWITZERLAND'S FEDERAL ACT ON THE PROTECTION OF NATURE AND CULTURAL HERITAGE AND FEDERAL ACT ON PATENTS FOR INVENTIONS BY HYPOTHETICALLY APPLYING THEM TO U.S. PATENT NUMBER 5,137,870

I. INTRODUCTION

Disclosure requirements have been characterized as simple transparency requirements, which will not be burdensome to patent applicants. Our review of disclosure requirements, however, suggest that these requirements will be difficult for applicants to satisfy, and that applicants will be required to disclose many sources of any genetic resource (GR) used at some point in making the invention, as well as those which could have been used. As a result of the enormous information potentially required, we question the feasibility of a disclosure mandate providing transparency and have concerns that it could be burdensome. Also, we question whether this requirement would discourage applicants from filing patent applications on certain inventions, thus further decreasing transparency.

To better understand disclosure requirements, we have chosen the law of Switzerland as a first example. If this exercise improves our understanding of the disclosure requirement in Switzerland, then we plan to undertake the same exercise using the laws of other World Intellectual Property Organization (WIPO) Members.

To comply with its access and benefit sharing (ABS)-user measures, Switzerland requires, among other things: (1) due diligence of compliance with domestic ABS provisions, (2) notification of said due diligence, and (3) disclosure of the source of GRs in patent applications. The sanctions for failing to comply with these provisions are significant fines, court-ordered publications of the judgments, and the possibility of a rejected patent. To better understand the application of the above laws, and to stimulate a factual discussion, the above laws are applied in this document to a selected patent.

II. LEGAL FRAMEWORK

Switzerland's ABS provisions on due diligence and notification can be found in the Federal Act on the Protection of Nature and Cultural Heritage ("NCHA"), Articles 23n and 23o, respectively. NCHA Article 24a describes the penalties for their non-compliance. Additionally, Article 49 of the Federal Act on Patents for Inventions ("Patents Act") mandates disclosure in patent applications. Violators of this provision are subject to the pre-grant penalties of Article 59a and post-grant sanctions of Article 81a.

1. **Federal Act on the Protection of Nature and Cultural Heritage Requirements**

A. **Due Diligence Requirement**

Article 23n of the NCHA includes:

1. Any person who in accordance with the Nagoya Protocol utilises genetic resources or benefits directly from their utilisation (users) must apply due diligence appropriate to the circumstances to ensure that:

a. the resources have been accessed lawfully; and

b. mutually agreed terms for the fair and equitable sharing of the benefits have been established.

2. Genetic resources are not subject to the due diligence requirement if they:

a. originate from a country that is not a Party to the Nagoya Protocol;

b. originate from a country that has no domestic access and benefit-sharing regulatory requirements;

…

5. If the requirements of paragraph 1 letters a and b are not met, users must ensure that they are met subsequently, or must refrain from utilising the genetic resources concerned or from benefiting directly from their utilisation.

B. **Notification/Market Authorization Requirement**

Art. 23o of the NCHA includes:
1. Notification of compliance with the due diligence requirement must be given to the FOEN before market authorisation has been obtained or, if such authorisation is not required, before the commercialisation of products developed on the basis of utilised genetic resources.

…

C. **Criminal Penalties**

Art 24a of the NCHA reads:
…
2. Any person who wilfully fails to provide information or provides false information under Article 23*o* shall be liable to a fine not exceeding 100,000 Swiss francs; in cases of negligence, the penalty shall be a fine not exceeding 40,000 Swiss francs. The court may order the publication of the judgment.
…

2. **Federal Act on Patents for Inventions Requirements**

A. Source of Genetic Resources

Art. 49a of the Patents Act reads:

1 The patent application must contain information on the source:

a. of the genetic resource to which the inventor or the patent applicant had access, provided the invention is directly based on this resource;

b. of traditional knowledge of indigenous or local communities of genetic resources to which the inventor or the patent applicant had access, provided the invention is directly based on this knowledge.

2 If the source is unknown to the inventor or the patent applicant, the patent applicant must confirm this in writing.

B. Pre-Grant Penalties

Art. 59 of the Patents Act reads:

…

2 If the patent application does not meet the other requirements of this Act or the Ordinance, the Institute shall set a time limit for the patent applicant by which the deficiencies must be remedied.
…

Art. 59a of the Patents Act reads:

…

3 The Institute shall reject the patent application if: …

b. the deficiencies mentioned in Article 59 paragraph 2 have not been remedied.

C. Post-grant Penalties

Art. 81a of the Patents Act reads:

1 Any person who wilfully provides false information under Article 49*a* is liable to a fine of up to 100,000 francs.
2 The court may order the publication of the judgment.
…

III. **PATENT INTRODUCTION**

The patent that will be used in this analysis is U.S. Patent 5,137,870 (“‘870 Patent”), entitled “Didemnins and Nordidemnins.” The ‘870 Patent was filed in the U.S. on February 20, 1990 and claims domestic priority to parent application 186,932, which was filed on September 12, 1990. The inventor was Kenneth L. Rinehart (“Rinehart”) and the assignee the University of Illinois at Urbana.

The Brief Summary of the Invention reads:

Novel antibiotics didemnin A, didemnin B, didemnin C, nordidemnin A, nordidemnin B, and nordidemnin C are extracted from a marine tunicate of the family Didemnidae, and tentatively identified as a Tridemnum sp. These antibiotics are active against DNA viruses, for example, herpes simplex virus types 1 and 2, and vaccinia virus; RNA viruses, for example, coxsackie virus and equine rhinovirus; and B388 leukemia in mice. Thus these antibiotics can be used to treat infections in humans, animals and plants caused by these viruses and other DNA and RNA viruses. Didemnin A and didemnin B also inhibit L1210 mouse leukemia cells in vitro. Acid addition salts and acyl derivatives of the didemnins can be made and used for the same biological purposes are the parent compounds.

…

The Detailed Description of the Invention reads:

*S*pecific locations from which these organisms have been obtained are as follows:
(1) Southwest side of Long Cay, Lighthouse Reef, Belize, 17.degree. 11.8' N by 87.degree. 36.5' W at a depth of 50 to 100 feet; (2) Rada el Cove, Isla San Andres**, Colombia**, 12.degree. 31'46" N by 81.degree. 44'5" W at 25 to 33 feet; (3) Palancar Reef, Isla de Cozumel, **Mexico**, 20.degree. 18.2' N by 87.degree. 2.5' W at 60 to 100 feet; (4) on the west side of the southern tip of Turneffe Island**, Belize,** 17.degree. 11.3' N by 87.degree. 55.6' W at 50 to 75 feet; (5) Punta Oeste, Coxen's Hole Harbor, Isla Roatan, **Honduras**, 16.degree. 15' N by 86.degree. 38' W at 10 to 70 feet; (6) on the leeward side of the western-most Holandes Cay, Isla San Blas, **Panama**, 9.degree. 35.6' N by 78.degree. 47' W at 60 feet.
…

Claim 1 of the '870 Patent reads:

A process for treating an animal or human hosting leukemia comprising: administering an effective amount of a didemnin selected from the group consisting of didemnin A, didemnin B, and didemnin C or a pharmaceutically acceptable salt thereof, to said host.

IV. **ANALYSIS**

If the subject matter of the '870 Patent is filed in Switzerland in 2016, what would the patent applicant be required to do in order to comply with Swiss law?

A. **Due Diligence in Mexico, Panama, Honduras, Colombia, and Belize**

As noted in the Specification of the '870 Patent, the inventor obtained samples of marine tunicate from the waters of Mexico, Panama, Colombia, Belize, and Honduras. According to the NCHA, a person who utilizes GRs or benefits directly from their utilization must apply due diligence appropriate under the circumstances. Thus, a first question is whether the inventor of the '870 patent utilized GRs or benefited directly from their utilization. The Nagoya Protocol ("NP") defines "'Utilization of genetic resources' means to conduct research and development on the genetic and/or biochemical composition of GRs, including through the application of biotechnology as defined in Article 2 of the Convention." Assuming that for purposes of Swiss law, "utilization" has the same meaning, the inventor did "utilize" the genetic resources because research and development was conducted on the samples.

To meet the due diligence requirements in Switzerland under Art 23n of the NCHA, it appears that Rinehart would need to comply with the access provisions for those countries that are both party to the NP and have domestic ABS laws. Of the above nations, only Mexico and Panama fall under this category.

Mexico:

Mexican access laws are complex and depend on whether access is being sought on land or in water. To obtain access in federal aquatic areas for research purposes, applicants have to obtain a permit from the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food ("SAGARPA"). Permit applicants must provide the following:[1]

- Names of the supervisors and technical leaders of the project;
- Objectives of the study;
- Application of the results;
- Common and scientific names of the organisms to be researched;
- Location at local, municipal and state levels;
- Geographical coordinates; and
- Justification of the chosen site.

The law also mandates permit holders provide status reports to SAGARPA and be compliant with other Mexican regulations including the Fisheries Act and Fisheries Act Administrative Rules.

Here, Rinehart obtained tunicates from the Palancar Reef in Cozumel, Mexico, an area that is administered by the Arrecifes de Cozumel National Park. He would therefore need to obtain a permit from SAGARPA and complete the numerous application requirements listed above. Rinehart would also have to follow other state and federal rules and regulations.

[1] http://www.fao.org/fishery/legalframework/nalo_mexico/en.

Panama:

Panama is also a member of the NP, and its domestic ABS rules can be found in Executive Decree No. 25 of April 2009 ("ED 25"). Under ED 25, Panama requires applicants seeking access to the country's genetic heritage, including its marine and coastal environments,[2] to receive permission from the Unit for Access to Genetic Resources (UNARGEN). Applicants must obtain the following:

- Accessory Contract granting Free Prior Informed Consent (FPIC) from owner of the resource;
- Access Contract from UNARGEN; and
- Benefits Contract from UNARGEN.

Applicants must also maintain compliance with various provisions of ED 25 throughout the life of the Access Contract.

As disclosed in the '870 Patent, Rinehart obtained tunicates from the Holandes Cay, Isla San Blas, in Panama. Therefore, he would have to receive FPIC from the owner of the research site. Then he must acquire Access and Benefits Contracts from UNARGEN. Finally, he must comply with additional Panamanian regulations while accessing the site on Holandes Cay.

Honduras, Colombia, Belize:

Rinehart also extracted tunicates from Honduras, Colombia, and Belize. Of these, only Honduras is a party to the NP, but it does not have domestic ABS laws. Switzerland, therefore, would not require Rinehart to provide due diligence of his activities in these countries. Suppose, however, he obtained tunicates from 10 other countries that are party to the NP and have ABS laws. Clearly, for each additional country, he would have added requirements, which could discourage research.

B. **Notification**

After performing due diligence for potentially many countries, applicants in Switzerland are required to submit notification of said due diligence prior to obtaining market authorization or commercialization under Article 23o of the NCHA. This provision mandates that applicants notify the Federal Office for the Environment (FOEN) that it conducted due diligence. In this case, Rinehart would have to notify FOEN that he conducted due diligence for Mexico and Panama.

C. **Market Authorization for Medicinal and Therapeutic Products**

After providing notification to FOEN, certain applicants seeking to do business in Switzerland may need to obtain market authorization. For new medicines and therapeutic products, applicants must receive authorization from Swissmedic, which will ascertain the products' safety before they are allowed to be sold in Switzerland. Here, because Rinehart's research was done in connection with his job at the University of Illinois, it is unlikely the University would market any products containing the didemnin found in the tunicates. However, any commercial applicant would have to obtain authorization from FOEN before marketing pharmaceuticals derived from GRs.

D. **For Patent Applicants, Source of GRs Must Be Disclosed**

In addition to the above requirements, Switzerland mandates its patent applicants include the source of GRs in their patent applications. Section 49a of the Patents Act requires that "The patent application must contain information on the source: of the genetic resource to which the inventor or the patent application had access, provided the invention is **directly based** on this resource; of traditional knowledge of indigenous or local communities of genetic resources to

[2] Panama, Executive Decree No. 25 of April 2009, Article 6(g).

which the inventor or the patent applicant had access, provided the invention is directly based on this knowledge" (emphasis added). Section 49b goes on to provide that if the source of the GR is unknown then the inventor or applicant must state so in writing.

Our understanding is that with regard to the '870 Patent, Rinehart would need to disclose each location where he obtained the tunicates. Based on the statute, he would first have to determine whether his invention was "directly based" on the tunicates. The definition of "directly based," however, is not clear from the text and a patent applicant cannot assume that he or she understands its meaning but would need to consult with a local patent attorney or research the Swiss patent law. If such research concluded that this phrase has the meaning afforded by the Swiss Delegation at the Twenty-Ninth IGC Session "that [an invention] would not exist without the GR or the TK,"[3] it would appear to necessitate the disclosure of the source for all of the GRs that were used. Almost every GR described in a specification could be implicated, not just the ones in the claims. For example, applicants often use many GRs in the experimental process before completing the actual invention, even though these GRs are not themselves part of the invention. In the case of the '870 Patent, Rinehart performed experiments on plants, animals, humans, DNA viruses and RNA viruses to determine the viability of his invention. Without these, the invention "would not exist" because Rinehart would not know whether it was effective in the first place. But the "would not exist" test raises the question as to what other GRs should be recognized. For example, but for the discovery of the thermophilic bacterium *Thermus aquaticus* in the United States Yellowstone National Park, biotechnology techniques used in Rinehart's invention, the invention would not exist. Should the source of this GR be disclosed as well?

Table 1 provides an overview of the requirements under the Swiss Patents Act, including the GRs in Rinehart's Specification that would need to be considered whether they fall under Switzerland's definition of "directly based."

TABLE 1. OVERVIEW OF REQUIREMENTS UNDER PATENTS ACT FOR '870 PATENT APPLICANT

Genetic Resource/Traditional Knowledge	**Location in Specification**	**'870 Applicant Meet Disclosure?**
Within the scope of the claim, but not obtained	Claims	No?
Tunicates, Belize Location #1	Col. 1, lines 53-55	Yes
Tunicates, Colombia	Col. 1, lines 55-56	Yes
Tunicates, Mexico	Col. 1, lines 56-58	Yes
Tunicates, Belize Location #2	Col. 1, lines 58-60	Yes
Tunicates, Honduras	Col. 1, lines 60-62	Yes
Tunicates, Panama	Col. 1, lines 62-64	Yes
Experimental Animals	Col. 1, line 30	**Needed**
Experimental Humans	Col. 1, line 30	**Needed**
Experimental Plants	Col. 1, line 30	**Needed**
DNA Viruses	Col. 1, line 31	**Needed**
RNA Viruses	Col. 1, line 31	**Needed**
Traditional knowledge	Not disclosed	**Needed**

[3] Draft Report, Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore, Twenty-Ninth Session, Geneva, February 15 to 19, 2016, Paragraphs 231-232.

E. **Penalties for NCHA and Patents Act Violations**

Switzerland imposes strict penalties on those violating the due diligence, notification and disclosure requirements. Failing to provide proper notification of due diligence could result in criminal fines of up to CHF100,000.[4] In addition, courts may order the publication of the judgment. Thus, in Rinehart's case, for any country for which he does not properly provide notification of due diligence could mean a fine of CHF100,000. On top of that, the court may order publication of the judgment for any violation of the notification requirement by Rinehart.

On the patent side, the penalty for providing false disclosure information in a patent application is a fine up to CHF100,000.[5] Here too, courts may order publication of the judgment.[6] If information on the source of the GR is missing from the patent application, it must be provided by a deadline or applicants risk denial of the patent. While Rinehart disclosed the source of the tunicates, this was done in 1990, many years before Switzerland had ABS and disclosure requirements on the books. Moreover, under today's law he could be penalized for not disclosing the source of the animals, plants, DNA viruses or RNA viruses on which he performed experiments.

The below table indicates the kind of penalties Rinehart would encounter today. As shown, he could be face up to CHF200,000 in fines, multiple court-ordered published judgments, and at least one denied patent.[7] Clearly, this would have enormous implications on an applicant's decision to file for a patent in Switzerland today.

TABLE 2. OVERVIEW OF PENALTIES UNDER SWISS LAW FOR '870 PATENT APPLICANT

Swiss Provision	**'870 Applicant Meet?**	**Max. Fines**	**Judgment Published**	**Patent Denied**
Due Diligence (Art. 23n NCHA)				
Belize Location #1	Not Needed	NA	NA	NA
Colombia	Not Needed	NA	NA	NA
Mexico	**Needed**	NA	NA	NA
Belize Location #2	Not Needed	NA	NA	NA
Honduras	Not Needed	NA	NA	NA
Panama	**Needed**	NA	NA	NA
Subtotal	**2 statutory requirements**	**NA**	**NA**	**NA**
Notification (Art. 23o NCHA)				
Belize Location #1	Not Needed	No	No	NA
Colombia	Not Needed	No	No	NA
Mexico	**Needed**	Yes	Yes	NA
Belize Location #2	Not Needed	No	No	NA
Honduras	Not Needed	No	No	NA
Panama	**Needed**	Yes	Yes	NA
Subtotal	**2 statutory requirements**	**CHF100,000 max (Criminal)**	**1 Judgment Published**	**NA**
Market Authorization	Not needed but would need if	Varies	NA	NA

[4] Federal Act on the Protection of Nature and Cultural Heritage, Art. 24a.
[5] Federal Act on Patents for Inventions, Art. 81(a(1)).
[6] Federal Act on Patents for Inventions, Art. 81(a(2)).
[7] If Rinehart files several related patent applications, for example, multiple patents would be denied in Switzerland.

(Federal Law on Medicinal Products and Medical Devices)	commercial exploitation of GR			
Disclosure (Patents Act 49a)				
Belize Location #1	Yes	No	No	No
Colombia	Yes	No	No	No
Mexico	Yes	No	No	No
Belize Location #2	Yes	No	No	No
Honduras	Yes	No	No	No
Panama	Yes	No	No	No
Experimental Animals	**Needed**	Yes	Yes	Yes
Experimental Plants	**Needed**	Yes	Yes	Yes
Experimental Humans	**Needed**	Yes	Yes	Yes
DNA Viruses	**Needed**	Yes	Yes	Yes
RNA Viruses	**Needed**	Yes	Yes	Yes
Subtotal	**5 Statutory Requirements**	**CHF100,000 max**	**1 Judgment Published**	**1 + Patent Rejected (depending on number of related patent applications filed)**
Overall Total	**9 Statutory Requirements**	**CHF200,000**	**2 Judgments Published**	**1+ Patent Rejected**

Some may argue that the solution for avoiding heavy fines in Switzerland is for applicants to not willfully submit false origin or notification information. However, there is still a large CHF 40,000 fine for negligence with respect to notification, and the act remains a crime regardless of intent.

Moreover, the potential publication of judgments (criminal in the case of false or negligent notification) and a potential refused patent are major deterrents to small research applicants like Rinehart, who would be discouraged from filing a patent application in Switzerland.

Overall, it is clear that the Swiss disclosure requirement is not a simple “check box” requirement and considerable time would be required to determine whether and how to meet the requirement.

[Appendix follows]

US005137870A

United States Patent [19]

Rinehart

[11] **Patent Number: 5,137,870**

[45] **Date of Patent: Aug. 11, 1992**

[54] **DIDEMNINS AND NORDIDEMNINS**

[75] Inventor: **Kenneth L. Rinehart,** Urbana, Ill.

[73] Assignee: **The Board of Trustees of the University of Illinois,** Urbana, Ill.

[21] Appl. No.: **482,372**

[22] Filed: **Feb. 20, 1990**

Related U.S. Application Data

[60] Division of Ser. No. 137,484, Dec. 23, 1987, Pat. No. 4,950,649, which is a continuation-in-part of Ser. No. 894,442, Jul. 31, 1986, abandoned, which is a continuation of Ser. No. 663,824, Oct. 22, 1984, abandoned, which is a continuation of Ser. No. 449,296, Dec. 13, 1982, abandoned, which is a continuation of Ser. No. 299,894, Sep. 18, 1981, abandoned, which is a division of Ser. No. 217,768, Dec. 18, 1980, abandoned, which is a continuation-in-part of Ser. No. 186,932, Sep. 12, 1980, abandoned.

[51] **Int. Cl.**[5] **A61K 37/00**
[52] **U.S. Cl.** **514/10;** 514/11
[58] **Field of Search** 514/10, 11

[56] **References Cited**

PUBLICATIONS

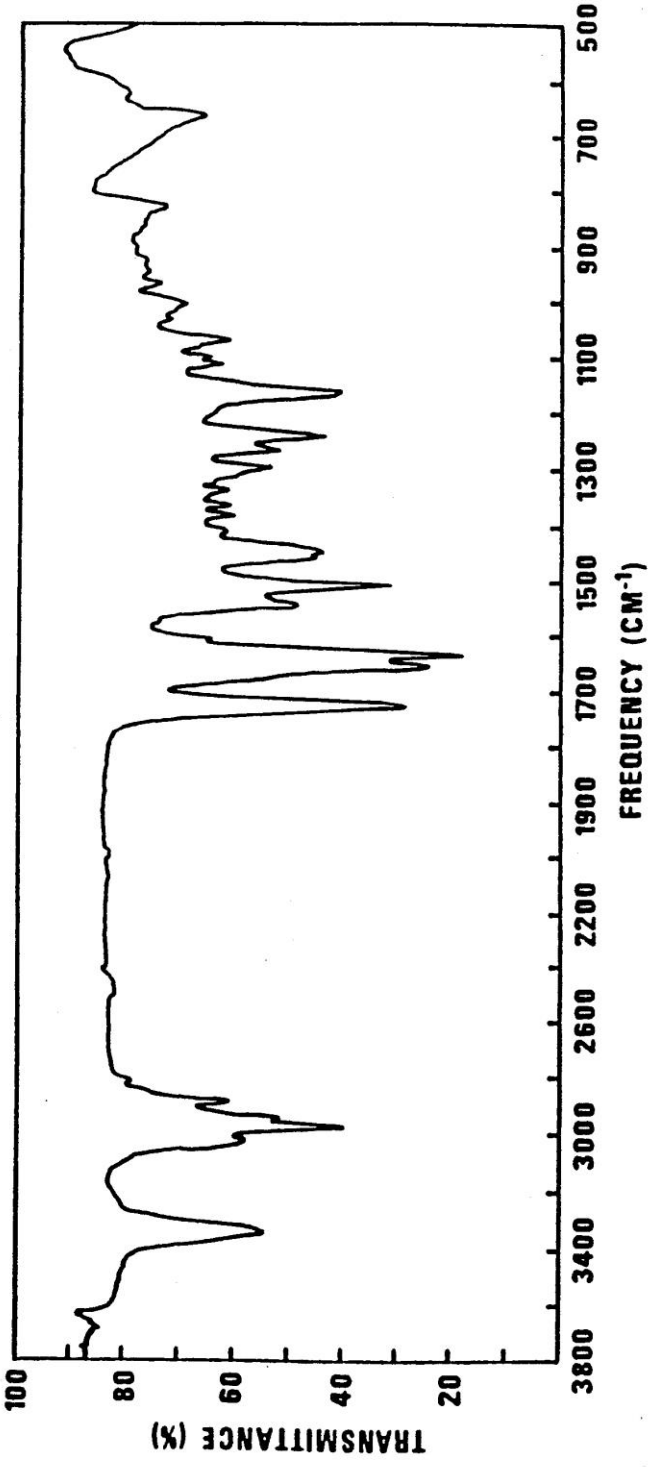
Morishima, et al., Jr. of Antibiotics, vol. XXIII, No. 5, May 1970, pp. 263–265.
Dorr, et al., Clinical Pharmacology (Adult & Pediatric) (Proceedings of Amer. Soc. Clin. Oncol., vol. 5, Mar. 1986), p. 39 (No. 151).
Stewart, et al., Clinical Pharmacology (Adult & Pediatric) (Proceedings of Amer. Soc. Clin. Oncol., vol. 5, Mar. 1986), p. 33 (No. 128).
Jiang, et al., Cancer Chemotherapy & Pharmacology, 1983, vol. 11, No. 4?, pp. 1–4.

Primary Examiner—Jerome D. Goldberg
Attorney, Agent, or Firm—Ernest V. Linek

[57] **ABSTRACT**

Novel antibiotics didemnins A, B and C (didemnins), and nordidemnins A, B and C (nordidemnins) which can be obtained from a marine organism. These antibiotics are active against a variety of DNA and RNA viruses; thus, they can be used in various environments to control or eradicate these viruses. Further, these antibiotics can be used to treat animals and humans hosting a neoplastic disease.

2 Claims, 2 Drawing Sheets


TRANSMITTANCE (%)
100
80
60
40
20
FREQUENCY (CM^{-1})
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3400
3000
2600
2200
1900
1700
1500
1300
1100
900
700
500

FIGURE 1

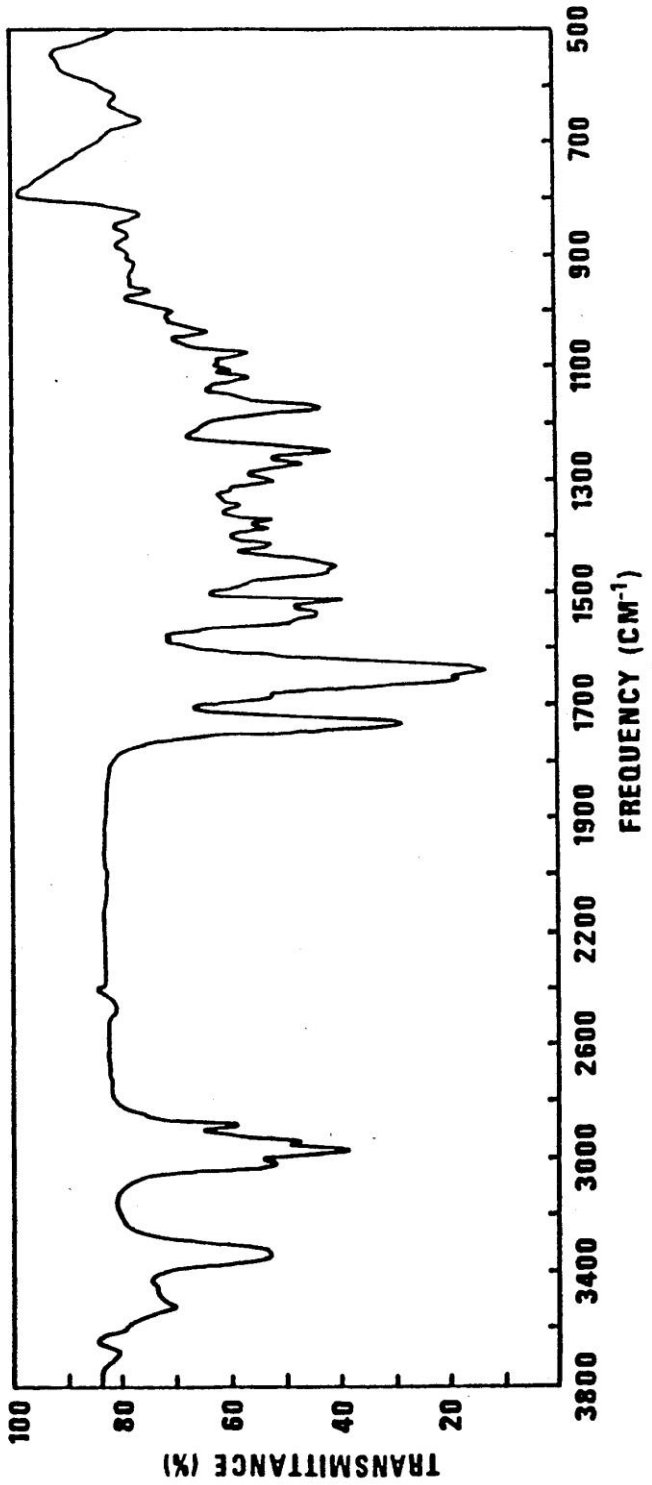

TRANSMITTANCE (%)
100
80
60
40
20
FREQUENCY (CM^{-1})
3800
3400
3000
2600
2200
1900
1700
1500
1300
1100
900
700
500

FIGURE 2

DIDEMNINS AND NORDIDEMNINS

CROSS REFERENCE TO RELATED APPLICATION

This application is a divisional of U.S. Ser. No. 07/137,484 filed Dec. 23, 1987, now U.S. Pat. No. 4,950,649, which is a continuation-in-part of U.S. Ser. No. 06/894,442 filed Jul. 31, 1986, abandoned, which is a continuation of Ser. No. 06/663,824 filed Oct. 22, 1984, abandoned, which is a continuation of Ser. No. 06/449,296 filed Dec. 13, 1982, abandoned, which is a continuation of Ser. No. 06/299,894 filed Sept. 8, 1981, abandoned, which is a divisional of Ser. No. 06/217,768 filed Dec. 18, 1980, abandoned, which is a continuation-in-part of Ser. No. 06/186,932 filed Sept. 12, 1980, abandoned.

BRIEF SUMMARY OF THE INVENTION

Novel antibiotics didemnin A, didemnin B, didemnin C, nordidemnin A, nordidemnin B, and nordidemnin C are extracted from a marine tunicate of the family Didemnidae, and tentatively identified as a Tridemnum sp. These antibiotics are active against DNA viruses, for example, herpes simplex virus types 1 and 2, and vaccinia virus; RNA viruses, for example, coxsackie virus and equine rhinovirus; and B388 leukemia in mice. Thus these antibiotics can be used to treat infections in humans, animals and plants caused by these viruses and other DNA and RNA viruses. Didemnin A and didemnin B also inhibit L1210 mouse leukemia cells in vitro. Acid addition salts and acyl derivatives of the didemnins can be made and used for the same biological purposes are the parent compounds.

DETAILED DESCRIPTION OF THE INVENTION

The organism from which didemnins and nordidemnins (A, B and C) are extracted is a colonial marine tunicate of the family Didemnidae Tridemnum sp. These organisms are in the suborder Aplousobranchia of the order Enterogona of the class Ascidiacea of the subphylum Urochordata of the phylum Chordata. They can be readily obtained by scuba techniques at depths of 10 to 100 feet where they encrust rocks, sponges, gorgonians, etc., in colony sizes up to 3 feet in diameter and ½ inch in thickness. They vary in color depending on location from green-white to purple-white to brown-white to orange-white.

Specific locations from which these organisms have been obtained are as follows:

(1) Southwest side of Long Cay, Lighthouse Reef, Belize, 17° 11.8′ N by 87° 36.5′ W at a depth of 50 to 100 feet; (2) Rada el Cove, Isla San Andres, Colombia, 12° 31′46″ N by 81° 44′5″ W at 25 to 33 feet; (3) Palancar Reef, Isla de Cozumel, Mexico, 20° 18.2′ N by 87° 2.5′ W at 60 to 100 feet; (4) on the west side of the southern tip of Turneffe Island, Belize, 17° 11.3′ N by 87° 55.6′ W at 50 to 75 feet; (5) Punta Oeste, Coxen's Hole Harbor, Isla Roatan, Honduras, 16° 15′ N by 86° 38′ W at 10 to 70 feet; (6) on the leeward side of the western-most Holandes Cay, Isla San Blas, Panama, 9° 35.6′ N by 78° 47′ W at 60 feet.

A variety of methods can be used to isolate and purify the didemnins and nordidemnins from samples of the tunicate organisms, for example, solvent extraction, partition chromatography, silica gel chromatography,

liquid-liquid distribution in a Craig apparatus, adsorption on resins, and crystallization from solvents.

The following examples describe preferred processes, but are not to be construed as limiting.

EXAMPLE 1

Tunicate sample AHCE #614 was collected on the southwest side of Long Cay, Lighthouse Reef, Belize, 17° 11.8′ N by 87° 36.5′ W at a depth of 50 to 100 feet. The sample was placed in isopropanol and stored at −10° C. until it was extracted by the following procedure.

500 Grams of tunicate were homogenized in a Waring blender with a total of 2.4 liters of 3:1 MeOH:toluene and the residue was filtered with suction to give a dark olive green solution. 500 Ml of the aqueous isopropanol from the storage container was evaporated down to ~40 ml of a mostly aqueous oily solution. This material was suspended in 400 ml of 3:1 methanol:toluene and combined with the 2.4 liters above.

The resulting dark green solution was partitioned versus 1400 ml of 1N $NaNO_3$ solution to give an aqueous phase and a toluene phase. The aqueous phase was extracted with chloroform (1×500 ml, 1×300 ml, 2×250 ml and 1×100 ml) to give a cloudy grass green chloroform solution which was evaporated under reduced pressure, taken up in dry $CHCl_3$, and filtered to remove salt. Evaporation of the chloroform yielded 878 mg of a dark green flaky solid.

108 Mg of this material was loaded onto a silica gel (Brinkmann, particle size 0.05–0.2 mm) column of dimensions 1.7 cm×46 cm, which was packed in chloroform. The column was eluted in step gradient fashion with 150 ml $CHCl_3$, 150 ml $CHCl_3$:MeOH, 99:1; 150 ml $CHCl_3$:MeOH, 97:3; and 250 ml $CHCl_3$:MeOH, 95:5. Three-ml fractions were collected, and the content of each fraction was evaluated by thin-layer chromatography developed with 9:1 $CHCl_3$:MeOH.

Relatively impure didemnin C (Rf 0.66–0.72) was obtained as an oil in trace amounts as the first major non-pigment to elute. It was contained in the last 30 ml of the 3% methanol in chloroform wash. Essentially pure didemnin C can be obtained by use of preparative thin-layer chromatography, as described for preparing didemnin A, below. Essentially pure didemnin B (Rf 0.59–0.64) was obtained as a yellow-white amorphous solid (6.1 mg). In most cases, didemnin B was already pure enough (by TLC) for data accumulation and testing. Didemnin A (Rf 0.46–0.52) was obtained as a greenish-white solid (34.7 mg) containing substantial impurities of both higher and lower Rf. Didemnins B and A were contained in the first 100 ml of the 5% methanol in chloroform wash. When an essentially pure sample was desired (for mass spectroscopy, etc.), didemnin A was purified by preparative thin-layer chromatography on 0.25 mm TLC plates employing 9:1 $CHCl_3$:MeOH as the eluent. In many cases, the best fractions of didemnin A collected from later columns had fewer impurities, which allowed immediate use for reactions and spectral data accumulation.

EXAMPLE 2

Salts of Didemnins

Since the didemnins are weakly basic, they form salts with mineral acids such as HCl, H_2SO_4, H_3PO_4, and the like. Such salts can be prepared by suspending the didemnins in water, adding a dilute acid until the pH of

the solution is about 3 to 4, and freeze-drying the solution to provide a dried residue of the didemnin salt. Salts of the didemnins can be used for the same biological purposes as the parent compounds.

EXAMPLE 3

Derivatives of Didemnins

The didemnins have free amino and hydroxyl groups available for derivatization. Thus, acyl amides and esters of the didemnins can be prepared by methods well known to those skilled in the art. Acyl derivatives of the didemnins can be used for the same biological purposes as the parent compounds.

Acids which can be used in the acylation of a didemnin include

(a) saturated or unsaturated, straight or branched chain aliphatic carboxylic acids, for example, acetic, propionic, butyric, isobutyric, tert-butylacetic, valeric, isovaleric, caproic, caprylic, decanoic, dodecanoic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, stearic, acrylic, crotonic, undecylenic, oleic, hexynoic, heptynoic, octynoic acids, and the like; (b) saturated or unsaturated, alicyclic carboxylic acids, for example, cyclobutanecarboxylic acid, cyclopentanecarboxylic acid, cyclopentenecarboxylic acid, methylcyclopentenecarboxylic acid, cyclohexanecarboxylic acid, dimethylcyclohexanecarboxylic acid, dipropylcyclohexanecarboxylic acid, and the like; (c) saturated or unsaturated, alicyclic aliphatic carboxylic acids, for example, cyclopentaneacetic acid, cyclopentanepropionic acid, cyclohexaneacetic acid, cyclohexanebutyric acid, methylcyclohexaneacetic acid, and the like; (d) aromatic carboxylic acids, for example, benzoic acid, toluic acid, naphthoic acid, ethylbenzoic acid, isobutylbenzoic acid, methylbutylbenzoic acid, and the like; and (e) aromatic-aliphatic carboxylic acids, for example, phenylacetic acid, phenylpropionic acid, phenylvaleric acid, cinnamic acid, phenylpropiolic acid and naphthlacetic acid, and the like. Suitable halo-, nitro-, hydroxy-, keto-, amino-, cyano-, thiocyano-, and lower alkoxyhydrocarbon carboxylic acids include hydrocarboncarboxylic acids as given above which are substituted by one or more of halogen, nitro, hydroxy, keto, amino, cyano, or thiocyano, or loweralkoxy, advantageously loweralkoxy of not more than six carbon atoms, for example, methoxy, ethoxy, propoxy, butoxy, amyloxy, hexyloxy, and isomeric forms thereof. Examples of such substituted hydrocarbon carboxylic acids are:

mono-, di-, and trichloroacetic acid;
α- and β-chloropropionic acid;
α- and γ-bromobutyric acid;
α- and δ-iodovaleric acid; mevalonic acid;
2- and 4-chlorocyclohexanecarboxylic acid; shikimic acid;
2-nitro-1-methyl-cyclobutanecarboxylic acid;
1,2,3,4,5,6-hexachlorocyclohexanecarboxylic acid;
3-bromo-2-methylcyclohexanecarboxylic acid;
4- and 5-bromo-2-methylcyclohexanecarboxylic acid;
5- and 6-bromo-2-methylcyclohexanecarboxylic acid;
2,3-dibromo-2-methylcyclohexanecarboxylic acid;
2,5-dibromo-2-methylcyclohexanecarboxylic acid;
4,5-dibromo-2-methylcyclohexanecarboxylic acid;
5,6-dibromo-2-methylcyclohexanecarboxylic acid;
3-bromo-3-methylcyclohexanecarboxylic acid;
6-bromo-3-methylcyclohexanecarboxylic acid;
1,6-dibromo-3-methylcyclohexanecarboxylic acid;
2-bromo-4-methylcyclohexanecarboxylic acid;
1,2-dibromo-4-methylcyclohexanecarboxylic acid;
3-bromo-2,2,3-trimethylcyclopentanecarboxylic acid;
1-bromo-3,5-dimethylcyclohexanecarboxylic acid;
homogentisic acid, o-, m-, and p-chlorobenzoic acid;
anisic acid;
salicylic acid;
p-hydroxybenzoic acid;
β-resorcylic acid;
gallic acid;
veratric acid;
trimethoxybenzoic acid;
trimethoxycinnamic acid;
4,4'-dichlorobenzilic acid;
o-, m-, and p-nitrobenzoic acid;
cyanoacetic acid;
3,4- and 3,5-dinitrobenzoic acid;
2,4,6-trinitrobenzoic acid;
thiocyanoacetic acid;
cyanopropionic acid;
lactic acid;
ethoxyformic acid (ethyl hydrogen carbonate);
malic acid;
citric acid;
isocitric acid;
6-methylsalicylic acid;
mandelic acid;
levulinic acid;
pyruvic acid;
glycine;
alamine;
valine;
isoleucine;
leucine;
phenylalanine;
proline;
serine;
threonine;
tyrosine;
hydroxyproline;
ornithine;
lysine;
arginine;
histidine;
hydroxylysine;
phenylglycine;
p-aminobenzoic acid;
m-aminobenzoic acid;
anthranilic acid;
aspartic acid;
glutamic acid;
aminoadipic acid;
glutamine;
asparagine;
and the like.

EXAMPLE 4

Isolation of Nordidemnins

Nordidemnins (A, B and C) are minor homologs of each didemnin in which norstatine replaces statine. The nordidemnins can be separated from the didemnins and isolated in essentially pure form by reverse phase high pressure liquid chromatography, monitoring the N,O-dimethyltyrosine chromophore by ultraviolet spectroscopy at 275 nm. Usable solvent systems for these separations include combinations of methanol, water and triethylamine; methanol, water and 2-propanol; or ethanol, water, and 2-propanol. Nordidemnins A. B and C

give molecular ions at m/z 928, 1097, and 1000, respectively. Hydrolysis in acid, derivatization and gas chromatography/mass spectrometry identified norstatine as replacing statine. Salts and derivatives of the nordidemnins can be made in accord with the procedures disclosed in Examples 2 and 3. These compounds, as well as the nordidemnins themselves, can be used for the same biological purposes as disclosed for the didemnins.

CHARACTERIZATION OF DIDEMNINS

Solubilities

Didemnins A, B and C are soluble in methanol, ethanol, isopropanol, dioxane, ethyl acetate, and chloroform. They are only sparingly soluble in toluene and insoluble in water. Nordidemnins A, B and C have a similar solubility pattern.

Acid Hydrolysis of Didemnins

The didemnin samples were hydrolyzed in 6N HCl at 110° C. for 24 hours. The resulting amino acids were identified by field desorption mass spectrometry (FDMS) of the mixture, as well as by gas chromatography (GC)/MS of the amino acids' trifluoroacetyl n-butyl ester derivatives. They were also quantitated by GC, and their identities confirmed by coinjection with derivatives of authentic samples.

Didemnin A contains a mole each of leucine; N-methylleucine, threonine; proline; N,O-dimethyltyrosine; and statine [see H. Morishima et al., Journal of Antibiotics 23:263 (1970) for a description of statine (from pepstatin)]. Statine was assigned as the threo isomer by its co-elution with the synthetic R,S-isomer, while gas chromatography on an optically active column indicated Leu, MeLeu, and Me_2Tyr to have the L-configuration.

Didemnin B contains a mole of each of the above six amino acids plus a mole of lactic acid and an additional mole of proline. Didemnin C contains a mole of each of the above six amino acids plus a mole of lactic acid. In addition to the above amino acids, each didemnin contains a hydroxy-isovalerylpropionyl group.

The order of linkage of these seven units was established by the fragment ions identified in high resolution electron impact mass spectra.

Each of the didemnins (A, B, C) is accompanied by a minor but varying amount of a homolog. The nature of the homology was clarified by the observation that GC traces of the derivatized amino acids always contain minor amounts of an amino acid identified by GC/MS as a homolog of statine, for which we propose the name norstatine [$(CH_3)_2CHCHNH_2CHOHCH_2COOH$]. The homologous peptides can then properly be designated as nordidemnins A, B and C, in which norstatine replaces the statine unit of each didemnin.

Mass Spectra

Didemnin A has the following mass spectral peaks: a molecular ion at 942.5678 ($C_{49}H_{78}N_6O_{12}$) and fragments at m/z 886, 843, 800, 756, 611, 583, 557, 523, 501, 499, 480, 455, 401, 383, 356, 313, 310, 298, 288, 262, 210, 183, 178, 154, 139, 134, 128, 122, 121, 100.

The field desorption mass spectrum of didemnin B gives an M+H ion at 1112.6442 ($C_{57}H_{90}N_7O_{15}$). The electron impact mass spectrum of didemnin B contains ions at m/z 942, 924, 913, 886, 844, 843, 797, 756, 611, 593, 557, 523, 425, 396, 383, 313, 307, 288, 262, 224, 210, 183, 154, 121, 100.

The field desorption mass spectrum of didemnin C contains an M+H ion at 1014.5873 ($C_{52}H_{82}N_6O_{14}$). In addition, the mass spectrum of didemnin C obtained by electron impact gives ions at m/z 958, 887, 859, 842, 693, 675, 578, 547, 536, 494, 368, 283, 262, 237, 224, 200, 172, 154, 121, 100. The minor homolog of didemnin C gives an M+H ion at 1000.5714 ($C_{51}H_{80}N_6O_{14}$). Its electron impact mass spectrum contains peaks at m/z 944, 873, 845 and 828 indicative of the homology.

NMR Spectra

Didemnin A had the following proton NMR peaks, in ppm from tetramethylsilane: 8.3, 7.8, 7.5, 7.1, 6.9, 5.2, 5.0, 4.9, 4.8, 4.6, 4.2, 4.1, 4.0, 3.8, 3.7, 3.6, 3.4, 3.2, 3.1, 2.9, 2.6 (singlet methyl), 2.6 (doublet of doublets), 2.4 (singlet methyl), 2.4 (multiplet), 2.1, 1.8, 1.6, 1.4, 1.35, 1.30, 1.2, 0.9 (several overlapping methyl doublets).

Didemnin B had the following proton NMR peaks, in ppm from tetramethylsilane: 8.0, 7.8, 7.4, 7.2, 7.0, 5.5, 5.4, 5.3, 4.9, 4.8, 4.6, 4.5, 4.4, 4.2, 3.9, 3.8, 3.5, 3.3 (doublet), 3.3 (singlet methyl), 3.1, 2.8, 2.7 (singlet methyl), 2.5, 2.3, 2.1, 1.9, 1.7, 1.5, 1.4, 1.3, 1.0 (several overlapping methyl doublets).

Didemnin C had the following proton NMR peaks, in ppm from tetramethylsilane: 7.7, 7.4, 7.2, 7.0, 5.2, 5.0, 4.8, 4.5, 4.2, 4.0, 3.7, 3.5, 3.3, 3.1, 3.0, 2.9, 2.8 (singlet methyl), 2.4 (singlet methyl), 2.3, 2.2, 2.1, 1.8, 1.5, 1.4–1.25, 1.1–0.8 (several overlapping methyls).

Didemnin A had the following ^{13}C-NMR signals, relative to tetramethylsilane in $CDCl_3$: 205.1, 175.3, 172.3, 171.4, 170.4, 169.9, 169.6, 168.6, 158.7, 130.4 (2 carbons), 129.9, 114.2 (2 carbons), 81.5, 71.1, 67.6, 66.2, 63.2, 57.4, 55.4, 55.3, 54.7, 49.9, 49.5, 47.1, 42.5, 41.3, 38.6 (2 carbons), 35.4, 34.2, 34.0, 31.2, 27.9, 27.0, 25.1 (2 carbons), 24.9, 23.7, 22.9, 22.3, 21.0, 18.7, 16.9, 15.4, 14.9 (2 carbons), 11.7.

Didemnin B has the following ^{13}C-NMR signals relative to tetramethyl silane in $CDCl_3$: 204.9, 174.0, 172.9, 172.4, 171.8, 171.3, 170.6, 169.7, 169.4, 168.4, 158.7, 130.3 (2 carbons) 130.1, 114.2 (2 carbons), 81.5, 70.5, 68.0, 66.5, 66.0, 57.7, 57.2, 56.7, 55.5, 55.3, 54.9, 49.6 (2 carbons), 47.0 (2 carbons), 41.4, 38.9, 38.7, 36.2, 34.0 (2 carbons), 31.3 (2 carbons), 28.4, 27.9, 27.2, 26.0, 24.9 (3 carbons) 23.8, 23.4, 21.4, 21.0, 20.3, 18.6, 16.9, 16.3, 15.2, 14.7, 11.7.

Infrared Spectra

The didemnins were dissolved in chloroform and examined in a Beckman IR-12 double beam spectrophotometer vs. a chloroform reference.

The spectrum of didemnin A is shown in FIG. 1. Peaks are observed at the following wavelengths:

Band Frequency (Wave Number cm^{-1})	Intensity (% T)
3680	84
3600	82 sh
3520	70
3340	54
3020	57
2970	39
2940	51
2880	60
2810	78
2460	82
1725	27.5
1650	23

-continued

Band Frequency (Wave Number cm^{-1})	Intensity (% T)
1630	17
1605	64
1540	48
1505	31
1455	45
1445	43
1405	61
1380	59
1360	60
1335	60.5
1325	64
1310	60 sh
1295	53
1265	51
1240	43
1195	63 sh
1160	40
1110	62
1100	63.5
1080	66 sh
1065	60
1030	71
1000	68
965	73
940	75
920	76
900	78
825	72
660	65
620	79

sh = shoulder

The spectrum of didemnin B is shown in FIG. 2. Peaks are observed at the following wavelengths:

Band Frequency (Wave Number cm^{-1})	Intensity (% T)
3680	80
3600	79
3340	52.5
3020	51.5
2970	38
2940	47
2880	58
2460	81
1725	28
1650	17
1640	13
1630	15 sh
1605	51 sh
1540	44
1514	38.5
1465	41
1455	40
1415	52
1390	52.5
1370	52
1345	57.5
1300	51
1270	46
1250	40.5
1170	42
1120	56
1105	59
1075	56
1040	63.5
1000	70
965	73.5
940	77
910	76.5
900	78
865	78
830	76
660	75

-continued

Band Frequency (Wave Number cm^{-1})	Intensity (% T)
615	80

sh = shoulder

The infrared absorption spectrum for didemnin C when pressed into a KBr disc is as follows:

Band Frequency	Intensity	Type
3672.9	73	SH
3562.9	50	SH
3501.2	40	SH
3479.0	38	SH
3421.1	35	SH
3339.1	22	BRD
3214.7	56	SH
3065.2	60	BRD
3031.4	59	BRD
2962.0	12	AVG
2935.0	19	AVG
2875.2	33	AVG
2794.2	71	SH
2499.0	79	BRD
2063.1	80	BRD
1733.2	11	AVG
1663.8	6	AVG
1639.6	3	AVG
1583.7	49	AVG
1544.2	22	AVG
1514.3	13	SHP
1488.2	33	AVG
1453.5	16	AVG
1413.0	31	AVG
1400.4	33	BRD
1386.0	25	AVG
1369.6	29	AVG
1342.6	32	AVG
1318.5	20	AVG
1305.0	24	AVG
1261.5	11	AVG
1250.0	14	AVG
1220.1	21	AVG
1169.0	14	AVG
1116.9	21	SH
1106.3	16	AVG
1092.8	17	AVG
1077.3	16	AVG
1033.9	19	AVG
1022.3	20	SH
966.4	54	AVG
938.4	63	AVG
924.0	63	AVG
862.2	58	AVG
802.4	20	AVG
755.2	60	AVG
720.4	57	AVG
704.1	58	AVG
650.0	54	AVG
615.3	53	AVG

Band Frequency in wavenumbers (cm^{-1}) Intensity in percen transmittance (%T) Data type in local peak region: BRD=Broad, AVG=Average, SH=Shoulder
This peak list is unedited

% T	Frequency
3	1639.5
6	1663.7
11	1733.1
11	1261.5
12	2962.0
13	1514.2
14	1250.0
14	1169.0
16	1453.5

-continued

% T	Frequency
16	1106.2
16	1077.2
17	1092.7
19	2935.0
19	1033.8
20	1318.5
20	1022.2
20	802.3
21	1220.0
21	1116.8
22	3339.0
22	1544.1
24	1305.0
25	1386.0
29	1369.5
31	1413.0

The IR spectra for nordidemnin A, B and C are essentially the same as for didemnin A, B and C, respectively.

Antiviral Activity

The antiviral activities of didemnins A, B and C are shown in the following table. The test method is as follows:

Costar 96 well trays were seeded with 0.2 ml cell suspension and incubated at 37° for 24 hours. The medium was removed and the wells were treated with serial 2-fold dilutions of drug (150–1.5 μg/ml) in 0.05 ml. Diluted virus (0.05 ml) or medium (BME-3% fbs) was added to each well and the cultures were returned to 37°. After overnight incubation, the cells were stained with aqueous crystal violet (0.5%), and washed thoroughly. Drug concentrations resulting in 50% cell destruction (ID_{50}) were determined visually for those cultures infected with virus (antiviral) as well as those cultures serving as toxicity controls. Ratios are the cytotoxic concentration ÷ antiviral concentration.

The cells used for the herpes simplex virus (HSV) type 1 and type 2 were Vero. ML cells were used for the assays with coxsackie (COX) A21 and equine rhinovirus (ER).

	ID_{50}		
	A	B	C
RNA Viruses			
Toxicity-ML cells	25	<1.5	6
Antiviral-cox virus	1.5	<1.5	<1.5
Ratio	17		>4
Toxicity-ML cells	25	<1.5	6
Antiviral-ER virus	1.5	<1.5	<1.5
Ratio	17		>4
DNA Viruses			
Toxicity-Vero cells	50	12	50
Antiviral-HSV-1 virus	3	1.5	<1.5
Ratio	17	8	>33
Toxicity-Vero cells	50	12	50
Antiviral-HSV-2 virus	1.5	1.5	<1.5
Ratio	33	8	>33

Antileukemia Activity

Didemnins A and B inhibit the growth of L1210 mouse leukemia cells in vitro as shown in the following table. The L1210 tube dilution assay is described in detail in a publication by L. H. Li, et al., Cancer Research 39:4816 (1979). ID_{50} and ID_{90} refer to the concentration of didemnins needed to inhibit cell growth by 50 and 90 percent, respectively.

L1210 Tube Dilution Assay

	L1210 Tube Dilution Assay	
	ID_{50} μg/ml	ID_{90} μg/ml
Didemnin A	0.019	0.058
Didemnin B	0.0018	0.0059

Didemnins A and B were also active in vitro against P388 leukemia in mice. The P388 mouse leukemia test is described in detail in a publication by G. L. Neil, et al., Cancer Treatment Reports 63, 1971–1978 (1979). The results of two P388 mouse leukemia tests using different dosage schedules is shown below.

In Vivo Testing of Didemnins Against P388 Leukemia

In Vivo Testing of Didemnins Against P388 Leukemia				
Compound[a]	Dose (μg/kg/injection	Median Survival Time[b] (day)	T/C (%)	Weight Change (g)
Didemnin A	63	10.7	104	+0.9
	125	11.9	115	+1.4
	250	12.2	119	+1.0
	500	13.2	129	+0.9
	1000	16.2	158	+0.3
	2000	16.0	155	−0.2
Didemnin B	63	14.8	143	+0.1
	125	14.0	136	−0.6
	250	14.2	138	−2.2
	500	T[c]	T	−3.4
	1000	T	T	T
	2000	T	T	T

[a]Schedule of Injection: daily intraperitoneal injection for 9 days following tumor implantation.
[b]Median survival time of control animals = 10.3 days.
[c]Toxic.

Compound	Dose (μg/kg/injection)	Median Survival Time[b] (day)	T/C (%)	Weight Change (g)
Didemnin A	250	11.1	109	+1.7
	500	12.1	118	+1.6
	1000	12.1	118	+1.8
	2000	12.4	121	+2.0
	4000	13.3	130	+1.3
	8000	14.3	140	+0.8
Didemnin B	30	12.8	125	+1.6
	60	14.3	140	+1.2
	120	15.0	147	+0.2
	250	16.0	157	−0.5
	500	18.0	176	−1.7
	1000	20.3	199	−3.8

[a]Schedule of injection: Intraperitoneal injection on Day 1, 5, and 9, following Tumor inoculation.
[b]Median survival time of Control (no drug) animals: 10.2 days.

The administration of didemnins A, B or C and nordidemnins A, B or C is useful prophylactically and therapeutically for treating neoplastic diseases e.g., leukemia, and viral infections. For example, pharmaceutical compositions containing the active ingredients are useful in prophylactic or therapeutic treatment of humans and animals infected or likely to be infected with viruses, e.g., hepatitis virus, rubella, rubeola, influenza, encephalitis viruses (i.e., arboviruses such as western or eastern equine encephalitis virus, Semliki Forest virus), herpes viruses (types 1 or 2 herpes simplex virus, cytomegalovirus, varicella-zoster and infectious bovine rhinotracheitis virus), rabies, enteroviruses (picornaviruses, echoviruses, coxsackie viruses), parainfluenza viruses, respiratory syncytial virus, sendai virus,

poliomyelitis viruses, yellow fever, Epstein-Barr virus (infectious mononucleosis), small pox, Dengue virus, common cold virus (rhinoviruses, coronaviruses, etc.), adenoviruses, polyomaviruses, papovaviruses, RNA-tumor viruses (e.g., feline leukemia virus, avian leukosis virus, avian sarcoma viruses), B virus, aleutians disease of mink, arena viruses, blue tongue virus of sheep, bovine viral diarrhea-mucosal disease virus, canine distemper virus, canine hepatitis virus, canine herpesvirus, equine abortion virus, infectious equine anemia virus, fowl pox virus, hog cholera virus, Marek's disease, mink enteritis virus, Newcastle disease virus, porcine enterovirus, pseudorabies virus, foot and mouth disease virus, reoviruses, and all other viruses or diseases of viral origin (for example, slowly progressing diseases that may be of viral origin such as multiplesclerosis) that are sensitive to the antiviral action of the didemnins or nordidemnins.

The dosage administered will be dependent upon the identity of the viral infection or.neoplastic disease, the type of host involved, its age, health, weight, kind of concurrent treatment, if any, frequency of treatment and therapeutic ratio.

Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.01 to about 20 mg/kg; intraperitoneal, 0.01 to about 100 mg/kg; subcutaneous, 0.01 to about 100 mg/kg; intramuscular, 0.01 to about 100 mg/kg; orally, 0.01 to about 200 mg/kg, and preferably about 1 to 100 mg/kg; intranasal instillation, 0.01 to about 20 mg/kg; and aerosol, 0.01 to about 20 mg/kg of animal (body) weight.

Expressed in terms of concentration, an active ingredient can be present in the compositions of the present invention for localized use about the cutis, intranasally, pharyngolaryngeally, bronchially, broncholially, intravaginally, rectally, or ocularly in a concentration of from about 0.01 to about 50% w/w of the composition; preferably about 1 to about 20% w/w of the composition; and for parenteral use in a concentration of from about 0.05 to about 50% w/v of the composition and preferably from about 5 to about 20% w/v.

The compositions of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, suppositories, sterile parenteral solutions or suspensions, sterile non-parenteral solutions or suspensions, and oral solutions or suspensions and the like, containing suitable quantities of an active ingredient.

For oral administration either solid or fluid unit dosage forms can be prepared.

Powders are prepared quite simply by comminuting the active ingredient to a suitably fine size and mixing with a similarly comminuted diluent. The diluent can be an edible carbohydrate material such as lactose or starch. Advantageously, a sweetening agent or sugar is present as well as a flavoring oil.

Capsules are produced by preparing a powder mixture as hereinbefore described and filling into formed gelatin sheaths. Advantageously, as in adjuvant to the filling operation, a lubricant such as a talc, magnesium stearate, calcium stearate and the like is added to the powder mixture before the filling operation.

Soft gelatin capsules are prepared by machine encapsulation of a slurry of active ingredients with an acceptable vegetable oil, light liquid petrolatum or other inert oil or triglyceride.

Tablets are made by preparing a powder mixture, granulating or slugging, adding a lubricant and pressing into tablets. The powder mixture is prepared by mixing an active ingredient, suitably comminuted, with a diluent or base such as starch, lactose, kaolin, dicalcium phosphate and the like. The powder mixture can be granulated by wetting with a binder such as corn syrup, gelatin solution, methylcellulose solution or acacia mucilage and forcing through a screen. As an alternative to granulating, the powder mixture can be slugged, i.e., run through the tablet machine and the resulting imperfectly formed tablets broken into pieces (slugs). The slugs can be lubricated to prevent sticking to the tablet-forming dies by means of the addition of stearic acid, a stearic salt, talc or mineral oil. The lubricated mixture is then compressed into tablets.

Advantageously the tablet can be provided with a protective coating consisting of a sealing coat or enteric coat of shellac, a coating of sugar and methylcellulose and polish coating of carnauba wax.

Fluid unit dosage forms for oral administration such as syrups, elixirs and suspensions can be prepared wherein each teaspoonful of composition contains a predetermined amount of active ingredient for administration. The water-soluble forms can be dissolved in an aqueous vehicle together with sugar, flavoring agents and preservatives to form a syrup. An elixir is prepared by using a hydroalcoholic vehicle with suitable sweeteners together with a flavoring agent. Suspensions can be prepared of the insoluble forms with a suitable vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

For parenteral administration, fluid unit dosage forms are prepared utilizing an active ingredient and a sterile vehicle, water being preferred. The active ingredient, depending on the form and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the water-soluble active ingredient can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampule and sealing. Advantageously, adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. Parenteral suspensions are prepared in substantially the same manner except that an active ingredient is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The active ingredient can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

In addition to oral and parenteral administration, the rectal and vaginal routes can be utilized. An active ingredient can be administered by means of a suppository. A vehicle which has a melting point at about body temperature or one that is readily soluble can be utilized. For example, cocoa butter and various polyethylene glycols (Carbowaxes) can serve as the vehicle.

For intranasal instillation, fluid unit dosage forms are prepared utilizing an active ingredient and a suitable pharmaceutical vehicle, water being preferred, or by dry powder for insufflation.

The active ingredients can also be admixed in animal feed. The active ingredients can conveniently be prepared in the form of a food premix. The food premix can comprise an active ingredient in admixture with an edible pharmaceutical diluent such as starch, oatmeal, flour, calcium carbonate, talc, dried fish meal and the

like nontoxic, orally acceptable pharmaceutical diluents. The prepared premix is then conveniently added to the regular feed.

For use as aerosols the active ingredients can be packaged in a pressurized aerosol container together with a gaseous or liquefied propellant, for example, dichlorodifluoromethane, carbon dioxide, nitrogen, propane, and the like, with the usual adjuvants such as cosolvents and wetting agents, as may be necessary or desirable.

The term "unit dosage form" as used in the specification and claims refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the novel unit dosage forms of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitation inherent in the art of compounding such an active material for therapeutic use in humans, as disclosed in this specification, these being features of the present invention. Examples of suitable unit dosage, forms in accord with this invention are tablets, capsules, troches, suppositories, powder packets, wafers, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampuls, vials, segregated multiples of any of the foregoing, and other forms as herein described.

The active ingredients to be employed as anti-viral can be easily prepared in unit dosage form with the employment of pharmaceutical materials which themselves are available in the art and can be prepared by established procedures. The following preparations are illustrative of the preparation of the unit dosage forms of the present invention, but are not intended to be limiting.

EXAMPLE 5

HARD-GELATIN CAPSULES

One thousand two-piece hard gelatin capsules for oral use, each capsule containing 100 mg of a didemnin or a nordidemnin, are prepared from the following types and amounts of ingredients:

didemnin or nordidemnin, micronized	100 gm
Lactose	100 gm
Corn Starch	20 gm
Talc	20 gm
Magnesium stearate	2 gm

The didemnin or nordidemnin, finely divided by means of an air micronizer, is added to the other finely powdered ingredients, mixed thoroughly and then encapsulated in the usual manner.

The foregoing capsules are useful for preventing or treating viral infection by the oral administration of one or two capsules one to four times a day.

Using the procedure above, capsules are similarly prepared containing didemnin or a nordidemnin in 50, 250 and 500 mg amounts by substituting 50 gm, 250 gm and 500 gm of didemnin for the 100 gm used above.

EXAMPLE 6

Soft Gelatin Capsules

One-piece soft gelatin capsules for oral use, each containing 250 mg of a didemnin or a nordidemnin (finely divided by means of an air micronizer), are prepared by first suspending the compound in 0.5 ml of corn oil to render the material capsulatable and then capsulating in the above manner.

The foregoing capsules are useful for preventing or treating viral infection by the oral administration of one or two capsules one to four times a day.

EXAMPLE 7

Tablets

One thousand tablets, each containing 500 mg of a didemnin or a nordidemnin are prepared from the following types and amounts of ingredients:

didemnin or nordidemnin, micronized	500 gm
Lactose	75 gm
Corn starch	50 gm
Magnesium stearate	4 gm
Light liquid petrolatum	5 gm

The didemnin or nordidemnin, finely divided by means of an air micronizer, is added to the other ingredients and then thoroughly mixed and slugged. The slugs are broken down by forcing through a Number Sixteen screen. The resulting granules are then compressed into tablets, each tablet containing 500 mg of the didemnin.

The foregoing tablets are useful for preventing or treating viral infection by the oral administration of one or two tablets one to four times a day.

Using the procedure above, tablets are similarly prepared containing a didemnin or a nordidemnin in 250 mg and 100 mg amounts by substituting 250 gm and 10 gm of a didemnin for the 500 gm used above.

EXAMPLE 8

Oral Suspension

One thousand ml of an aqueous suspension for oral use, containing in each teaspoonful (5 ml) dose, 500 mg of a didemnin or a nordidemnin, is prepared from the following types and amounts of ingredients:

didemnin or nordidemnin, micronized	500 gm
Citric acid	2 gm
Benzoic acid	1 gm
Sucrose	700 gm
Tragacanth	5 gm
Lemon Oil	2 gm
Deionized water, q.s. 1000 ml.	

The citric acid, benzoic acid, sucrose, tragacanth and lemon oil are dispersed in sufficient water to make 850 ml of suspension. The didemnin, finely divided by means of an air micronizer, is stirred into the syrup until uniformly distributed. Sufficient water is added to make 1000 ml.

The composition so prepared is useful for preventing or treating viral infection at a dose of 1 tablespoonful (15 ml) three times a day.

EXAMPLE 9

A sterile aqueous suspension for parenteral injection, containing in 1 ml 300 mg of a didemnin is prepared from the following types and amounts of ingredients:

A didemnin, micronized	300 gm

-continued

Polysorbate 80	5 gm
Methylparaben	2.5 gm
Propylparaben	0.17 gm
Water for injection, q.s. 1000 ml.	

All the ingredients, except the didemnin, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized didemnin, finely divided by means of an air micronizer, and the final suspension is filled into sterile vials and the vials sealed.

The composition so prepared is useful for preventing or treating viral infection at a dose of 1 milliliter (1M) three times a day.

EXAMPLE 10

Suppository, Rectal and Vaginal

One thousand suppositories, each weighing 2.5 gm and containing 150 mg of a didemnin or a nordidemnin are prepared from the following types and amounts of ingredients:

didemnin or nordidemnin, micronized	150 gm
Propylene glycol	150 gm
Polyethylene glycol #4000, q.s.	2,500 gm

The didemnin or nordidemnin, is finely divided by means of an air micronizer and added to the propylene glycol and the mixture passed through a colloid mill unit uniformly dispersed. The polyethylene glycol is melted and the propylene glycol dispersion added slowly with stirring. The suspension is poured into unchilled molds at 40° C. The composition is allowed to cool and solidify and then removed from the mold and each suppository foil wrapped.

The foregoing suppositories are inserted rectally or vaginally for preventing or treating viral infection.

EXAMPLE 11

Intranasal Suspension

One thousand ml of a sterile aqueous suspension for intranasal instillation, containing in each ml 150 mg of a didemnin or a nordidemnin, is prepared from the following types and amounts of ingredients:

didemnin or nordidemnin, micronized	150 gm
Polysorbate 80	5 gm
Methylparaben	2.5 gm
Propylparaben	0.17 gm
Deionized water, q.s. 1000 ml.	

All the ingredients, except the didemnin or nordidemnin, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized didemnin, finely divided by means of an air micronizer, and the final suspension is aseptically filled into sterile containers.

The composition so prepared is useful for preventing or treating viral infection, by intranasal instillation of 0.2 to 0.5 ml given one to four times per day.

EXAMPLE 12

Animal Feed

One thousand grams of feed premix is prepared from the following types and amounts of ingredients:

A didemnin (or nordidemnin)	20 gm
Soybean meal	400 gm
Fish meal	400 gm
Wheat germ oil	50 gm
Sorghum molasses	130 gm

The ingredients are mixed together and pressed into pellets.

The premix can be fed directly to laboratory animals, i.e., rats and mice, for preventing or treating viral infection.

For larger animals the premix can be added to the animal's regular feed in an amount calculated to give the desired dose of didemnin. For example, one part of premix is added to 2.5 parts of a cat's regular feed to provide the desired dose of 200 mg/kg/day for a cat of 2.5 kg.

An active ingredient can also be present, as shown in Examples 12–15 in the undiluted pure form for use locally about the cutis, intranasally, pharyngolaryngeally, bronchially, broncholially or orally.

EXAMPLE 13

Powder

Five hundred grams of a didemnin or a nordidemnin in bulk form is finely divided by means of an air micronizer. The micronized powder is placed in a shaker-type container.

The foregoing composition is useful for preventing or treating viral infection, at localized sites by applying the powder one to four times per day.

EXAMPLE 14

Oral Powder

One thousand grams of a didemnin or a nordidemnin in bulk form is finely divided by means of an air micronizer. The micronized powder is divided into individual doses of 250 mg and packaged.

The foregoing powders are useful for preventing or treating viral infection, by the oral administration of one or two powders suspended in a glass of water, one to four times per day.

EXAMPLE 15

Insufflation

One thousand grams of a didemnin or a nordidemnin in bulk form is finely divided by means of an air micronizer.

The foregoing composition is useful for preventing or treating viral infection, by the inhalation of 30 to 75 mg one to four times per day.

EXAMPLE 16

Hard Gelatin Capsules

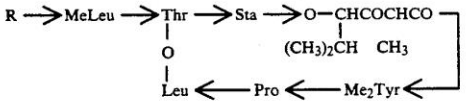
One thousand two-piece hard gelatin capsules for oral use, each capsule containing 100 mg of a didemnin or a nordidemnin.

The didemnin is finely divided by means of an air micronizer and encapsulated in the usual manner.

The foregoing capsules are useful for preventing or treating viral infection, by the oral administration of one or two capsules one to four times a day.

Using the procedure above, capsules are similarly prepared containing didemnin or a nordidemnin in 50, 250 and 500 mg amounts by substituting 50 gm, 250 gm and 500 gm of a didemnin for the 100 gm used above.

Data shows that the tentative structures for didemnin A, B and C can be shown as follows:



Didemnin A: R = H

Didemnin B: R = $CH_3CHOHCO$ → Pro →

Didemnin C: R = $CH_3CHOHCO$ →

Where

MeLeu = → N(CH3) — CH(CH2CH(CH3)2) — CO →

Thr = → NH — CH(CHCH3) — CO →

-continued

Sta = → NH — CH(CH2CH(CH3)2) — CHOH — CH2 — CO →

Me2Tyr = → N(CH3) — CH(CH2 — C6H4(OCH3)) — CO →

Pro = → N — CH — CO →

Leu = → NH — CH(CH2CH(CH3)2) — CO →

I claim:

1. A process for treating an animal or human hosting leukemia comprising: administering an effective amount of a didemnin selected from the group consisting of didemnin A, didemnin B, and didemnin C or a pharmaceutically acceptable salt thereof, to said host.

2. A process for treating an animal or human hosting leukemia comprising: administering an effective amount of a nordidemnin selected from the group consisting of nordidemnin A, nordidemnin B, and nordidemnin C, to said host.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,137,870
DATED : August 11, 1992
INVENTOR(S) : Rinehart

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

column 1, line 3, insert the following:

"This invention was made with Government support under Grant No. AI04769 awarded by the National Institutes of Health. The Government has certain rights in the invention."

Signed and Sealed this

Twenty-fifth Day of April, 1995

Attest:

Bruce Lehman

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

[End of Appendix and of document]