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SPRUSON & FERGUSON

Australia

Patents Act 1990

NOTICE OF ENTITLEMENT

634247

I, John David O'Connor, of 31 Market Street, Sydney, New South Wales, Australia, being authorised by the Applicant/Nominated Person in respect of Application No 58351/90 state the following:-

The Applicant/Nominated Person has entitlement from the actual inventors as follows:-

The Applicant/Nominated Person is the assignee of the actual inventors.

The Applicant/Nominated Person is the depositor of the following deposit with the Fermentation Research Agency of Industrial Science and Technology:

Microorganism:	Streptoverticillium sp. BA-13793
Deposit Date:	20 January 1989
Accession No.:	FERM BP-2785

26 November 1992

John David O'Connor

9345T/jj

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ANTITUMOR SUBSTANCE BE-13793C

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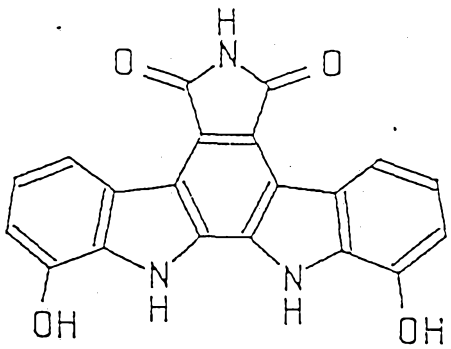
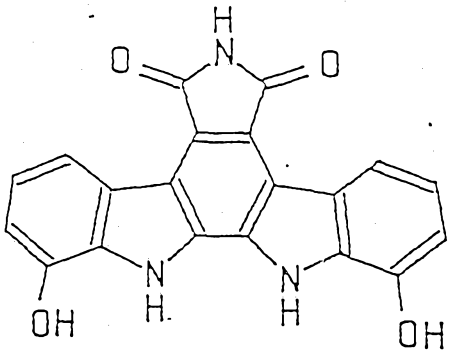
(56) Prior Art Documents
AU 29607/89 C07D 487/14

(57) Claim

1. An antitumor substance BE-13793C or a pharmaceutically acceptable salt thereof, which is represented by the following formula:



3. A method of producing an antitumor substance BE-13793C or a pharmaceutically acceptable salt thereof, which is represented by the formula:



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which comprises culturing a microorganism or a mutant thereof capable of producing said antitumor substance BE-13793C and harvesting the antitumor substance BE-13793C thus accumulated.

5. A microorganism which produces an antitumor substance BE-13793C and belongs to the genus Streptoverticillium, or a mutant thereof.

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AOJP DATE 13/02/92 PCT NUMBER PCT/JP90/00812

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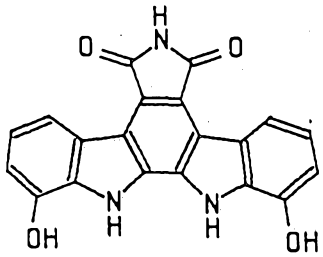
(81) 指定国
AU, HU, KR, NO.

添付公開書類 国際調査報告書

(54) Title: ANTITUMOR SUBSTANCE BE-13793C

(54) 発明の名称 抗腫瘍性物質BE-13793C



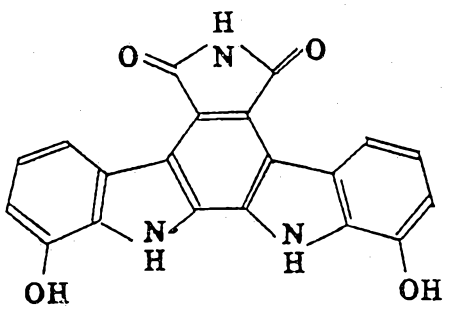
(I)

(57) Abstract

A new substance BE-13793C having chemical structure (I), pharmaceutically acceptable salts thereof, and Streptoverticillium sp. BA-13793 as a new strain of the genus Streptoverticillium and a variant thereof. The above substance can be obtained from the culture medium of the above new strain. Since the above substance and salts thereof have an antitumor activity, they are useful for treating tumors of warm-blooded mammals including man.

(57) 要約

下記の化学構造式



で表される新規物質ＢＥ－１３７９３Ｃ 又はその医薬として許容しうる塩及びストレプトバーティシリウム（Streptoverticillium）属に属する新規な菌株、ストレプトバーティシリウム・エスピー ＢＡ－１３７９３ 株又はその変異株。

上記化合物は前記新菌株を培養し、得られた培養物から採取される。

新規物質ＢＥ－１３７９３Ｃ 又はその医薬として許容しうる塩は、抗腫瘍作用を有しており、人を含む温血哺乳動物の腫瘍の治療剤として有用である。

情報としての用途のみ
PCTに基づいて公開される国際出願のパンフレット第1頁にPCT加盟国を同定するために使用されるコード

AT オーストリア
AU オーストラリア
BB バルバードス
BE ベルギー
BF ブルキナ・ファソ
BG ブルガリア
BJ ベナン
BR ブラジル
CA カナダ
CF 中央アフリカ共和国
CG コンゴー
CH スイス
CI コート・ジボアール
CM カメルーン
CS チェコスロバキア
DE ドイツ
DK デンマーク
ES スペイン
FI フィンランド
FR フランス
GA ガボン
GI ギニア
GB イギリス
GR ギリシャ
HU ハンガリー
IT イタリー
JP 日本
KP 朝鮮民主主義人民共和国
KR 大韓民国
LI リヒテンシュタイン
LK スリランカ
LU ルクセンブルグ
MC モナコ
MG マダガスカル
ML マリ
MN モンゴル
MR モーリタニア
MW マラウイ
NL オランダ
NO ノルウェー
PL ポーランド
RO ルーマニア
SD スーダン
SE スウェーデン
SN セネガル
SU ソビエト連邦
TD チャード
TG トーゴ
US 米国

SPECIFICATION

ANTITUMOR SUBSTANCE BE-13793C

TECHNICAL FIELD

The present invention relates to a novel compound which is of value in the field of medicine. More particularly, the invention relates to a novel substance which inhibits the growth and proliferation of tumor cells to produce an antitumor effect, a method of producing the novel substance, uses for the substance, and a novel microorganism belonging to the genus Streptoverticillium which produces the substance.

BACKGROUND OF ART

In the field of cancer chemotherapy, a variety of microbial metabolites such as bleomycins or adriamycin have been used in clinical practice. However, many of these substances are not sufficiently effective for many of tumors which are clinically encountered and, moreover, the acquisition of resistance of tumor cells to these drugs, which is being made increasingly clear, has been interfering with their use in clinical cases (the Proceedings of the 47th Congress of the Japanese Cancer Association, pages 12 to 15, 1988).

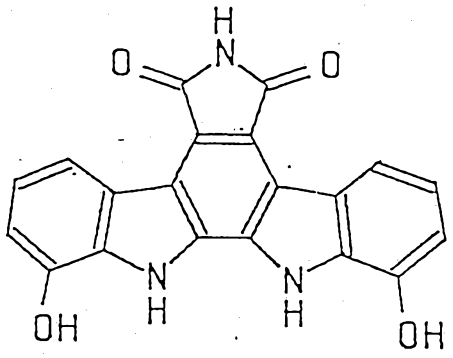
Under these circumstances, there is naturally a constant demand for the development of new anticancer agents. Thus, a strong demand exists for a substance which would overcome the resistance of various types of tumors to the existing anticancer agents and be effective even in those cases which do not respond to the anticancer drugs heretofore

available.

The inventors of the present invention screened a variety of microbial metabolites in search of candidate antitumor agents. As a result, it has been found that a novel compound of the following formula has an excellent antitumor activity. The present invention has been achieved on the basis of the above finding.

DISCLOSURE OF INVENTION

Accordingly, the present invention provides a novel antitumor substance, designated as BE-13793C, which is represented by the following formula:



or a pharmaceutically acceptable salt thereof. In further aspects, the present invention relates to a method of producing the antitumor substance BE-13793C, use of the substance BE-13793C as an antitumor agent, and a novel microorganism belonging to the genus Streptoverticillium which produces the substance BE-13793C.

The physicochemical properties of the novel antitumor substance BE-13793C of the present invention are as follows.

Physicochemical properties of BE-13793C

Description: a yellowish orange amorphous solid or crystal.

Molecular formula: $C_{20}H_{11}N_3O_4$.

Elemental analysis: Calcd. C, 67.23 %; H, 3.10 %

Found C, 67.21 %, H, 3.12 %.

Melting point:

showing no obvious decomposition point (melting point) up to 295 °C.

Solubility:

hardly soluble in water, soluble in methanol and highly soluble in an organic solvent such as tetrahydrofuran or dimethyl sulfoxide.

Acidity/neutrality/basicity: acidic.

Rf: 0.45 (developer: chloroform/methanol; 5 : 1 v/v), (Kieselgel 60 F_{254}, Merck).

Color reaction:

Potassium permanganate: positive.

Mass spectrum (FAB-MS) (m/z): 357 $[M]^+$

Ultraviolet (UV) absorption spectrum (λ_{max}^{MeOH}, nm):

245, 298, 307, 327, 400

Infrared (IR) absorption spectrum ($\nu_{cm^{-1}}^{KBr}$):

3430, 3270, 1743, 1710, 1590, 1485, 1408, 1335, 1290, 1245, 1060, 815, 800, 765

^{1}H-NMR (DMSO-d_6, δ ppm):

6.98 (2H, br d, J=7.6Hz), 7.13 (2H, t, J=7.6Hz), 8.42 (2H, br d, J=7.6Hz), 10.19 (2H, br s), 10.87 (1H, br s), 11.57 (2H, br s)

^{13}C-NMR (DMSO-d_6, δ ppm):

110.9 (d), 115.2 (d), 115.5 (s), 119.7 (s), 120.6 (s), 123.0 (s), 128.7 (s), 130.0 (s), 143.3 (s), 171.2 (s)

Biological activity of BE-13793C

In vitro activity tests were performed for evaluating the inhibitory activities of the antitumor substance BE-13793C on mouse tumor cells. In the in vitro antitumor assay using P388 tumor cells, the test substance was first dissolved in dimethyl sulfoxide and the obtained solution was serially diluted with a cell culture medium containing 20 % of dimethyl sulfoxide (20 % v/v DMSO-RPMI-1640 medium). Then, 2 µl of the dilution was added to 200 µl of a cell culture medium (10 % v/v fetal calf serum-RPMI-1640 medium) containing 2×10^4 or 3×10^4 tumor cells. Next, each mixture was incubated at 37 °C under 5 % CO_2 for 72 hours. The viable cells were then counted with a Coulter counter. The result was compared with the control data. As a result, the antitumor substance BE-13793C showed an intense inhibitory effect on the growth of the P388 tumor cells. The concentration (IC_{50}) of the antitumor substance BE-13793C causing 50 % inhibition on P388/S tumor cell growth was 0.7 µM, while that on P388/V cell growth was

0.7 μM.

The P388 cells are commonly employed mouse leukemia cells while the P388/V cells are a strain of P388 leukemia cells which have acquired resistance to the anticancer agent vincristine.

Furthermore, the antitumor substance BE-13793C inhibited the growth of P388/A cells, which had acquired resistance to the anticancer agent adriamycin, and the 50 % inhibitory concentration (IC_{50}) thereof was 1.0 μM.

The compound of the present invention called BE-13793C showed an antitumor effect on transplanted mouse Ehrlich tumor cells (ascites type). In this assay, 10^6 (lethal dose) tumor cells per mouse were intraperitoneally administered. Then the test substance was serially diluted and intraperitoneally administered. Table 1 summarizes the results.

Table 1

Effect of BE-13793C on Ehrlich ascites cancer[1,2]

Substance	Dosage, i.p.[3] (mg/kg/injection)	MST[4] (day)	MST[5,6] (% T/C)
BE-13793C	50	28.6	218
	20	26.4	202
	8	16.0	122
Control group	0	13.1	100

(Footnotes to Table 1)

1. Inoculum: 10^6 Ehrich ascites cancer cells, intraperitoneal.

2. Host: Female ICR mice.

3. Treatment schedule: BE-13793C was intraperitoneally administered once a day from the 1st to the 10th day.

4. MST: Mean survival time (in days).

5. % T/C: (Treated MST/control MST) x 100.

6. Criteria: When % T/C $\geq$ 125, the test compound was considered to produce a marked antitumor effect at the particular dose.

With regard to the acute toxicity of the antitumor substance BE-13793C on female ICR mice, no death was found on the 5th day when 100 mg/kg of said substance was intraperitoneally administered once.

As described above, the antitumor substance BE-13793C of the present invention remarkably inhibits the growth of mouse cancer cells. Therefore, it is valuable as a therapeutic agent for mammalian tumors including leukemia and many tumors such as lung, stomack, colon cancers and others.

Furthermore, the present invention relates to the uses of the compound of the present invention as an anticancer drug which is in the indipendent form or in the form of a pharmaceutical composition comprising an effective amount of the compound of the present invention optionally together with inert and pharmaceutically acceptable carrier(s).

Such a pharmaceutical composition may be produced by using the compound of the present invention in combination with

an inert and pharmaceutically acceptable carrier and provided in various dosage forms of oral, parenteral or topical administration. Suitable dosage forms include solid oral preparations (for example, tablet, capsule, pill, powder, granules) and liquid oral preparations (for example, solution, suspension, emulsion). Furthermore, sterile compositions which are extemporaneously reconstituted with sterile water, physiological saline or other sterile solvent for injection can also be provided. The composition may contain 10 to 100% w/w of the compound of the present inention.

The compound of the present invention may be used in the form of any salt thereof so long as it is pharmaceutically acceptable. Examples of the salt include those obtained by using inorganic or organic bases (for example, sodium hydroxide, potassium hydroxide, sodium hydrogen carbonate, triethylamine or 2-aminoethanol).

The clinically preferred dosage of the compound of the present invention depends on the specific compound to be used, type of formulating agent, frequency of administration, therapeutic target site, and characteristics of the host and of the tumor. By way of illustration, the daily dose per adult human ranges from 10 to 500 mg for oral administration and from 10 to 100 mg for parenteral administration, preferably intravenous administration. Although the frequency of administration varies depending on the administration method and the patient's

conditions, it is commonly sufficient to administer the compound of the present invention one to five times per day.

The method for production of BE-13793C is described hereunder. The microorganisms and mutants thereof, which are used in the production of the antitumor substance BE-13793C of the present invention, are not limited so long as they can produce the antitumor substance BE-13793C. For example, microorganism strains having the following bacteriological characteristics may be used therefor.

1. Morphology:

Under a microscope, the strain shows well-developed aerial hyphae from which whirls are formed at almost constant intervals. Further, 5 to 8 secondary branches, each having 5 to 10 terminal spore chains, are observed.

Each spore is in the form of a cylinder (0.5 x 1 to 1.5 μm) and has a smooth surface.

Neither any special organ (for example, sporangium, flagella spore or sclerotium) nor fragmentation of the hyphae is observed.

2. Cultural characteristics:

Table 2 shows the cultural characteristics on various agar plate media at 28 °C for 14 days.

Table 2

Medium	Growth	Aerial Hypha	Color of Basal Hypha	Soluble Pigment
yeast-malt-agar (ISP-2)	very good flat	poor, cotton white	light brown	none
oatmeal-agar (ISP-3)	very good flat	poor, cotton white	yellow	none
starch-inorganic salt-agar (ISP-4)	very good flat	good, cotton grayish white	pale yellowish orange	none
glycerin-asparagine-agar (ISP-5)	very good rising	good, powder yellowish white	yellowish orange	none
peptone-yeast iron-agar (ISP-6)	very good wrinkled	good, powder grayish white	pale brown	none
tyrosine-agar (ISP-7)	very good rising	good, powder yellowish white	light brown	none
nutrient agar	very good flat	good, powder white	pale yellowish brown	none
sucrose-nitrate-agar	poor	little	colorless	none
glucose-asparagine-agar	poor	little	yellowish orange	none

3. Growth temperature (yeast-malt-agar medium, 14 days):

12 °C: Poor growth and no formation of aerial hypha.

20 °C: Poor growth and no formation of aerial hypha.

28 °C: Good growth and good formation of aerial hyphae.

37 °C: Good growth but poor formation of aerial hyphae.

45 °C: No growth.

4. Physiological characteristics:

(1) Liquefaction of gelatin: negative.
(glucose-peptone-gelatin medium)

(2) Hydrolysis of starch: positive.
(starch-inorganic salt-agar medium)

(3) Coagulation and peptonization of skim milk: negative.
(skim milk medium)

(4) Production of melanoid pigments: negative.

(5) Resistance to common salt: growing at a common salt content below 4 % W/V.
(yeast-malt-agar medium)

5. Utilization of carbon sources:

The following sugars are added to a Pridham-Gottlieb agar base medium and the strain is cultured therein at 28 °C for 14 days. Table 3 shows the results.

Table 3

D-glucose	+
D-xylose	-
L-arabinose	+
L-rhamnose	±
D-fructose	+
D-galactose	+
raffinose	+
D-mannitol	-
inositol	+
salicin	-
sucrose	-

Note: +: available; ±: uncertain; -: unavailable.

6. Amino acid composition of cell wall:

LL-diaminopimelic acid and glycine are detected.

These bacteriological characteristics suggest that the strain belongs to the genus Streptoverticillium. Reference to relevant literature inclusive of Bergey's Manual of Determinative Bacteriology 8th Edition (1974) and Hosenkin no Dotei Jikken-ho (ed. by The Society for Actinomycetes, Japan) revealed that this strain is closely relates to Streptoverticillium mobaraense. However, the strain differs therefrom in the utilization of raffinose and sucrose. Further, Streptoverticillium mobaraense shows green hyphae on an agar

medium, different from the strain. These facts indicate that the strain is a novel one. Thus, it was named Streptoverticillium sp. BA-13793.

This strain has been deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan under the accession number FERM P-10489 on January 20, 1989, after conversion to deposition under Budapest Treaty, FERM BP-2785 on March 1, 1990.

For the purposes of the present invention, all variants and mutants of the antitumor substance BE-13793C-producing microorganism may be used. Such mutants may be derived from the parent strains by the known techniques such as irradiation with X-ray or ultraviolet light, treatment with a chemical mutagen (for example, nitrogen mustard, azaserine, nitrous acid, 2-aminopurine or N-methyl-N'-nitro-N-nitrosoguanidine (NTG)), or routine transformation techniques (for example, contacting with phages, transformation, transduction or conjugation).

In order to produce the antitumor substance BE-13793C of the present invention, the BE-13793C-producing strain BA-13793 is cultured in a nutrient medium under aerobic conditions so as to give a cultured broth containing the antitumor substance BE-13793C. The nutrients to be included in the medium may be those which are commonly employed in the culture of Actinomycetes. For example, a carbon source may be

selected from among commercially available glucose, glycerol, maltose, starch, sucrose, molasses, dextrin and a mixture thereof. A nitrogen source may be selected from among commercially available soybean flour, corn gluten meal, corn steep liquor, meat extract, yeast extract, cotton-seed flour, peptone, wheat germ, fish meal, inorganic ammonium salts, sodium nitrate and a mixture thereof. As inorganic salts, commercially available calcium carbonate, sodium chloride, potassium chloride, magnesium sulfate or various phosphates may be used in alone or combination. In addition, a trace amount of a heavy metal salt (for example, iron, copper, cobalt, molybdenum, manganese, zinc salts) may be used, if required. Moreover, if foaming is copious, an antifoam such as various vegetable oils (for example, soybean oil or linseed oil), higher alcohols (for example, octadecanol) and various silicone compounds may be optionally added to the medium. In addition, any other medium components (for example, boron compounds, 3-(N-morpholino)propanesulfonic acid) may be used so long as the strain can utilize them so as to promote the production of the antitumor substance BE-13793C.

The strain can be cultured by the same procedures as those commonly used in the production of microbial metabolites. Thus either solid culture or liquid culture may be employed. In the case of liquid culture, either stationary culture, stirring culture, shake culture or submerged aerobic culture may be conducted, though shake culture and submerged aerobic

culture under agitation are particularly preferable. The culture temperature may range from 20 to 37 °C, preferably from 25 to 30 °C. The pH value of the medium preferably ranges from 4 to 8. The culture may be conducted for 24 to 192 hours, preferably 48 to 120 hours.

The desired antitumor substance BE-13793C may be harvested from the cultured broth by a separation procedure commonly employed in the recovery of a microbial metabolite from a cultured broth. Since BE-13793C is contained in the culture filtrate and in the cells, it may be purified by combining conventional separation procedures employed for the recovery from a culture filtrate and cells (for example, solvent extraction, ion exchange chromatography, affinity chromatography, partition chromatography, or gel filtration). Furthermore, high performance liquid chromatography and thin layer chromatography may be used therefor.

A preferred method of separation and purification is as follows. The culture broth is first centrifuged to recover the cells, which are then extracted with an organic solvent such as methanol or acetone. The extract is concentrated under reduced pressure and the obtained concentrate is extracted with an organic solvent such as ethyl acetate. This extract is concentrated to thereby give a crude product containing BE-13793C. Next, the crude product is purified by, for example, column chromatography with Sephadex LH-20. Thus, BE-13793C

can be obtained in the form of a yellowish orange crystalline substance.

The following Examples are merely intended to illustrate the invention in further detail and should by no means be construed to limit it. The present invention should be considered to encompass all modifications of the example given herein as well as all the known production, concentration, extraction and purification processes which may be applied by those skilled in the art to BE-13793C in view of the properties of BE-13793C disclosed in this specification.

EXAMPLE

Four 500 ml conical flasks each containing 100 ml of a culture medium (pH 6.7) comprising 0.1 % of glucose, 2.0 % of dextrin, 1.0 % of corn gluten meal, 0.5 % of fish meal, 0.1 % of yeast extract, 0.1 % of sodium chloride, 0.05 % of magnesium sulfate, 0.05 % of calcium chloride, 0.0002 % of ferrous sulfate, 0.00004 % of cupric chloride, 0.00004 % of manganese chloride, 0.00004 % of cobalt chloride, 0.00008 % zinc sulfate, 0.00008 % of sodium borate, 0.00024 % of ammonium molybdate and 0.5 % of 3-(N-morpholino)propanesulfonic acid were inoculated with Streptoverticillium BA-13793 strain grown on an agar slant medium. Each flask was then incubated on a rotary shaker (180 rpm) at 28 °C for 72 hours. One-milliliter aliquots of the culture were inoculated into 50 conical flasks of 500 ml capacity each containing 100 ml of the above-mentioned medium

and incubated on a rotary shaker (180 rpm) at 28 °C for 120 hours. The resulting broth (about 5 ℓ) was filtered and the cells thus obtained were washed with 500 ml of deionized water. Then, 2.5 ℓ of methanol was added thereto and the mixture was stirred at room temperature for 1 hour. After filtering, a methanol extract was obtained. The extraction with methanol was repeated. The methanol extracts (about 5 ℓ) were combined and concentrated to about 800 ml. The concentrate thus obtained was extracted with 3 ℓ of ethyl acetate and the ethyl acetate extract was concentrated to dryness. The obtained residue was washed with 500 ml of chloroform. Thus, 720 mg of a crude product containing BE-13793C was obtained. This crude product was dissolved in 2 ℓ of methanol and concentrated. The orange precipitate thus formed was filtered to thereby give 546 mg of a product containing BE-13793C. This product was dissolved in a solvent mixture (methanol/tetrahydrofuran; 1 : 1 v/v) and subjected to column chromatography with the use of Sephadex LH-20 (1.5x120 cm, Pharmacia) and developed with methanol/tetrahydrofuran (1 : 1 v/v). The BE-13793C fraction thus obtained was concentrated to thereby give 99 mg of BE-13793C in the form of a yellowish orange crystalline substance.

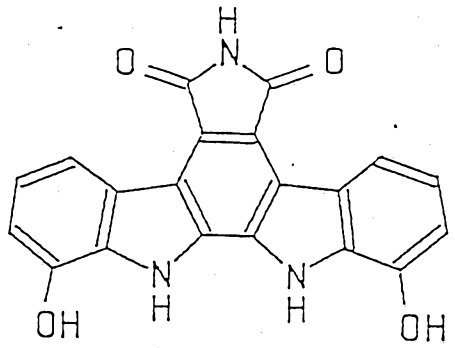
INDUSTRIAL APPLICABILITY

The antitumor substance BE-13793C of the present invention inhibits not only the growth of tumor cells showing no resistance to existing antitumor drugs but also the growth

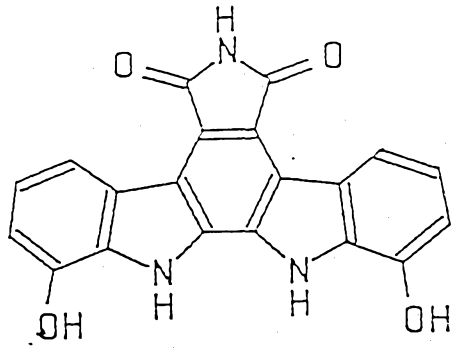
of those which have acquired the resistance against said antitumor drugs. Thus, it is highly valuable as a therapeutic agent for mammalian tumors including human tumors.

The claims defining the invention are as follows:

1. An antitumor substance BE-13793C or a pharmaceutically acceptable salt thereof, which is represented by the following formula:

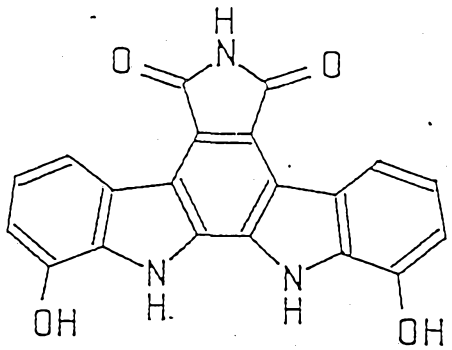


2. An antitumor composition which comprises an effective amount of an antitumor substance BE-13793C or a pharmaceutically acceptable salt thereof, which is represented by the following formula:



as an active ingredient together with a pharmaceutically acceptable carrier diluent, adjuvant and/or excipient.

3. A method of producing an antitumor substance BE-13793C or a pharmaceutically acceptable salt thereof, which is represented by the formula:



which comprises culturing a microorganism or a mutant thereof capable of producing said antitumor substance BE-13793C and harvesting the antitumor substance BE-13793C thus accumulated.

4. A method as claimed in Claim 3, wherein Streptoverticillium sp. BA-13793 strain or a mutant thereof is cultured.

5. A microorganism which produces an antitumor substance BE-13793C and belongs to the genus Streptoverticillium, or a mutant thereof.

6. A microorganism as claimed in Claim 5, which is Streptoverticillium sp. BA-13793 strain or a mutant thereof.

7. An antitumor substance comprising BE-13793C or a pharmaceutically acceptable salt thereof, substantially as hereinbefore described with reference to the Example.

8. A method of producing an antitumor substance comprising BE-13793C or a pharmaceutically acceptable salt thereof, substantially as hereinbefore described with reference to the Example.

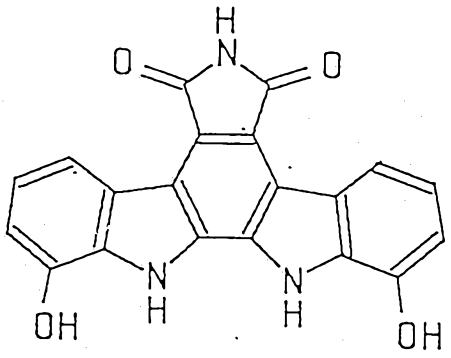
DATED this NINETEENTH day of NOVEMBER 1992

Banyu Pharmaceutical Co., Ltd.

Patent Attorneys for the Applicant
SPRUSON & FERGUSON

ABSTRACT

A novel antitumor substance BE-13793C or a pharmaceutically acceptable salt thereof, which is represented by the following formula:



an anticancer agent comprising the novel substance or a pharmaceutically acceptable salt thereof, a method of producing the novel substance, and a novel microorganism belonging to the genus Stereptoverticillium, which produces the novel substance, are disclosed.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP90/00812

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] C07D487/14, A61K31/40, C12P17/18, C12N1/20// (C12P17/18, C12R1:625) (C12N1/20, C12R1:625)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07D487/14, C12P17/18, A61K31/40, C12N1/20

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Biological Abstracts Data Base (BIOSIS)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 63-295589 (Kyowa Hakko Kogyo Co., Ltd.), 1 December 1988 (01. 12. 88), (Family: none)	1 - 6
A	BIOCHEMISTRY, Vol.29, No.1, (1990), WAKAMATSU K. et al. [COMPLEX FORMATION OF PEPTIDE ANTIBIOTIC RO-09-0198 WITH LYSOPHOSPHATIDYLETHANOLAMINE PROTON NMR ANALYSES IN DMSO SOLUTION], p.113-118	1 - 6

* Special categories of cited documents: [10]

"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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
September 13, 1990 (13. 09. 90)	October 1, 1990 (01. 10. 90)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

国際調査報告

国際出願番号PCT/JP90/00812

I. 発明の属する分野の分類

国際特許分類（IPC） Int. Cℓ' C07D487/14, A61K31/40, C12P17/18, C12N1/20//(C12P17/18, C12R1:625)(C12N1/20, C12R1:625)

II. 国際調査を行った分野

調査を行った最小限資料

分類体系	分類記号
IPC	C07D487/14, C12P17/18, A61K31/40, C12N1/20

最小限資料以外の資料で調査を行ったもの

Biological Abstracts Data Base (BIOSIS)

III. 関連する技術に関する文献

引用文献のカテゴリー※	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	請求の範囲の番号
A	JP, A, 63-295589（協和醗酵工業株式会社）, 1. 12月. 1988（01. 12. 88）,（ファミリーなし）	1-6
A	BIOCHEMISTRY, 第29巻, 第1号, (1990), WAKAMATSU K. et al「COMPLEX FORMATION OF PEPTIDE ANTIBIOTIC RO-09-0198 WITH LYSOPHOSPHATIDYLETHANOLAMINE PROTON NMR ANALYSES IN DMSO SOLUTION」, p.113-118	1-6
~~A~~	~~ANTIBIOTIKI, 第25巻, 第1号, (1980), POGOZHEVA V. V. et al「SELECTION OF ACTIVE STRAIN OF STREPTOVERTICILLIUM GRISEOCARNEUM VAR BLEOMYCINI PRODUCING BLEOMYCETIN~~	~~1-6~~

※引用文献のカテゴリー
「A」特に関連のある文献ではなく、一般的技術水準を示すもの
「E」先行文献ではあるが、国際出願日以後に公表されたもの
「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「O」口頭による開示、使用、展示等に言及する文献
「P」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「T」国際出願日又は優先日の後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」同一パテントファミリーの文献

IV. 認証

国際調査を完了した日	国際調査報告の発送日
13. 09. 90	01.10.90
国際調査機関	権限のある職員
日本国特許庁（ISA/JP）	4B 8931 特許庁審査官 石橋和美