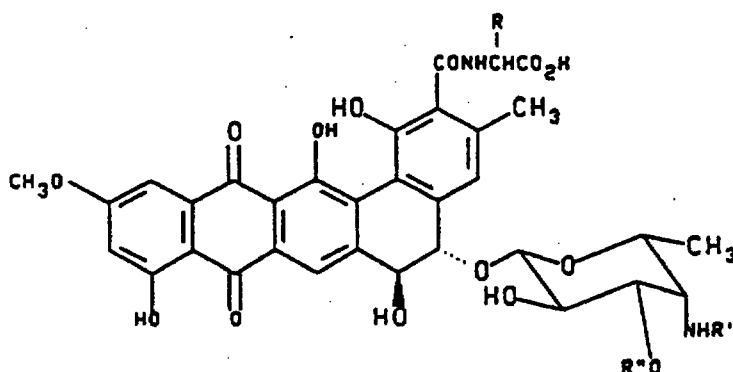


Description

The present invention relates to antifungal antibiotics, their production, their pharmaceutical use, and pharmaceutical compositions containing them. More particularly, the antibiotics are produced by *Actinomadura hibisca* and possess a benzo[a]naphthalene nucleus.

Few examples of benzo[a]naphthalene quinones derived from microbial sources have been reported and these include compounds designated G-2N and G-2A, and KS-619-1. While no biological activity was reported for G-2N and G-2A, KS-619-1 is disclosed as inhibitor of calcium ion and calmodulin-dependent cyclic nucleotide phosphodiesterase. Recently, EP-A-277,621 discloses antifungal antibiotics BU-3608 (Ia), BU-3608 B (Ib), and BU-3608 C (Ic). Antibiotics benanomycin A and B were reported in J. Antibiotics, 1988, 41:807-811; benanomycin B appears to be the same as BU-3608 C whereas benanomycin A has a hydroxyl group in place of the sugar amino group.



$R=CH_3$

Ia: $R'=CH_3$; $R''=\beta\text{-D-xylosyl}$

Ib: $R'=CH_3$; $R''=H$

Ic: $R'=H$; $R''=\beta\text{-D-xylosyl}$

$R=H$

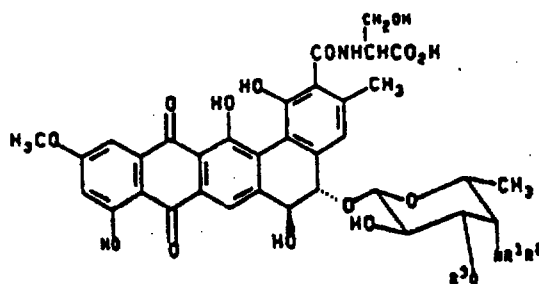
Id: $R'=CH_3$; $R''=\beta\text{-D-xylosyl}$

Ie: $R'=H$; $R''=\beta\text{-D-xylosyl}$

EP-A-345 735 discloses BU-3608 D (Id) and Bu-3608 E (Ie).

SUMMARY OF THE INVENTION

The present invention provides compounds of formula II



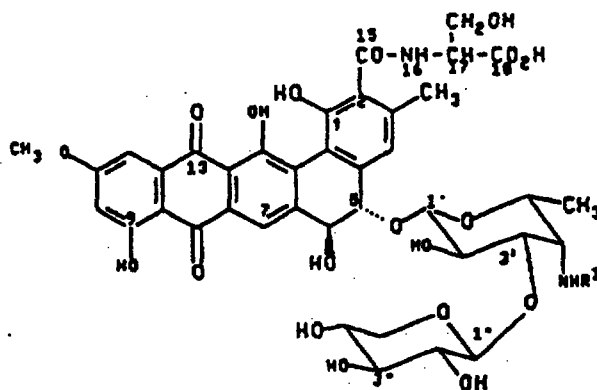
II

wherein the serine is D-serine; R^1 and R^2 are independently H or C_{1-6} alkyl; and R^3 is H or $\beta\text{-D-xylosyl}$; or a pharmaceutically acceptable salt thereof.

β -D-xylosyl is the fragment



Another aspect of the present invention provides a process for preparing a compound of formula III



III

BU-3608 FA-1: $R^1 = \text{CH}_3$

BU-3608 FA-2: $R^1 = \text{H}$

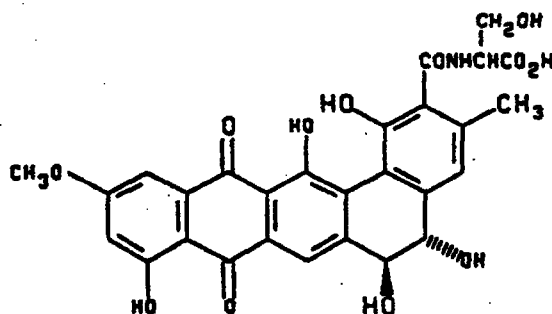
wherein R^1 is H or methyl, or a pharmaceutically acceptable salt thereof, which comprises cultivating a strain of *Actinomadura hibisca* capable of producing a compound of formula III in a medium containing assimilable sources of carbon and nitrogen and D- or DL-serine under aerobic condition, and recovering said compound of formula III from the cultured broth.

A further aspect of the invention provides a pharmaceutical composition comprising a compound of formula II and a pharmaceutically acceptable carrier.

A further aspect of the invention provides a method for treating fungal infections in a mammalian host comprising administering an antifungal effective dose of a compound of formula II to said host.

Yet another aspect of the invention provides strains of *Actinomadura hibisca* capable of producing antibiotics of formula III in a medium containing D- or DL-serine.

Also provided by the present invention is a compound having the formula IV or a salt thereof.



IV

The compound of formula IV represents the aglycone of BU-3608 FA-1 and FA-2 and is useful in the synthesis of the parent antifungal agents or derivatives thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 represents a 400 MHz proton nuclear magnetic resonance spectrum of BU-3608 FA-1 in DMSO- d_6 .

Fig. 2 represents a 400 MHz proton nuclear magnetic resonance spectrum of BU-3608 FA-2 in DMSO- d_6 .

DETAILED DESCRIPTION OF THE INVENTION

The antibiotics of the present invention are antifungal agents. Compounds of formula III, i. e. BU-3608 FA-1 and BU-3608 FA-2, are produced by cultivating a strain of Actinomadura hibisca capable of producing the antibiotics, or a variant thereof, or a mutant thereof, in a medium containing D- or DL-serine. As examples of antibiotic producing strains of Actinomadura hibisca mention can be made of strain P157-2 and mutant strains derived therefrom designated A2660, A2493 and B0012. Strain P157-2, which produces BU-3608 FA-1 preferentially in a medium supplemented with a source of D-serine, was isolated from a soil sample collected on Fiji Island in the South Pacific. A biologically pure culture of Actinomadura hibisca strain P157-2 has been deposited with the American Type Culture Collection in Rockville, MD and added to their permanent collection of microorganism as ATCC 53557. Detailed description of the cultural and physiological characteristics of strain P157-2 is disclosed in EP-A-277 621. The mutant strains A2660, A2493 and B0012, which are capable of producing both BU-3608 FA-1 and FA-2 in a medium containing a source of D-serine, were obtained from the parent strain P157-2 by exposing the parent strain to N-methyl-N'-nitro-N-nitrosoguanidine (1,000 μ g/ml) for an hour; the strains were selected on the basis of their ability to produce BU-3608 antibiotic complex, i.e. BU-3608, or the B, C, D and E components, in the absence of added source of D-serine. Biologically pure cultures of A2660, A2493 and B0012 have been deposited with the ATCC and assigned the accession numbers 53762 (A2660), 53815 (A2493) and 53816 (B0012).

Cultural characteristics of the above-mentioned antibiotic producing strains of Actinomadura hibisca are provided in Table I.

Table I. Cultural characterization of some producer strains

Media	Culture growth			
	P157-2	A-2660	A-2493	B-0012
Yeast extract-malt extract agar (ISP-2)	++	++	++	++
Glycerol-asparagine agar (ISP-5)	+	++	-	++
Sucrose nitrate agar	+	+	-	++
Glucose-asparagine agar	++	++	-	++
D-Ribose	++	++	-	++
D-Glucose	++	++	-	++
Sucrose	+	+	-	++
Cellobiose	++	++	-	++
Trehalose	++	++	-	++
Sol. starch	++	++	-	++
Inositol	+	+	-	+
Salicin	+	+	-	+
L-Rhamnose	+	+	-	NT
D-Mannose	+	+	-	-
++: Good growth +: Growth -: No growth NT: not tested				

It is to be understood that for the production of BU-3608 FA-1 and FA-2 the present invention is not limited to the particular organisms mentioned above, but includes the use of variants and mutants produced from these strains by various means such as x-ray radiation, UV-radiation and chemical mutagens. Other producers of antibiotics of the BU-3608 family and variants and mutants thereof capable of incorporating D-serine are also included.

The producing organism is grown in a nutrient medium containing a source of D-serine in addition to known nutritional sources for actinomycetes, i.e. assimilable sources of carbon and nitrogen plus optional inorganic salts and other known growth factors. Submerged aerobic conditions are preferably employed for the production of large quantities of antibiotic, although for production of limited amounts, surface cultures and bottles may also be used. The general procedures used for the cultivation of other actinomycetes are

applicable to the present application.

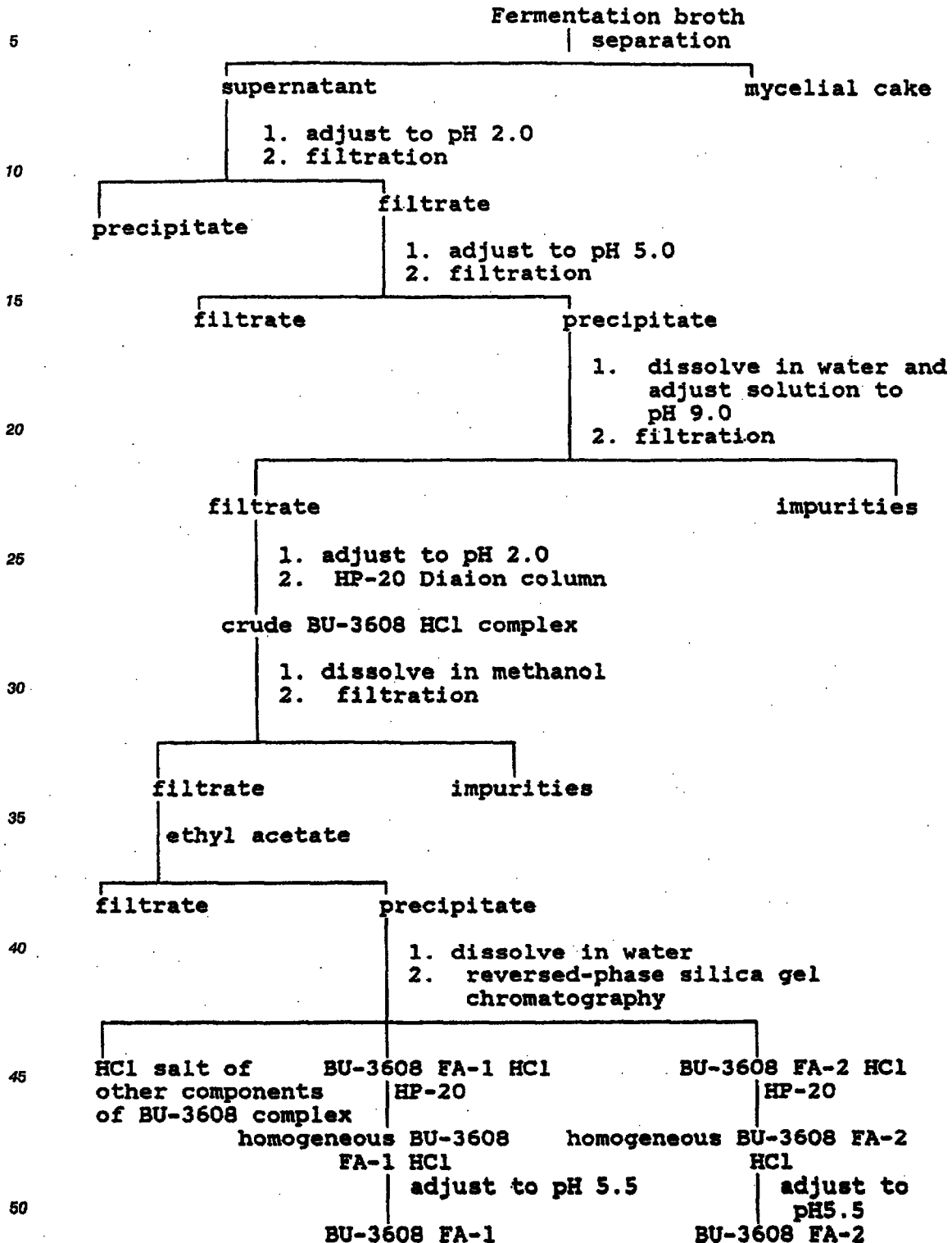
The nutrient medium should contain an appropriate assimilable carbon source such as ribose, glucose, sucrose, cellobiose. As nitrogen source, ammonium chloride, ammonium sulfate, urea, ammonium nitrate, sodium nitrate, etc. may be used either alone or in combination with organic nitrogen sources such as peptone, meat extract, yeast extract, corn steep liquor, soybean powder, cotton seed flour, etc. There may also be added if necessary nutrient inorganic salts to provide sources of sodium, potassium, calcium, ammonium, phosphate, sulfate, chloride, bromide, carbonate, zinc, magnesium, manganese, cobalt, iron, and the like. As a source of D-serine, either D-serine or DL-serine may be used.

Production of antibiotics BU-3608 FA-1 and FA-2 may be effected at any temperature conducive to satisfactory growth of the producing organism, e.g. 25 - 40°C and is most conveniently carried out at a temperature of around 27 - 32°C. Ordinarily, optimum antibiotic production is obtained in shake flasks after incubation periods of 5 - 8 days although a longer period may be necessary in certain cases. Aeration in shake flasks is achieved by agitation, e.g. shaking on a rotary shaker. If fermentation is to be carried out in tank fermentors, it is desirable to produce a vegetative inoculum in a nutrient broth by inoculating the broth culture from a slant culture or a lyophilized culture of the organism. After obtaining an active inoculum in this manner, it is aseptically transferred to the fermentation tank medium. Antibiotic production in tank fermentors usually reaches the optimum after 3 - 6 days of incubation. Agitation in the tank fermentor is provided by stirring and aeration may be achieved by injection of air or oxygen into the agitated mixture. Antibiotic production may be monitored using chromatographic or spectroscopic techniques, or by a conventional biological assay.

ISOLATION AND PURIFICATION OF ANTIBIOTICS

The antibiotics of the present invention may be recovered from the cultivated broth by any suitable method for such recovery. A general scheme for one such method for the isolation and purification of antibiotics BU-3608 FA-1 and FA-2 from the fermentation broth is shown below as Scheme I.

Scheme 1. Isolation of BU-3608 FA-1 and FA-2



To elaborate on the flow chart of Scheme 1, whole fermentation broth is separated into mycelial cake and supernatant by a conventional method such as centrifugation. The supernatant is acidified and the precipitate thus formed is removed. The filtrate is adjusted to pH 5 to deposit crude antibiotic which is redissolved in alkaline water and filtered to remove impurities. The filtrate is acidified to pH 2 and chromatographed on an adsorption column such as Diaion HP-20 to yield a crude BU-3608 HCl complex.

The crude antibiotic complex may be recrystallized e.g. from ethyl acetate, and the product separated into individual antibiotic components using reversed phase silica gel HPLC. Fractions containing BU-3608 FA-1 and BU-3608 FA-2 may be further purified by Diaion HP-20 chromatography. The hydrochloride salts of the antibiotics may then be converted to the zwitterionic form by adjusting the pH of an aqueous solution of the HCl salt to pH 5.5.

Antibiotics BU-3608 FA-1 and FA-2 are characterized by the following physico-chemical properties.

Table II. Physico-chemical properties of BU-3608 FA-1 and FA-2

		BU-3608 FA-1	BU-3608 FA-2
Nature	:	Dark red amorphous powder	Dark red amorphous powder
M.P. (dec.)	:	215 - 220°C	186 - 190°C
$[\alpha]_D^{24}$	:	+ 919° (c 0.1, 0.1N HCl)	+ 79° (c 0.1, 0.1N HCl)
SIMS m/z	:	857 (M+H) ⁺	843 (M+H) ⁺
Molecular formula	:	C ₄₀ H ₄₄ N ₂ O ₁₉	C ₃₉ H ₄₂ N ₂ O ₁₉
¹ H NMR	:	substantially as shown in Figure 1	substantially as shown in Figure 2
¹³ C NMR (100MHz DMSO-d ₆)	:	16.3(q), 20.3(q), 36.4(q), 54.9(d), 56.1(q), 61.6(t), 63.1(d), 65.9(q), 67.8(d), 69.3(d), 69.9(d), 71.7(d), 73.6(d), 75.8(d), 80.5(d), 82.1(d), 104.1(d), 104.2(d), 105.2(d), 106.0(d), 110.3(s), 111.4(d), 117.1(d), 119.0(s), 119.2(s), 126.3(s), 132.1(s), 133.0(s), 136.8(s), 137.6(s), 137.9(s), 143.5(s), 158.0(s), 163.6(s), 165.8(s), 166.2(s), 168.5(s), 172.2(s), 180.2(s), 187.3(s)	16.4(q), 20.4(q), 55.0(d), 56.2(q), 61.7(t), 54.3(d), 65.9(q), 67.3(d), 69.4(d), 69.7(q), 71.8(d), 73.5(d), 75.9(d), 79.1(d), 82.5(d), 104.2(d), 104.3(d), 105.1(d), 106.1(d), 110.4(s), 111.5(d), 117.2(d), 119.1(s), 119.3(s), 126.3(s), 132.1(s), 133.1(s), 136.8(s), 137.8(s), 138.0(s), 143.5(s), 158.2(s), 163.7(s), 165.8(s), 166.2(s), 168.6(s), 172.3(s), 180.1(s), 187.4(s)
UV _{λmax} nm (ε)			
in 50% MeOH	:	221(32,100), 276(27,400) 499(12,900)	223(27,700), 277(25,400) 499(12,200)
in 0.01N HCl-50% MeOH	:	234(37,400), 299(31,100) 460(12,900)	234(34,500), 296(28,900) 460(12,000)
in 0.01N NaOH-50% MeOH	:	244(34,100), 320(15,700) 498(14,900)	233(37,200), 319(16,600) 498(15,100)
IR (KBr)cm ⁻¹	:	3400, 2920, 1605, 1385, 1295, 1260, 1160, 1040	3400, 2920, 1600, 1385, 1295, 1255, 1160, 1040
TLC SiO ₂ R _f	:	0.26	0.22

(S-114, MeOAc-n-PrOH-28% NH₄OH = 45:105:60, v/v)

HPLC Rt (min)

8.61

7.65

(ODS, CH₃CN-0.15% KH₂PO₄, pH 3.5 (25:75))

5

Antibiotics BU-3608 FA-1 and FA-2 may be subjected to further chemical modification. Heating BU-3608 FA-1, FA-2, or a mixture thereof in an acidic medium for a period sufficient to cleave the xylosyl group provides the corresponding desxylosyl derivatives and small amounts of the aglycone IV. The solvent used may be for example dioxane, tetrahydrofuran, water, lower alkanol, or mixtures thereof; acid catalyst may be for example hydrochloric acid, sulfuric acid, and trifluoroacetic acid. The temperature may be from about 60 to about 100°C, or the reflux temperature of the solvent. Reaction time may be from about 0.5 to about 10 hrs and will depend on the reaction conditions employed. N,N-dimethyl BU-3608 FA-2, prepared by reductive alkylation procedure described below, may be converted to its corresponding desxylosyl compound in a similar fashion. It has been discovered that acid hydrolysis of N,N-dimethyl BU-3608 FA-2 results in a practical yield of the aglycone IV.

The amino group of BU-3608 FA-1, FA-2 or their corresponding desxylosyl derivatives may be alkylated by reductive alkylation which comprises first reacting the antibiotic starting material with an aldehyde or a ketone to form an imine, and subsequently reducing the imine thus formed. The condensation and reduction may be conducted in the same reaction vessel in one step, or in two separate steps. The primary amine group of BU-3608 FA-2 or its desxylosyl derivatives may be converted into a tertiary amine having two identical alkyl groups by treatment with at least two equivalents of the carbonyl compound relative to the antibiotic, followed by reduction; or a tertiary amine having two different alkyl substituents may be obtained by using a controlled amount of a first carbonyl reactant to convert the primary amine into a secondary amine which is then reacted with a second different carbonyl compound to give the product tertiary amine. If the second carbonyl compound is not added, a secondary amine is obtained.

The carbonyl reactant may be an aldehyde or a ketone having one to six carbon atoms, for example, formaldehyde, acetaldehyde, propionaldehyde, and acetone. Reduction of the imine may be accomplished by using reducing agents such as metal hydrides, for example, sodium borohydride, sodium cyanoborohydride, lithium aluminum hydride. The reaction is carried out in a polar organic solvent or a mixture thereof such as water, acetonitrile, lower alkanols, and dimethyl sulfoxide. The reaction temperature is not particularly restricted and may be from room temperature to about 100°C. In our experience, the alkylation reaction carried out at room temperature is usually complete within 24 hours. Optimum reaction conditions will of course depend on the nature and reactivity of the particular reactants used. It will be appreciated that N,N-dimethyl BU-3608 FA-2 may be obtained from BU-3608 FA-2, FA-1, or a mixture thereof without having to separate the two components. Similarly, N,N-dimethyl desxylosyl BU-3608 FA-2 may be obtained from desxylosyl BU-3608 FA-2, FA-1, or a mixture thereof.

BIOLOGICAL ACTIVITY

40

Antifungal activities of representative compounds of the present invention were evaluated both *in vitro* and *in vivo*. The minimum inhibitory concentrations (MICs) against various fungi were determined by serial agar dilution method using Sabouraud dextrose agar. Thus, approximately 0.003 ml of fungal suspension containing 10⁶ cells/ml was applied to the surface of agar plates containing the test antibiotics. The MIC values recorded after the cultures had been incubated for 40 hours at 28°C are set forth below in Table III.

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50

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Table III. *In vitro* antifungal activity in Sabouraud dextrose agar (pH 7.0)

Test organism	MIC (µg/ml)							
	BU-3608		N,N-dimethyl		Des-xylosyl		Des-xylosyl	
	FA-1	FA-2	BU-3608	FA-2	BU-3608	FA-1	BU-3608	Des-xylosyl N,N-dimethyl BU-3608 FA-2
<i>Candida albicans</i> IAM4888	6.3	6.3	6.3	3.1	3.1	3.1	3.1	6.3
<i>C. albicans</i> A9540	12.5	6.3	6.3	6.3	3.1	3.1	3.1	6.3
<i>Cryptococcus neoformans</i> D49	0.8	0.8	0.8	0.8	1.6	1.6	3.1	1.6
<i>C. neoformans</i> IAM4514	0.8	0.8	0.8	0.8	1.6	1.6	3.1	1.6
<i>Aspergillus fumigatus</i> IAM2530	3.1	3.1	3.1	3.1	6.3	6.3	12.5	6.3
<i>A. fumigatus</i> IAM2034	6.3	6.3	6.3	3.1	6.3	6.3	12.5	6.3
<i>A. flavus</i> FA21436	6.3	6.3	6.3	25.0	50	50	50	12.5
<i>Fusarium moniliforme</i> A2284	6.3	6.3	6.3	25.0	>100	>100	>100	50
<i>Trichophyton mentagrophytes</i> D155	6.3	6.3	6.3	12.5	12.5	12.5	12.5	6.3
<i>T. mentagrophytes</i> #4329	6.3	6.3	6.3	6.3	12.5	12.5	12.5	6.3
<i>Blastomyces dermatitidis</i> D40	3.1	3.1	3.1	6.3	6.3	6.3	6.3	3.1
<i>Sporothrix schenckii</i> IF08158	1.6	1.6	1.6	1.6	6.3	6.3	6.3	3.1
<i>Petridiellidium boydii</i> IF08073	12.5	25	25	ND	>100	>100	>100	100
<i>Hucor spinosus</i> IF05317	>100	>100	>100	ND	12.5	12.5	25	25

ND: Not determined

In vivo antifungal activities were evaluated against intravenous infections with *Candida albicans* A9540, *Cryptococcus neoformans* IAM4514 and *Aspergillus fumigatus* IAM2034 in mice. *Candida albicans* and *Cryptococcus neoformans* were cultured for 18 and 48 hours, respectively, at 28 °C in YGP medium [yeast extract(0.2%), glucose(1.5%), peptone(0.5%), K₂HPO₄(0.05%) and MgSO₄(0.05%)] and suspended in saline. *Aspergillus fumigatus* was cultured for 7 days at 28 °C on YGP agar slant and suspended in saline. Spores were collected by filtering fungal suspension through gauze. Male ICR mice weighing 20 to 24 g

were infected intravenously with about 10 times the median lethal dose of the fungi.

Test compounds were administered at various doses to groups of 5 mice each intravenously either once just after fungal challenge (day 0) or once daily for 5 days from day 0 to day 4 (qd X 5). The 50% protective dose (PD₅₀) was calculated from survival rates recorded on the 20th day after fungal challenge. All control animals died within 7 to 15 days after fungal infection. The results of in vivo studies are presented in Table IV.

Table IV

In vivo activity against <u>Candida</u> , <u>Cryptococcus</u> and <u>Aspergillus</u> intravenous infections						
	PD ₅₀ (mg/kg/inj. iv)					
	<u>C. albicans</u> A9540		<u>C. neoformans</u> IAM4514		<u>A. fumigatus</u> IAM2034	
	Single	qd x 5	Single	qd x 5	Single	qd x 5
BU-3608 FA-1	18	-	-	-	-	-
BU-3608 FA-2	7.4	-	-	-	-	-
N,N-dimethyl BU-3608 FA-2	9.0	7.5	11	2.8	36	15

The LD₅₀ for N,N-dimethyl BU-3608 FA-2 was determined in mice after a single intravenous dose. At 600 mg/kg, the highest dose test, neither lethal toxicity nor any significant toxic sign was observed.

For treatment of fungal infections in animals and human beings, the antibiotics of the present invention may be given in an antifungally effective amount by any accepted routes of administration; these include, but are not limited to, intravenous, intramuscular, oral, intranasal, and for superficial infections, topical administration. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions or emulsions. They may also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, physiological saline, or some other sterile injectable medium immediately before use. Oral formulation may be in the form of tablets, gelatin capsules, powders, lozenges, syrups, and the like. For topical administration, the compound may be incorporated into lotions, ointments, gels, creams, salves, tinctures, and the like. Unit dosage forms may be prepared using methods generally known to those skilled in the art of pharmaceutical formulations.

It will be appreciated that when treating a host infected with a fungus susceptible to the antibiotics of this invention, the actual preferred route of administration and dosage used will be at the discretion of the attending clinician skilled in the treatment of fungal infections, and will vary according to the causative organism, its sensitivity to the antibiotic, severity and site of the infection, and patient characteristics such as age, body weight, rate of excretion, concurrent medications, and general physical condition.

The following examples illustrate the present invention.

Example 1. Fermentation Production Of BU-3608 FA-1

(a) Agar Slant.

Actinomadura hibisca P157-2 (ATCC 53557) was grown on an agar slant of pH 7.0 having the composition

0.5% soluble starch

0.5% glucose

0.1% fish meat extract

0.1% yeast extract

0.2% NZ-case (digested casein product; Scheffield Chemical Co., USA)

0.2% NaCl

0.1% CaCO₃

1.6% agar

The culture was incubated at 28°C for 10 days.

(b) Seed Culture.

A portion of the microbial growth from the slant culture was transferred to a 500-ml Erlenmeyer flask containing 100 ml of vegetative medium pH 7.0 composed of

- 3% glucose
- 3% soybean meal
- 0.5% Pharmamedia
- 0.1% yeast extract
- 0.3% CaCO₃

The culture was incubated at 32 °C for 6 days on a rotary shaker set at 200 rpm.

(c) Production Culture.

Five ml of the microbial growth was transferred from the seed culture to a 500-ml Erlenmeyer flask containing 100 ml of sterile production medium composed of

- 3% glucose
- 3% soybean meal
- 0.5% Pharmamedia
- 0.1% yeast extract
- 0.3% CaCO₃
- 0.25% D-serine

The culture was incubated at 28 °C for 6 days on a rotary shaker set at 200 rpm. Antibiotic production reached 443 µg/ml and 72.9% of which was BU-3608 FA-1, 26.0% BU-3608 and 1.1% BU-3608C. Antibiotic production was determined by measuring the optical density of the liquid from the fermentation broth at 500 and 600 nm. Genuine optical density was obtained by subtracting the OD value at 600 nm from that at 500 nm. Antibiotic concentration is expressed as equivalent amount of BU-3608 free base. The antibiotics were identified using the HPLC procedure described in Example 5.

Example 2. Fermentation Production Of BU-3608 FA-1 and FA-2 Using Strain A-2493

Agar slant culture and seed culture of an arginine auxotroph mutant of *Actinomadura hibisca* P157-2 designated as strain A-2493 (ATCC 53815) were grown using media having the same compositions as those described in Example 1 and under conditions given in Example 1.

Production A.

Five ml of the microbial growth was transferred from the seed culture to each 500-ml Erlenmeyer flask (100 flasks) containing 100 ml of the same production medium described in Example 1(c). The culture was incubated at 28 °C for 6 days on a rotary shaker set at 200 rpm. Antibiotic production reached 261 µg/ml and was composed of 29.8% BU-3608 FA-1, 28.9% BU-3608 FA-2, 19.5% BU-3608C and 21.8% BU-3608.

Production B.

- Production of antibiotics by strain A2493 was also effected in a medium (pH 7.0) composed of
- 3% glucose
 - 3% Protein S (soybean flour, Ajinomoto)
 - 0.3% CaCO₃
 - 0.5% DL-serine

Antibiotic production reached 890 µg/ml after the culture had been incubated for 11 days at 28 °C. The ratio of components was BU-3608 FA-2 17.7%, FA-1 17.0%, BU-3608 33.5% and BU-3608 C 31.8%.

Example 3. Fermentation Production Of BU-3608 FA-1 And FA-2 Using Strain B-0012.Production A.

The conditions and culture media described in Example 1 parts (a), (b) and (c) were followed using the variant strain designated B-0012 (ATCC 53816) instead of the parent strain P157-2. Antibiotic production reached 1,150 µg/ml after 6 days and the ratio of components was BU-3608 FA-1 33.7% FA-2 21.2%, BU-

3608 24.0% and BU-3608 C 21.1%.

Production B.

5 A portion of microbial growth from the slant culture of strain B-0012 was also transferred to a 500-ml Erlenmeyer flask containing 100 ml of a medium pH 7.0 having the following composition

- 1% soluble starch
- 1% glucose
- 0.5% yeast extract
- 10 0.5% peptone
- 0.3% NaCl
- 0.2% CaCO₃

The seed culture was grown at 32°C for 6 days and 5 ml of the microbial growth was transferred to a 500-ml Erlenmeyer flask containing 100 ml of a production medium pH 7.0 composed of

- 15 3% glucose
- 3% Protein S (soybean flour, Ajinomoto)
- 0.3% CaCO₃
- 0.25% D-serine

20 The culture was incubated at 28°C for 11 days. Antibiotic production reached 1,970 µg/ml and the ratio of components was BU-3608 FA-2 20.0%, FA-1 10.0%, BU-3608 C 39.0% and BU-3608 31.0%.

Example 4. Fermentation Production Of BU-3608 FA-1 and FA-2 Using Strain A-2660.

25 Agar slant and seed cultures of *Actinomadura hibisca* mutant strain A-2660 (ATCC 53762) were produced using the same conditions and media given in Example 1 parts (a) and (b). Five ml of the seed culture was transferred to a 500-ml Erlenmeyer flask containing 100 ml of a production medium composed of

- 3% glucose
- 3% soybean meal
- 30 0.5% Pharmamedia
- 0.1% yeast extract
- 0.3% CaCO₃
- 0.5% DL-serine

35 The culture was incubated at 28°C for 7 days. Antibiotic production reached 620 µg/ml with the ratio of components as BU-3608 FA-1 17.0%, FA-2 15.6%, BU-3608 23.9%, BU-3608 C 24.7%, D 8.3% and E 10.5%.

Example 5. Isolation And Purification of BU-3608 FA-1 And FA-2 From Fermentation Broth.

40 Ten liters of fermentation broth obtained from the procedure described in Example 2, Production A, was separated into mycelial cake and supernatant by centrifugation. The supernatant was acidified to pH 2.0 using 6N HCl and the amorphous precipitate deposited was removed by filtration. The clear filtrate was adjusted to pH 5.0 using 6N NaOH, and kept at 5°C for two hours. The dark red precipitate deposited was collected by filtration. The precipitate was dissolved in 4.1 L of water adjusted to pH 9.0 with 6N NaOH and
45 the solution was filtered to remove insoluble impurities. The filtrate was adjusted to pH 2.0 and applied on a column of Diaion HP-20 (2.0 L). The column was washed with water and eluted with 60% aqueous acetone (pH 3.0). Concentration of the red eluate afforded amorphous solid of BU-3608 complex hydrochloride (3.1 g). The complex solid (3.0 g) was dissolved in methanol (120 ml) and filtered. To the stirred filtrate, 720 ml of ethyl acetate was added dropwise and the resulting solution was kept at 5°C for 15 hours. The
50 precipitate deposited was collected by filtration and dried (1.28 g).

The solid (1.28 g) was dissolved in water (100 ml) and subjected to reversed phase chromatography on a column of YMC GEL ODS A 60 (10 L, Yamamura Chemical Lab.) which had been equilibrated with a mixture of CH₃CN-0.15% KH₂PO₄, pH 3.5 (21:79). Elution was carried out with the same solvent mixture, and the eluate was collected in 1L-fractions. The fractions were analyzed by HPLC (column : YMC A-301-3,
55 4.6 mm I.D. x 100 mm 3µm, ODS Yamamura Chemical Lab., mobile phase : CH₃CN-0.15% KH₂PO₄, pH 3.5 (25:75), flow rate : 0.8 ml/min, detection : UV absorption at 254 nm, retention time : BU-3608 FA-2, 7.65 min; BU-3608 FA-1, 8.61 min ; BU-3608A, 19.11 min). The fractions containing homogeneous BU-3608 FA-2 or BU-3608 FA-1, were pooled and concentrated in vacuo to remove CH₃CN. Each concentrate was

desalted by Diaion HP-20 chromatography to yield nearly homogeneous BU-3608 FA-2 hydrochloride (75 mg) and BU-3608 FA-1 hydrochloride (50 mg).

In order to convert the hydrochloride salt to its free form and to remove contaminated inorganic salts, an aqueous solution of each salt was adjusted to pH 5.5 with 0.1 N NaOH to deposit pure zwitterionic form of BU-3608 FA-2 (48 mg) and BU-3608 FA-1 (11 mg).

Example 6. Preparation Of N,N-dimethyl BU-3608 FA-2

A mixture of BU-3608 FA-1 and BU-3608 FA-2 (45:55, 510 mg) was dissolved in 50 ml of water and the solution was adjusted to pH 7.9 by addition of 1N sodium hydroxide and diluted with 50 ml of acetonitrile. To this solution were added sequentially aqueous formaldehyde (>35%, 1.6 ml) and sodium cyanoborohydride (240 mg) at room temperature. The solution was stirred for 1 hour at room temperature and the progress of reaction monitored by HPLC. The organic solvent was removed in vacuo and the aqueous residue was adjusted to pH 10.9. The solution (40 ml) was added dropwise to 240 ml of acetone with stirring and kept at 5°C for 2 hours. The resulting precipitate was collected by centrifugation (3000 rpm) and redissolved in 40 ml of water. After removal of traces of the acetone in vacuo, the solution was adjusted to pH 5.0 and allowed to stand at 5°C for 24 hours. The precipitate deposited was collected by centrifugation, washed sequentially with water and acetone, and vacuum-dried at 60°C to afford 80 mg of zwitterionic N,N-dimethyl FA-2. M.p. 214 - 218°C (dec.);

UV λ_{max} 0.01N NaOH nm (ϵ)

232.8 (32,900), 320.0(15,500), 498.4(15,200).

Example 7. Preparation Of Desxylosyl BU-3608 FA-1

A solution of BU-3608 FA-1 (54 mg) in dioxane (5.4 ml) and 1N HCl (5.4 ml) was refluxed on a steam bath for 8 hr. The reaction mixture was diluted with water (30 ml) and charged on a short column of Diaion HP-20 (Mitsubishi Kasei, 1.8 x 25 cm). The column was washed with water and then eluted with acidic 80% acetone (pH 3, acidified with 1N HCl). The reddish orange eluate was collected and evaporated to give a deep red powder. The powder was dissolved in 35% acetonitrile/phosphate buffer (pH 3.5) and chromatographed on an ODS column (YMC-ODS, 2.1 x 25 cm, eluted with the same solvent). The fractions containing the desired compound were combined and passed through an HP-20 column. The column was washed with water and eluted with 80% acetone (pH 3). Evaporation of the eluate gave a dark red powder, which was dissolved in water (8 ml) and the solution was adjusted to pH 5.3 with 0.1 N NaOH. The resulting precipitate was collected by centrifugation, washed with acetone and dried to give a dark red powder (23.3 mg, 51%). MP > 180°C (dec). Purity by HPLC: >95%.

IR: ν_{max} (KBr) cm^{-1} : 3400, 1605, 1290, 1255, 1060.
 UV: λ_{max} (1/100N NaOH) nm (ϵ): 212(35,400), 319(15,300), 498(14,500).
¹H NMR: (400 MHz, DMSO- d_6): 1.26 (3H, d, J=6Hz, 6'-CH₃), 2.32 (3H, s, Ph-CH₃), 2.65 (3H, s, NCH₃), 3.75 (2H, m, OCH₂), 3.91 (3H, s, OCH₃), 4.68 (1H, d, J=8Hz, 1'-H), 6.72 (1H, d, J=2Hz, 10-H), 6.87 (1H, s, 4-H), 7.12 (1H, d, J=2Hz, 12-H), 7.71 (1H, s, 7-H).

Example 8. Preparation Of Desxylosyl BU-3608 FA-2

A solution of BU-3608 FA-2 (54 mg) in dioxane (5.4 ml) and 1N HCl (5.4 ml) was refluxed on a steam bath for 8 hr. The reaction mixture was diluted with water (30 ml), adsorbed on a short column of Diaion HP-20 (Mitsubishi Kasei, 1.8 x 25 cm), washed with water and eluted with 80% acetone (pH 3). The vermilion eluate was pooled and concentrated and the residue was dissolved in water (8 ml). The solution was adjusted to pH 5.3 by 0.1N NaOH and the resulting precipitate was collected by centrifugation, washed with acetone and dried in vacuo to give a dark red powder (37.7 mg 85%). MP >180°C dec. Purity by HPLC:>95%.

IR: ν_{max} (KBr) cm^{-1} : 3400, 1605, 1290, 1265, 1035
 UV: λ_{max} (1/100 N NaOH) nm (ϵ): 214(33,000), 234(32,300), 319(14,900), 498(14,100)
¹H NMR (DMSO- d_6): 1.15 (3H, d, J=7Hz, 6'-CH₃), 2.32 (3H, s, PhCH₃), 3.75 (2H, m,

OCH₂), 3.91 (3H, s, OCH₃), 4.67 (1H, d, J=8Hz, 1'-H), 6.72 (1H, d, J=3Hz, 10-H), 6.93 (1H, s, 4-H), 7.12 (1H, d, J=3Hz, 12-H), 7.71 (1H, s, 7-H).

5 Example 9. Preparation Of Desxylosyl N,N-dimethyl BU-3608 FA-2

Method A

A solution of N,N-dimethyl BU-3608 FA-2 (product of Example 6, 50 mg) in dioxane (5 ml) and 1N HCl (5 ml) was refluxed on a steam bath for 8 hr. The reaction mixture was cooled to ambient temperature and filtered to give 7.2 mg of a precipitate. The filtrate was charged on a dry silica gel column (Merck Kieselgel 60, 4 x 30 cm) and eluted with n-BuOH-AcOH-H₂O(3:1:1). The eluate was collected in 10-ml fractions. Fractions 2-14 and the precipitate earlier obtained were worked-up as described in Example 10. Fractions 21-32 were combined and charged on an HP-20 column (1.8 x 25 cm). The column was washed with water and eluted with 80% acetone (pH 3). Concentration of the vermilion eluate gave a solid, which was successively purified by ODS column chromatography (YMC-ODS, 2 x 37 cm, eluted with 20% CH₃CN/pH 3.5 phosphate buffer) and Diaion HP-20 chromatography (1.8 x 25 cm, eluted with 80% acetone at pH 3) to give 7.8 mg (17%) of the title compound as a dark red powder. MP >180 °C (dec.) Purity by HPLC >95%.

IR: ν_{max} (KBr) cm⁻¹: 3400, 1730, 1610, 1380, 1260, 1070.
 UV: λ_{max} (1/100N NaOH) nm (ϵ): 211(38,100), 318(14,200), 496(12,500).
¹H NMR (DMSO-d₆): 1.23 (3H, d, J=7Hz, 6'-CH₃), 2.29 (3H, s, PhCH₃), 2.75 (6H, s, NCH₃), 3.74 (2H, m, OCH₂), 3.91 (3H, s, OCH₃), 4.60 (1H, d, J=8Hz, 1'-H), 6.73 (1H, d, J=3Hz, 10-H), 6.89(1H, s, 4-H), 7.12 (1H, d, J=3Hz, 12-H), 7.77 (1H, s, 7-H).

Method B

A solution of desxylosyl BU-3608 FA-2 (product of Example 8, 71 mg) in water (7 ml) and acetonitrile (7 ml) was adjusted to pH 7 using 0.1N NaOH. To this solution was added aqueous formaldehyde (35%, 0.3 ml) and sodium cyanoborohydride (45 mg) at room temperature. The reaction mixture was stirred overnight and the organic solvent was removed in *vacuo*. The aqueous residue was adjusted to pH 10 with NaOH and then added dropwise to acetone (70 ml). The precipitate was filtered off and dissolved in water at pH 2.5. The solution was charged on a column of Diaion HP-20 (1.8 x 25 cm), washed with water, and developed with acidic acetone (pH 3, acidified with 1N HCl). The deep red eluate was pooled, concentrated to about 5 ml, adjusted to pH 5.3 with dilute NaOH, and then added dropwise to acetone (70 ml). The resulting precipitate was collected by filtration, and re-precipitated from aqueous acetone to give 66 mg (90%) of the title compound as a dark red powder which was identical in all respects to that obtained by Method A. Purity by HPLC: >95%.

40 Example 10. BU-3608 FA Aglycone

Fractions 2-14 of the eluate from the silica gel column in Example 9 (Method A) were combined and concentrated to dryness to give a powder. The powder and the precipitate of Example 9 (Method A) were dissolved in 0.01N NaOH, charged on a column of Diaion HP-20 (1.8 x 25 cm), washed with water and eluted with 80% acetone (pH 3). The vermilion eluate was pooled and acetone was evaporated in *vacuo* to give a suspension. The suspension was acidified with 1N HCl (pH 3) and extracted with butanol. Concentration of the butanol extract gave 11.5 mg of the aglycone (33%) as a dark red amorphous powder. MP >200 °C (dec.) Purity by HPLC >95%.

IR: ν_{max} (KBr) cm⁻¹: 3240, 1720, 1605, 1340, 1305, 1165.
 UV: λ_{max} (1/100N NaOH) nm (ϵ): 212(34,500), 319(15,200), 498(14,000).
¹H NMR (DMSO-d₆): 2.34 (3H, s, PhCH₃), 3.73 (2H, m, OCH₂), 3.91 (3H, s, OCH₃), 4.24 (2H, AB-q, J=11Hz, 5-H & 6-H), 4.46 (1H, m, N-CH-COOH), 6.92 (1H, d, J=2Hz, 10-H), 7.06 (1H, s, 4-H), 7.28 (1H, d, J=2Hz, 12-H), 8.08 (1H, s, 7-H).

Example 11

The general procedure described in Example 6 is followed using the reactants listed below to provide the corresponding alkylated analogs of BU-3608 FA-1 and FA-2.



10

ABII

BU-3608 FA-1

acetaldehyde (1 equiv)*

 $R^3 = \beta\text{-D-xylosyl}; R^1 = \text{CH}_3;$
 $R^2 = \text{CH}_3\text{CH}_2-$

"

propionaldehyde (1 equiv)

 $R^3 = \beta\text{-D-xylosyl}; R^1 = \text{CH}_3;$
 $R^2 = \text{CH}_3\text{CH}_2\text{CH}_2-$

20

"

acetone (1 equiv)

 $R^3 = \beta\text{-D-xylosyl}; R^1 = \text{CH}_3;$
 $R^2 = -\text{CH}(\text{CH}_3)_2$

BU-3608 FA-2

acetaldehyde (2 equiv)

 $R^3 = \beta\text{-D-xylosyl}; R^1 = R^2 =$
 CH_3CH_2-

25

"

acetone (1 equiv)

 $R^3 = \beta\text{-D-xylosyl}; R^1 = \text{H},$
 $R^2 = \text{CH}((\text{CH}_3)_2$

"

propionaldehyde (2 equiv)

 $R^3 = \beta\text{-D-xylosyl}; R^1 = R^2 =$
 $\text{CH}_3\text{CH}_2\text{CH}_2-$

30

"

butyraldehyde (1 equiv)

 $R^3 = \beta\text{-D-xylosyl}; R^1 = \text{H};$
 $R^2 = \text{CH}_3(\text{CH}_2)_3-$
Desxylosy BU-3608
FA-1

acetaldehyde (1 equiv)

 $R^3 = \text{H}; R^1 = \text{CH}_3; R^2 = \text{CH}_3\text{CH}_2-$

35

propionaldehyde (1 equiv)

 $R^3 = \text{H}; R^1 = \text{CH}_3; R^2 =$
 $\text{CH}_3\text{CH}_2\text{CH}_2-$

40

45

50

55

ABII

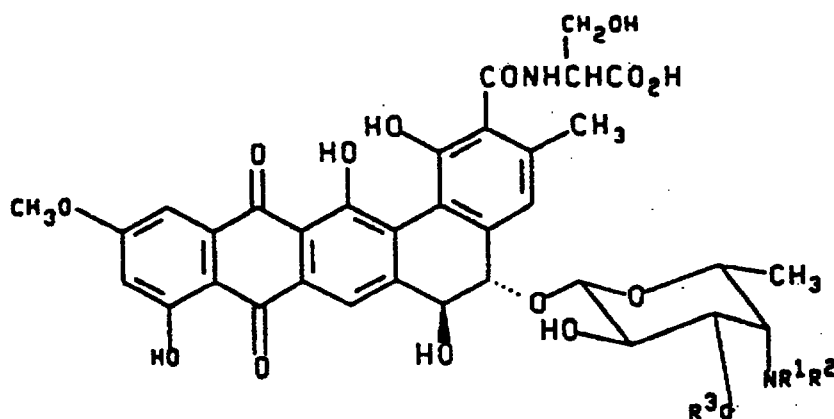
5	"	acetone (1 equiv)	$R^3=H; R^1=CH_3; R^2=-CH(CH_3)_2$
	Desxylosy BU-3608	acetaldehyde (2 equiv)	$R^3=H; R^1=R^2=CH_3CH_2-$
	FA-2		
10	"	acetone (1 equiv)	$R^3=H; R^1=H; R^2=-CH(CH_3)_2$
	"	propionaldehyde (2 equiv)	$R^3=H; R^1=R^2=CH_3CH_2CH_2-$
15	"	butyraldehyde (1 equiv)	$R^3=H; R^1=H; R^2=CH_3(CH_2)_3-$

*Minimum relative to BU-3608 reactant

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A compound having the formula II



II

wherein the serine is D-serine; R^1 and R^2 are independently H or C_{1-6} alkyl; and R^3 is H or β -D-xylosyl; or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein R^3 is β -D-xylosyl, or a pharmaceutically acceptable salt thereof.
3. A compound of claim 1 wherein R^3 is H, or a pharmaceutically acceptable salt thereof.
4. A compound of claim 1 wherein R^1 is H or methyl, and R^2 is H or methyl, or a pharmaceutically acceptable salt thereof.
5. A compound of claim 1 wherein R^1 and R^2 are independently C_{2-6} alkyl; or R^1 is H or methyl and R^2 is C_{2-6} alkyl, or a pharmaceutically acceptable salt thereof.

ABII

5

"

acetone (1 equiv)

 $R^3 = H; R^1 = CH_3; R^2 = -CH(CH_3)_2$

Desxylosy BU-3608

acetaldehyde (2 equiv)

 $R^3 = H; R^1 = R^2 = CH_3CH_2-$

FA-2

10

"

acetone (1 equiv)

 $R^3 = H; R^1 = H; R^2 = -CH(CH_3)_2$

15

"

propionaldehyde (2 equiv)

 $R^3 = H; R^1 = R^2 = CH_3CH_2CH_2-$

"

butyraldehyde (1 equiv)

 $R^3 = H; R^1 = H; R^2 = CH_3(CH_2)_3-$

20

*Minimum relative to BU-3608 reactant

25

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

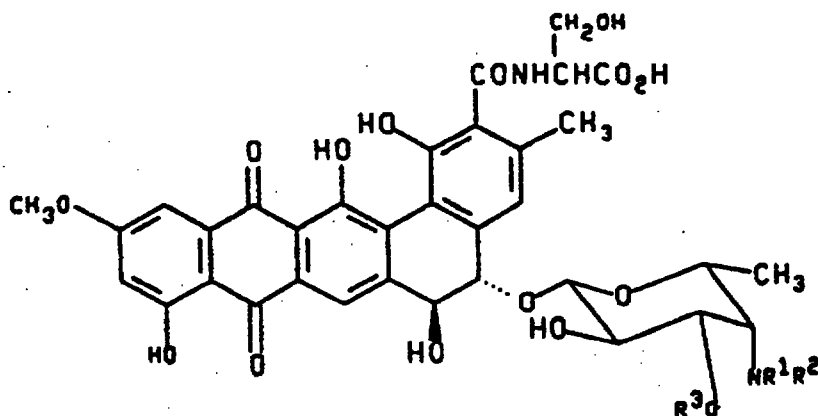
1. A compound having the formula II

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35

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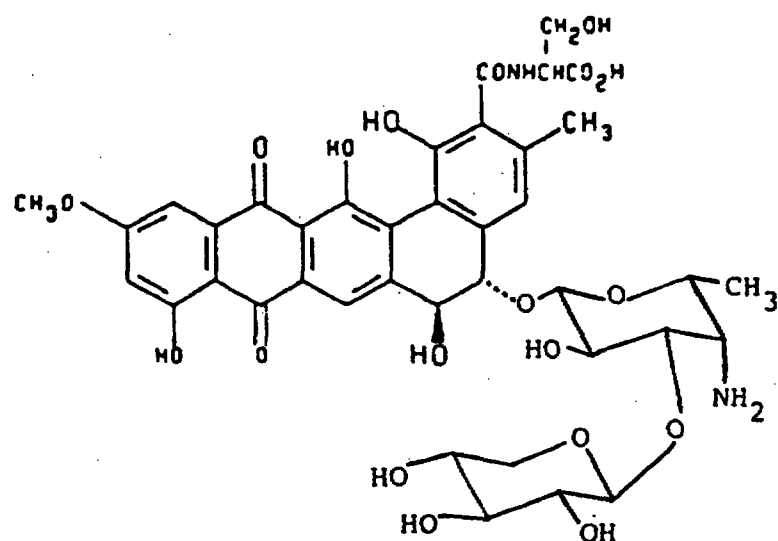
**II**

wherein the serine is D-serine; R^1 and R^2 are independently H or C_{1-6} alkyl; and R^3 is H or β -D-xylosyl; or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein R^3 is β -D-xylosyl, or a pharmaceutically acceptable salt thereof.
3. A compound of claim 1 wherein R^3 is H, or a pharmaceutically acceptable salt thereof.
4. A compound of claim 1 wherein R^1 is H or methyl, and R^2 is H or methyl, or a pharmaceutically acceptable salt thereof.
5. A compound of claim 1 wherein R^1 and R^2 are independently C_{2-6} alkyl; or R^1 is H or methyl and R^2 is C_{2-6} alkyl, or a pharmaceutically acceptable salt thereof.

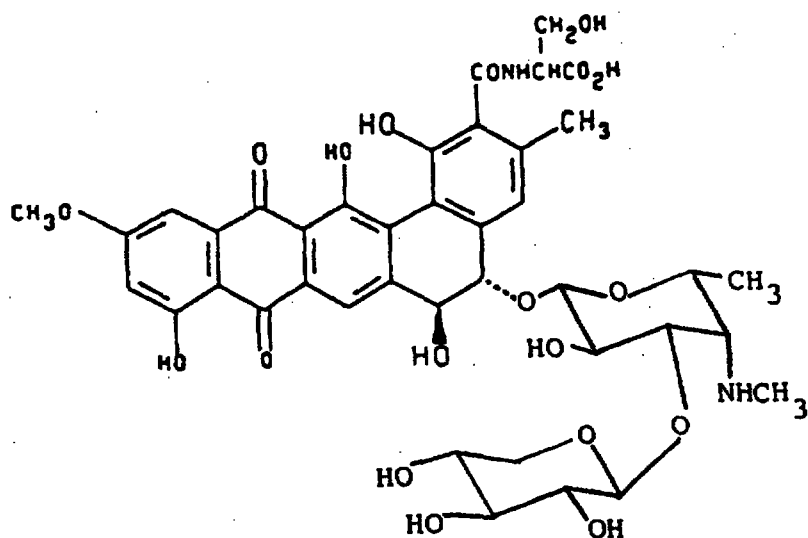


6. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

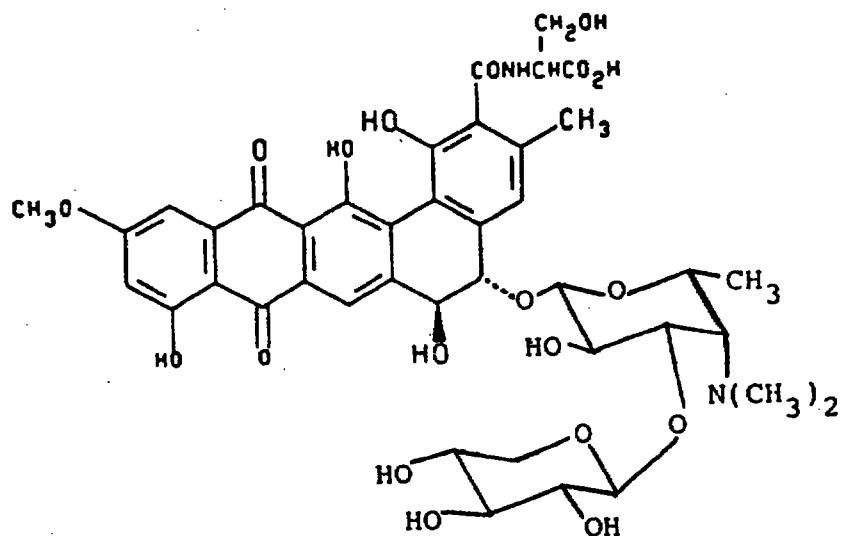
7. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

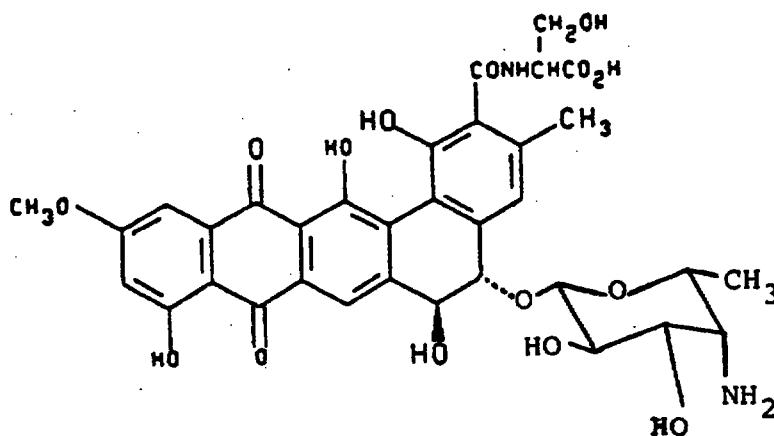


8. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

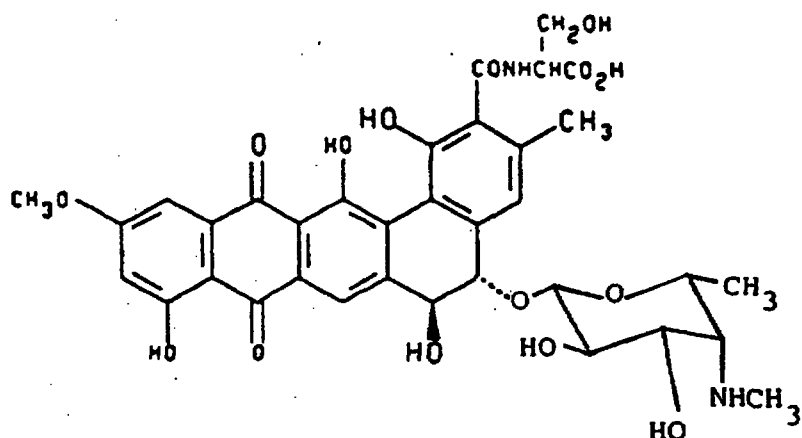
9. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

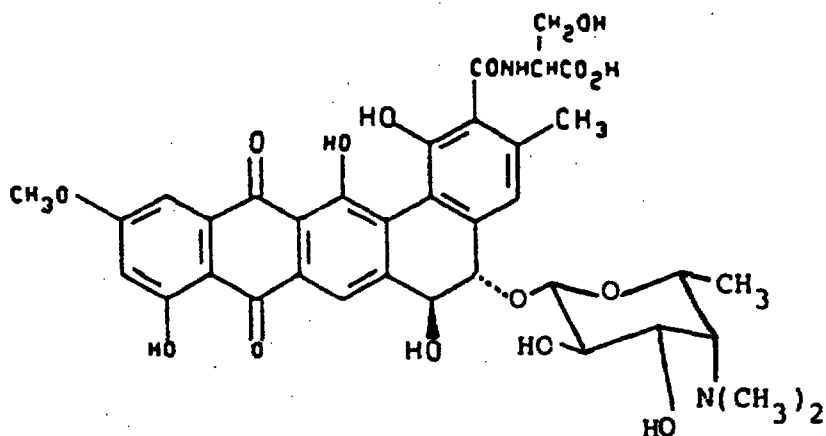


10. The compound of claim 1 having the formula



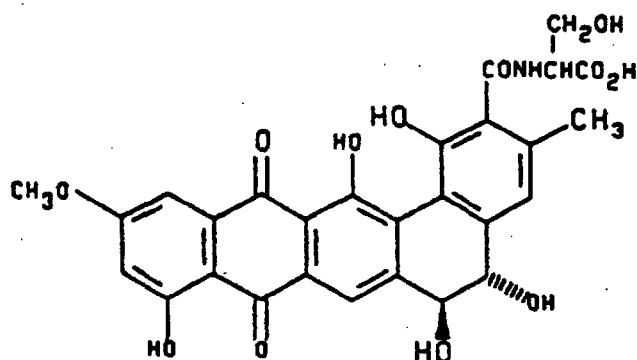
or a pharmaceutically acceptable salt thereof.

11. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

12. The compound of the formula



IV

wherein the serine is D-serine; or a salt thereof.

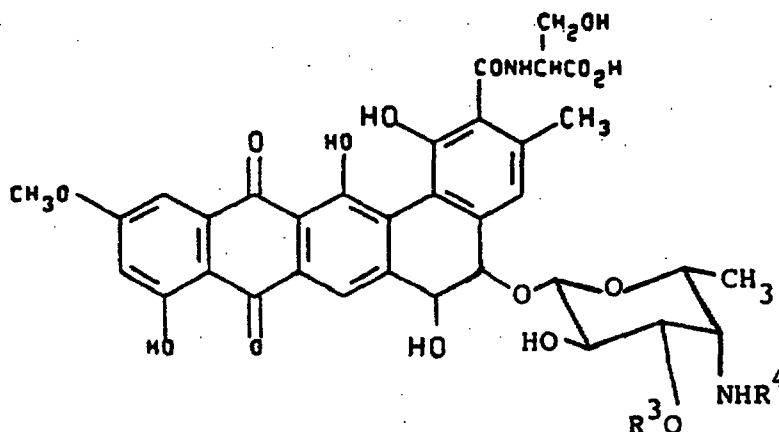


13. A process for preparing a compound of formula II according to claim 1 which comprises

A. cultivating a strain of *Actinomadura Hibisca* capable of producing said antibiotic in a medium containing assimilable sources of carbon and nitrogen, and D- or DL-serine under aerobic condition, and recovering said antibiotic from the cultured broth to obtain a compound of formula II wherein R¹ is H or methyl, R² is H and R³ is β -D-xylosyl;

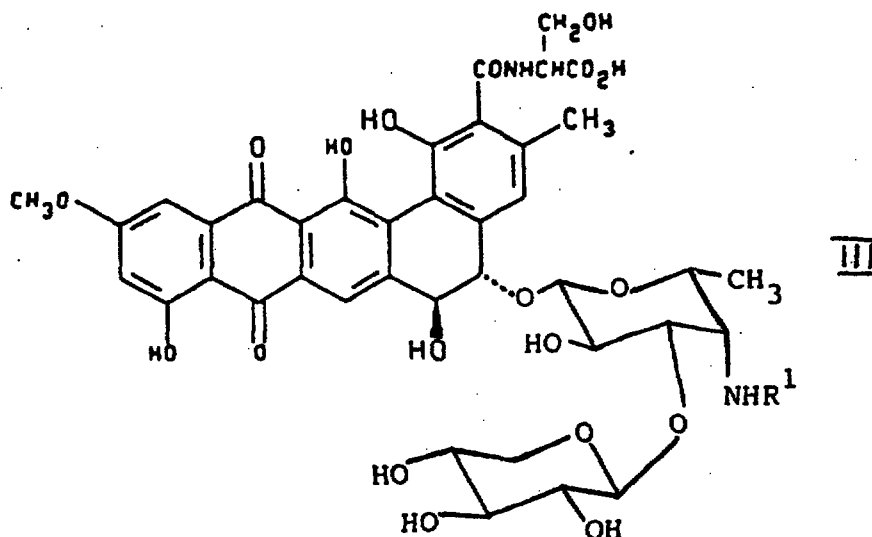
B. if desired, heating a compound of formula III in an acidic medium to obtain the corresponding desxylosyl derivative,

C. if desired, reacting a compound obtained according to step A or B, which corresponds to formula V:



wherein R³ is H or β -D-xylosyl and R⁴ is H or methyl, or a pharmaceutically acceptable salt thereof with an aldehyde or a ketone having 1 to 6 carbon atoms to form an imine and treating the imine with a reducing agent to obtain a compound of formula II, wherein R¹ and R² are independently C₁₋₆ alkyl and R³ is H or β -D-xylosyl or a pharmaceutically acceptable salt thereof.

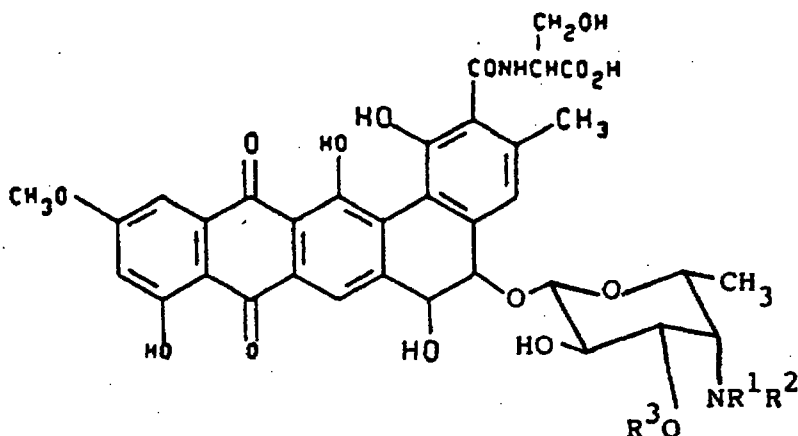
14. A process for preparing an antibiotic of formula III



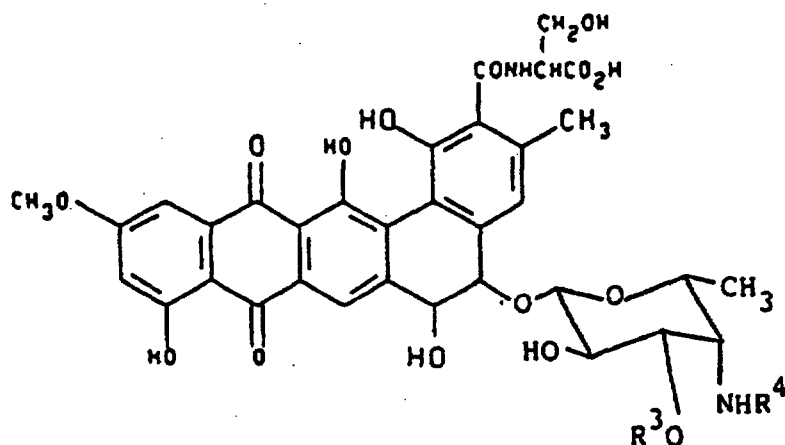
wherein R¹ is H or methyl, which comprises cultivating a strain of *Actinomadura hibisca* capable of producing said antibiotic in a medium containing assimilable sources of carbon and nitrogen, and D- or DL-serine under aerobic condition, and recovering said antibiotic from the cultured broth.



15. A process for preparing a compound having the formula



wherein R^1 and R^2 are independently C_{1-6} alkyl; or R^3 is H or β -D-xylosyl; or a pharmaceutically acceptable salt thereof, which comprises the steps of
(a) reacting a compound of formula



wherein R^3 is H or β -D-xylosyl and R^4 is H or methyl, or a pharmaceutically acceptable salt thereof with an aldehyde or a ketone having 1 to 6 carbon atoms to form an imine; and (b) treating the imine of (a) with a reducing agent.

16. A pharmaceutical composition which comprises at least one compound of claim 1 and a pharmaceutically acceptable carrier.

17. A process for preparing the composition of claim 16 which comprises incorporating at least one compound of claim 1 into a pharmaceutically acceptable carrier.

18. The use of at least one compound of claim 1 for preparing a pharmaceutical composition for treating fungal infections in a mammalian host.

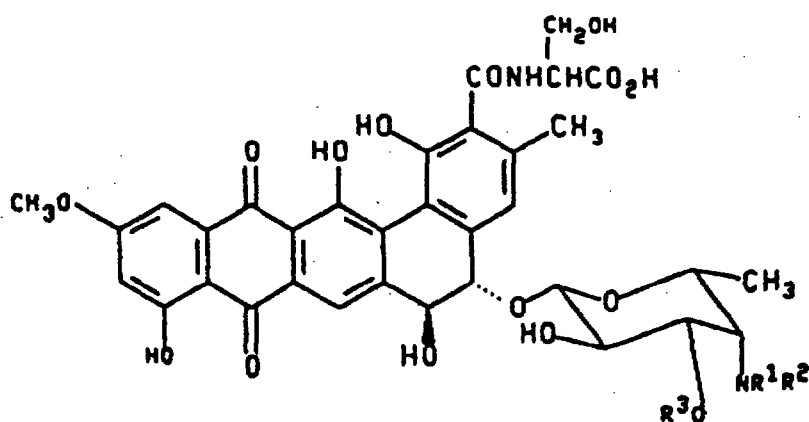
19. A biologically pure culture of the microorganism *Actinomadura hibisca* having the identifying characteristics of ATCC 53815 and which is capable of producing an antibiotic of formula III upon cultivation in an aqueous medium containing an assimilable source of carbon, nitrogen and D-serine.

20. A biologically pure culture of the microorganism *Actinomadura hibisca* having the identifying characteristics of ATCC 53816 and which is capable of producing an antibiotic of formula III upon cultivation in an aqueous medium containing an assimilable source of carbon, nitrogen and D-serine.



Claims for the following Contracting State : ES

1. A process for preparing a compound having the formula II

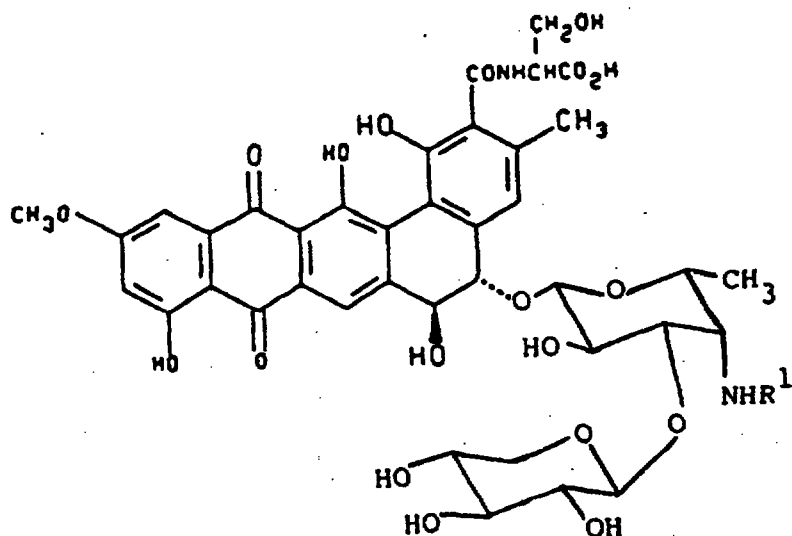


II

wherein the serine is D-serine; R¹ and R² are independently H or C₁₋₆ alkyl; and R³ is H or β-D-xylosyl; or a pharmaceutically acceptable salt thereof; which comprises

A. cultivating a strain of *Actinomadura Hibisca* capable of producing said antibiotic in a medium containing assimilable sources of carbon and nitrogen, and D- or DL-serine under aerobic condition, and recovering said antibiotic from the cultured broth to obtain a compound of formula II wherein R¹ is H or methyl, R² is H and R³ is β-D-xylosyl;

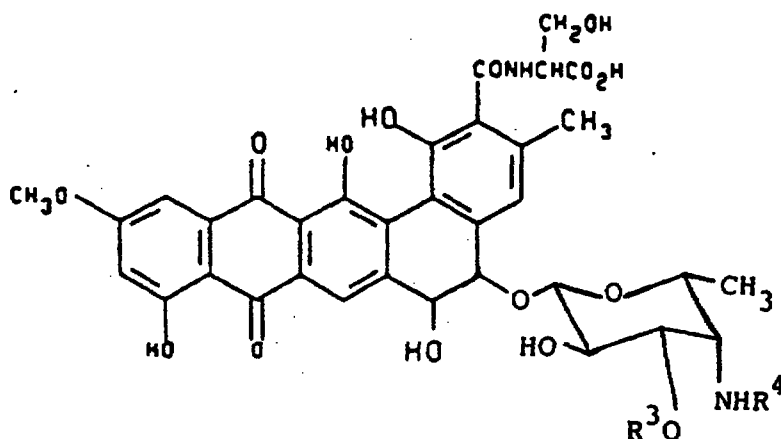
B. if desired, heating a compound of formula III



III

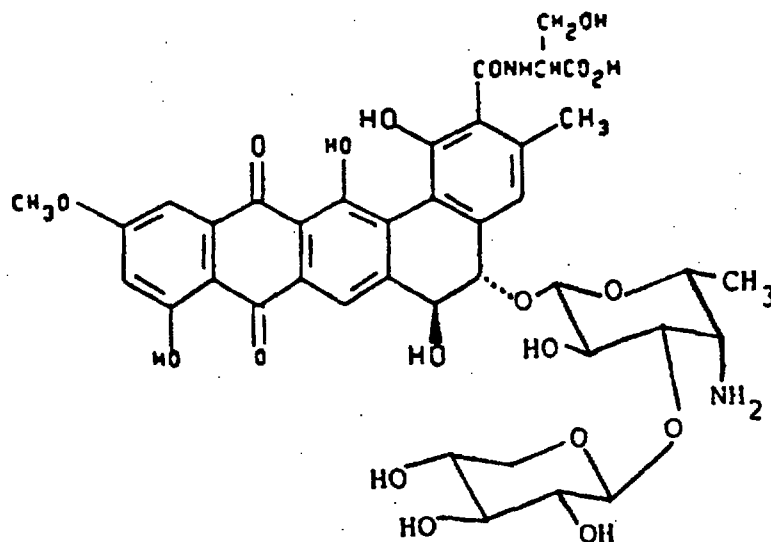
wherein R¹ is H or methyl, in an acidic medium to obtain the corresponding desxylosyl derivative, C. if desired, reacting a compound obtained according to step A or B, which corresponds to formula V:





wherein R^3 is H or β -D-xylosyl and R^4 is H or methyl, or a pharmaceutically acceptable salt thereof with an aldehyde or a ketone having 1 to 6 carbon atoms to form an imine and treating the imine with a reducing agent to obtain a compound of formula II, wherein R^1 and R^2 are independently C_{1-6} alkyl and R^3 is H or β -D-xylosyl or a pharmaceutically acceptable salt thereof.

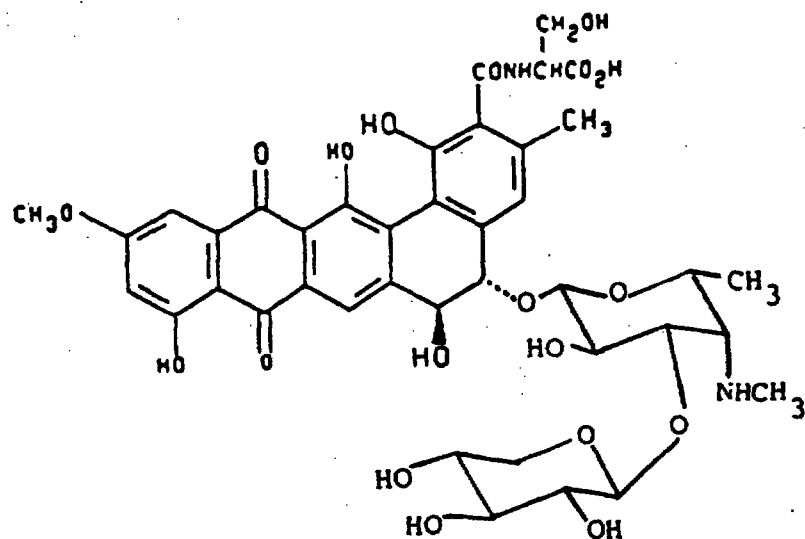
2. The process according to claim 1 wherein R^3 is β -D-xylosyl, or a pharmaceutically acceptable salt thereof.
3. The process according claim 1 wherein R^3 is H, or a pharmaceutically acceptable salt thereof.
4. The process according claim 1 wherein R^1 is H or methyl, and R^2 is H or methyl, or a pharmaceutically acceptable salt thereof.
5. The process according claim 1 wherein R^1 and R^2 are independently C_{2-6} alkyl; or R^1 is H or methyl and R^2 is C_{2-6} alkyl, or a pharmaceutically acceptable salt thereof.
6. The process according claim 1 wherein a compound of the formula



or a pharmaceutically acceptable salt thereof, is obtained.

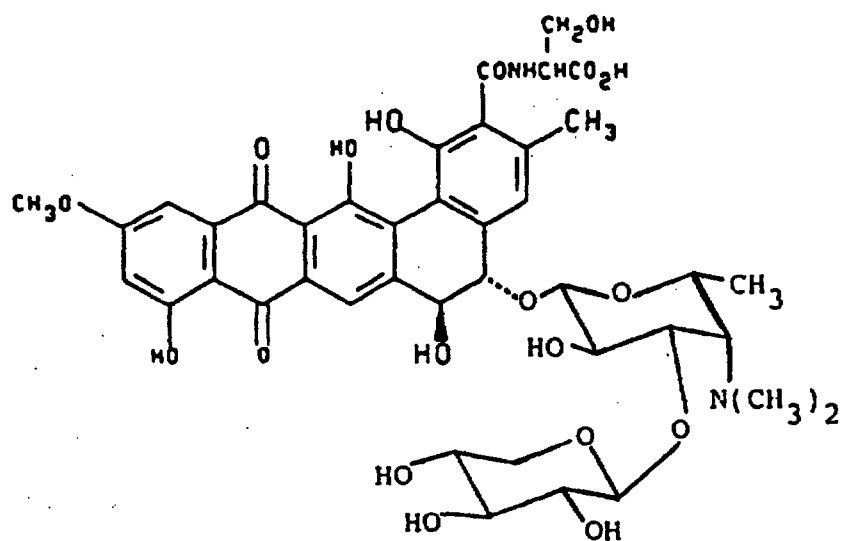


7. The process according claim 1 wherein a compound of the formula



or a pharmaceutically acceptable salt thereof, is obtained.

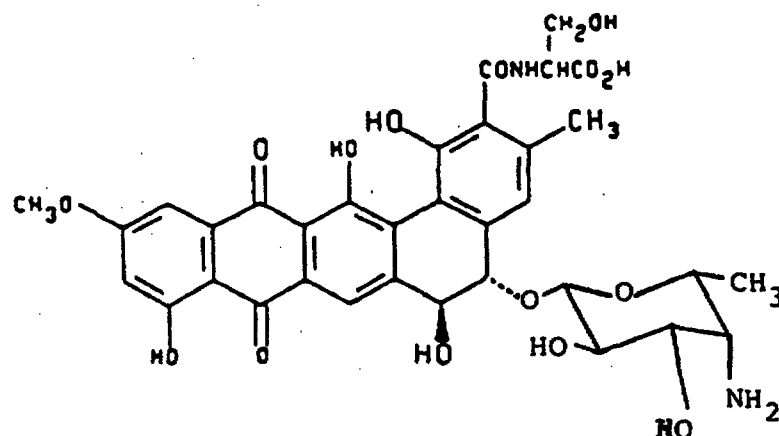
8. The process according claim 1 wherein a compound of the formula



or a pharmaceutically acceptable salt thereof, is obtained.

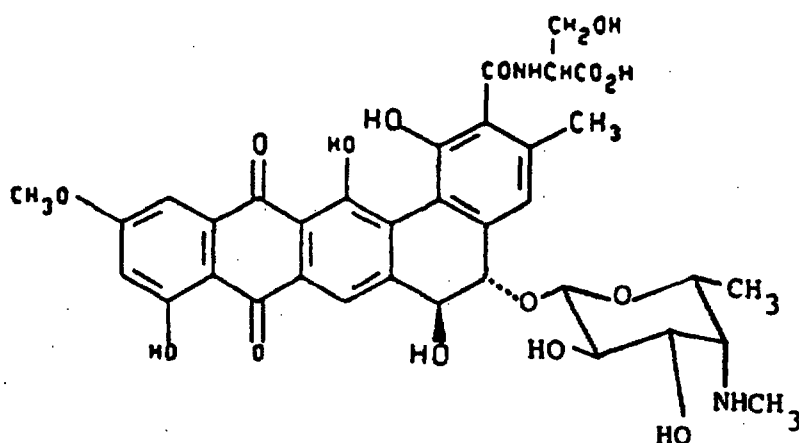


9. The process according claim 1 wherein a compound of the formula



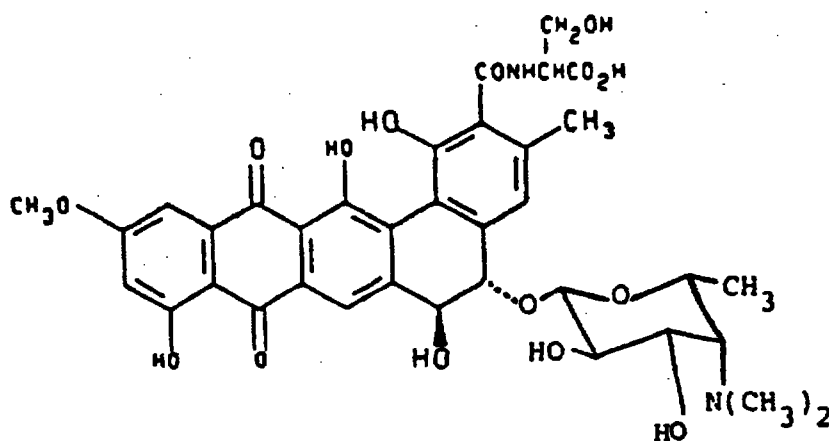
or a pharmaceutically acceptable salt thereof, is obtained.

10. The process according claim 1 wherein a compound of the formula



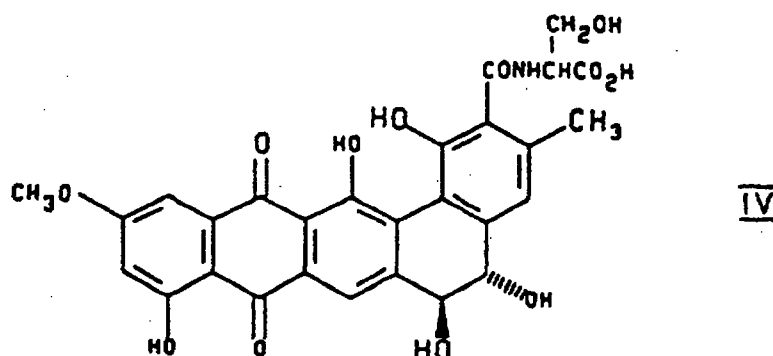
or a pharmaceutically acceptable salt thereof, is obtained.

11. The process according claim 1 wherein a compound of the formula



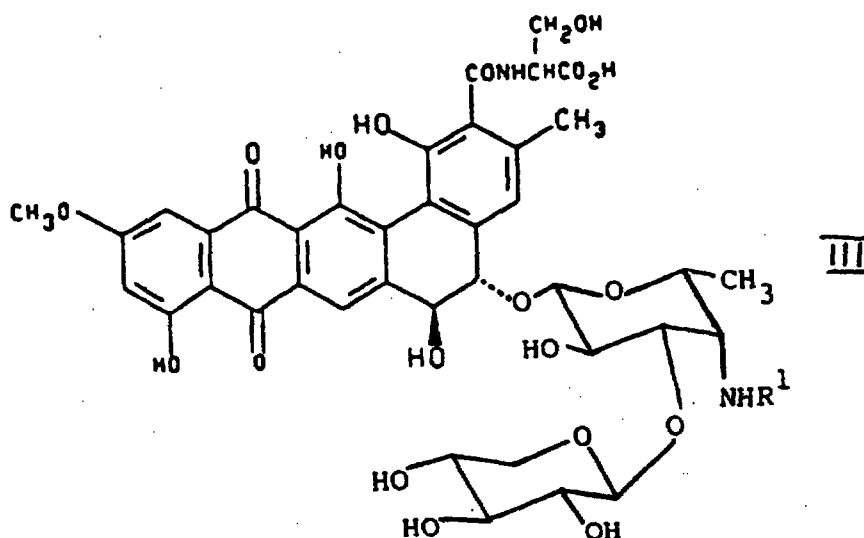
or a pharmaceutically acceptable salt thereof, is obtained.

12. The process for preparing a compound of the formula



wherein the serine is D-serine; or a salt thereof, which comprises acid hydrolysis of the compound of formula II, wherein R³ is β-D-xylosyl and R¹ and R² are methyl.

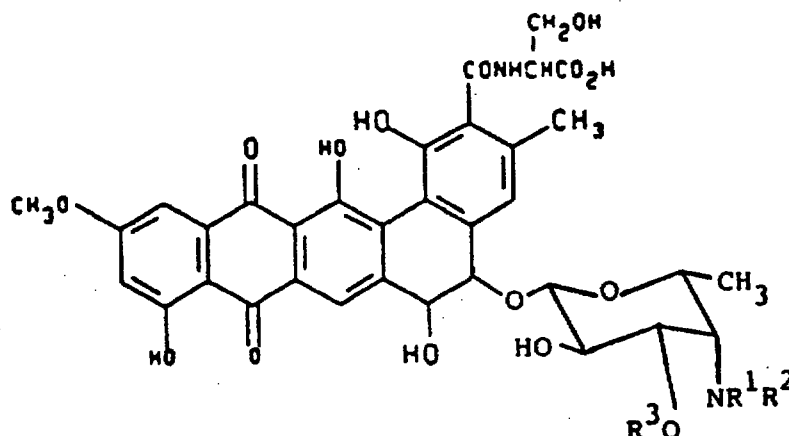
13. A process for preparing an antibiotic of formula III



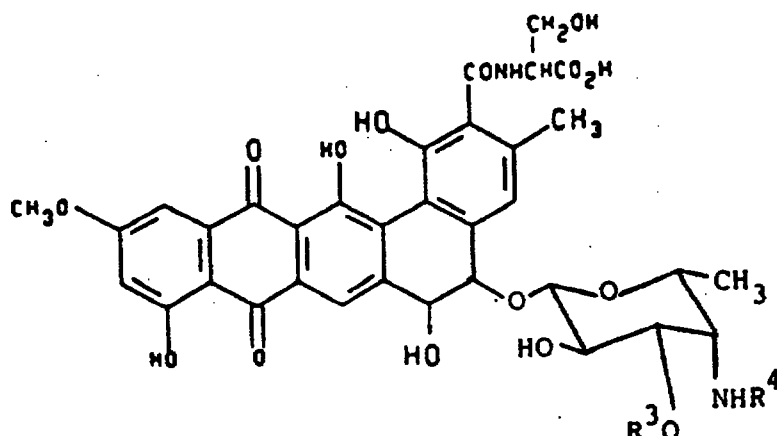
wherein R¹ is H or methyl, which comprises cultivating a strain of *Actinomadura hibisca* capable of producing said antibiotic in a medium containing assimilable sources of carbon and nitrogen, and S- or DL-serine under aerobic condition, and recovering said antibiotic from the cultured broth.



14. A process for preparing a compound having the formula



wherein R^1 and R^2 are independently C_{1-6} alkyl; or R^3 is H or β -D-xylosyl; or a pharmaceutically acceptable salt thereof, which comprises the steps of
(a) reacting a compound of formula



wherein R^3 is H or β -D-xylosyl and R^4 is H or methyl, or a pharmaceutically acceptable salt thereof with an aldehyde or a ketone having 1 to 6 carbon atoms to form an imine; and (b) treating the imine of (a) with a reducing agent.

15. A process for preparing a pharmaceutical composition which comprises incorporating at least one compound as defined in claim 1 into a pharmaceutically acceptable carrier.

16. The use of at least one compound of claim 1 for preparing a pharmaceutical composition for treating fungal infections in a mammalian host.

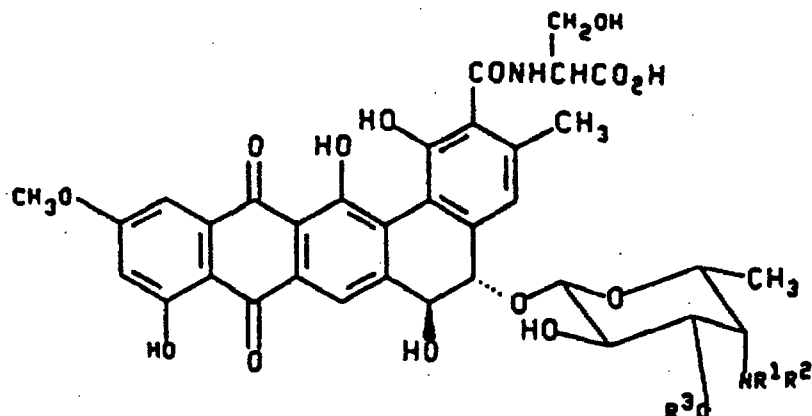
17. A biologically pure culture of the microorganism *Actinomadura hibisca* having the identifying characteristics of ATCC 53815 and which is capable of producing an antibiotic of formula III upon cultivation in an aqueous medium containing an assimilable source of carbon, nitrogen and D-serine.

18. A biologically pure culture of the microorganism *Actinomadura hibisca* having the identifying characteristics of ATCC 53816 and which is capable of producing an antibiotic of formula III upon cultivation in an aqueous medium containing an assimilable source of carbon, nitrogen and D-serine.



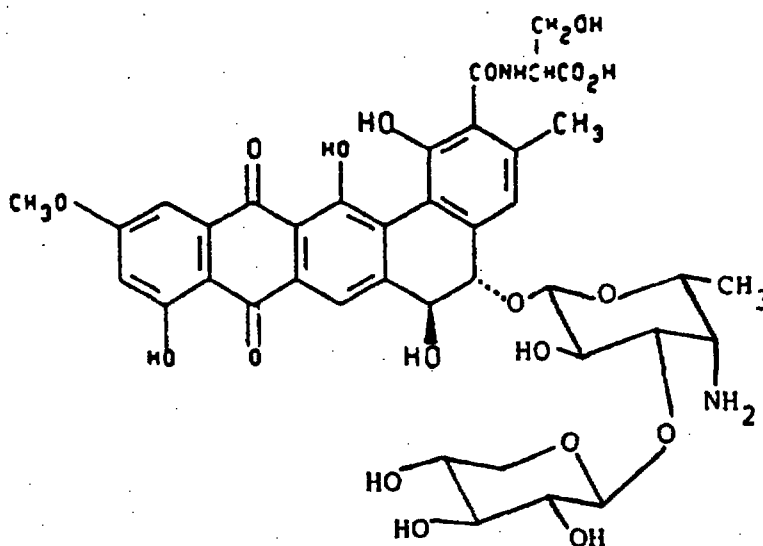
Claims for the following Contracting State : GR

- 1. A compound having the formula II**



II

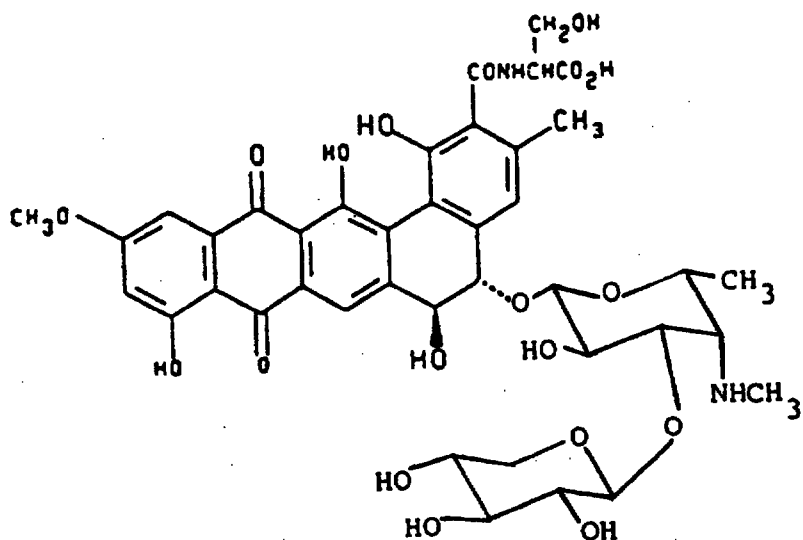
- wherein the serine is D-serine; R¹ and R² are independently H or C₁₋₆ alkyl; and R³ is H or β-D-xylosyl; or a pharmaceutically acceptable salt thereof.
2. A compound of claim 1 wherein R³ is β-D-xylosyl, or a pharmaceutically acceptable salt thereof.
3. A compound of claim 1 wherein R³ is H, or a pharmaceutically acceptable salt thereof.
4. A compound of claim 1 wherein R¹ is H or methyl, and R² is H or methyl, or a pharmaceutically acceptable salt thereof.
5. A compound of claim 1 wherein R¹ and R² are independently C₂₋₆ alkyl; or R¹ is H or methyl and R² is C₂₋₆ alkyl, or a pharmaceutically acceptable salt thereof.
6. The compound of claim 1 having the formula



- 55 or a pharmaceutically acceptable salt thereof.

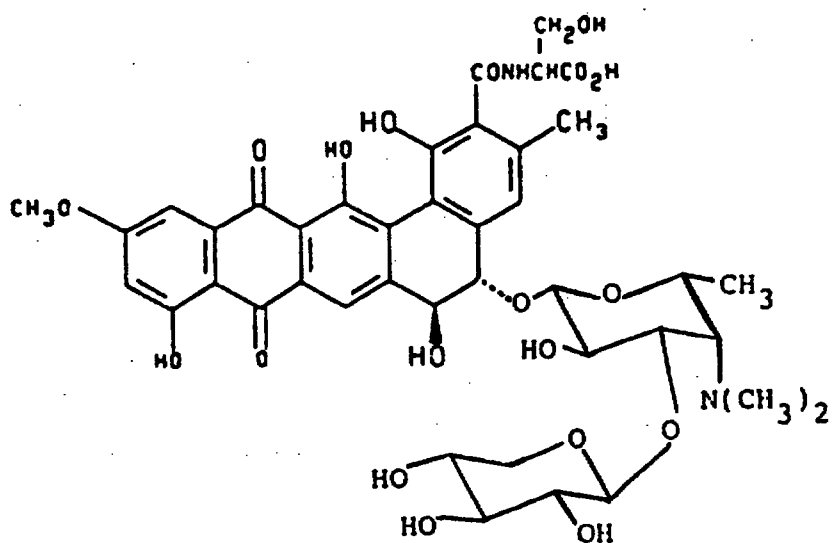


7. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

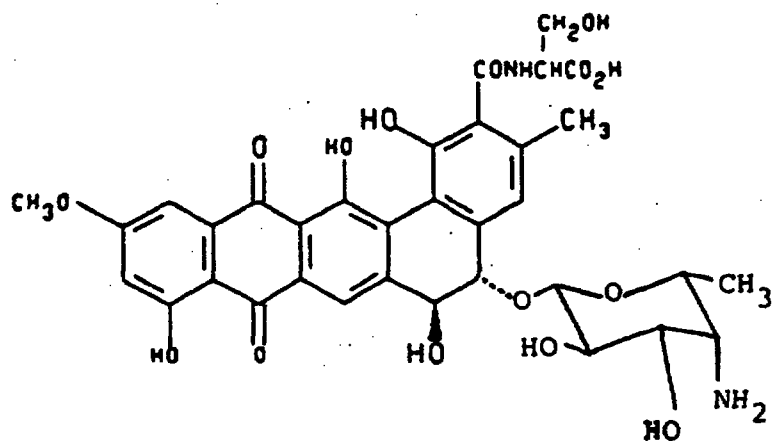
8. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

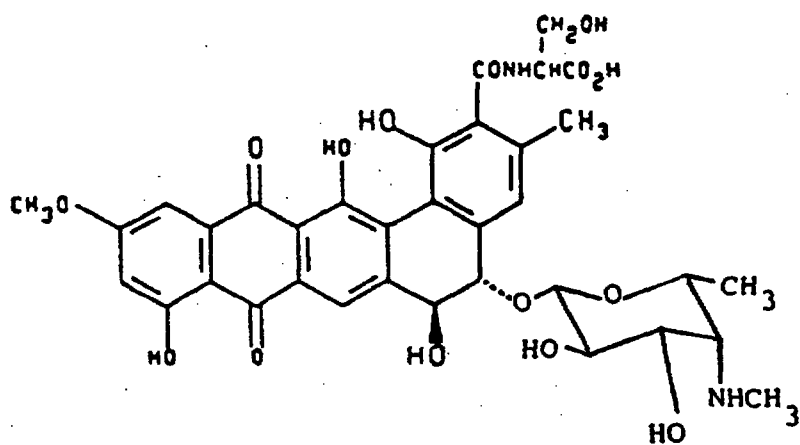


9. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

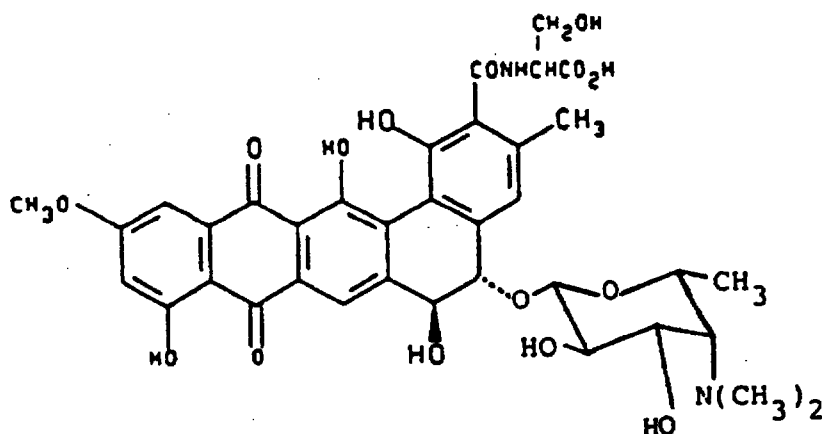
10. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

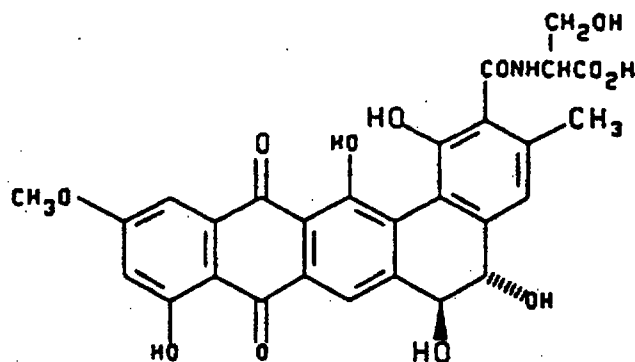


11. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

12. The compound of the formula



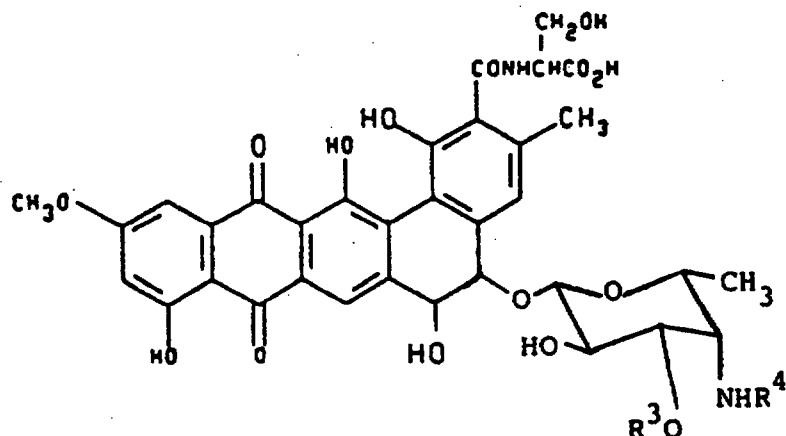
IV

wherein the serine is D-serine; or a salt thereof.

13. A process for preparing a compound of formula II according to claim 1 which comprises

- A. cultivating a strain of *Actinomadura Hibisca* capable of producing said antibiotic in a medium containing asimilable sources of carbon and nitrogen, and D- or DL-serine under aerobic condition, and recovering said antibiotic from the cultured broth to obtain a compound of formula II wherein R¹ is H or methyl, R² is H and R³ is β -D-xylosyl;
- B. if desired, heating a compound of formula III in an acidic medium to obtain the corresponding desxylosyl derivative,
- C. if desired, reacting a compound obtained according to step A or B, which corresponds to formula V:

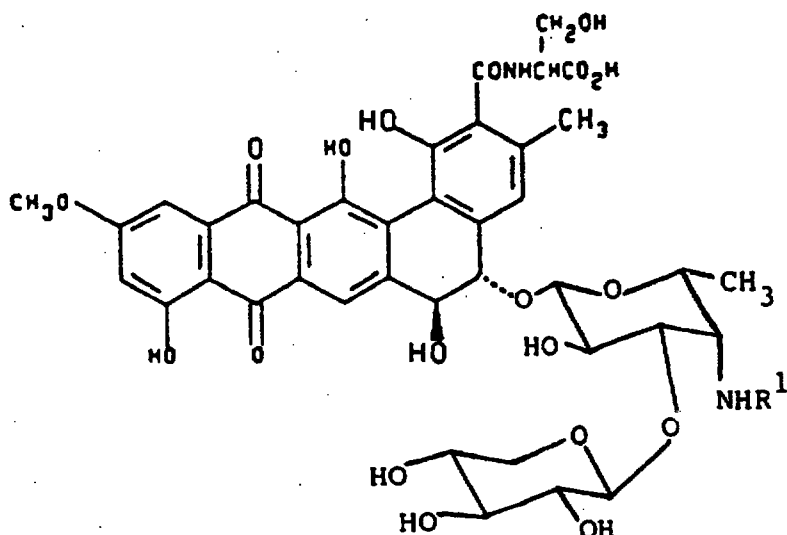




V

wherein R^3 is H or β -D-xylosyl and R^4 is H or methyl, or a pharmaceutically acceptable salt thereof with an aldehyde or a ketone having 1 to 6 carbon atoms to form an imine and treating the imine with a reducing agent to obtain a compound of formula II, wherein R^1 and R^2 are independently C_{1-6} alkyl and R^3 is H or β -D-xylosyl or a pharmaceutically acceptable salt thereof.

14. A process for preparing an antibiotic of formula III

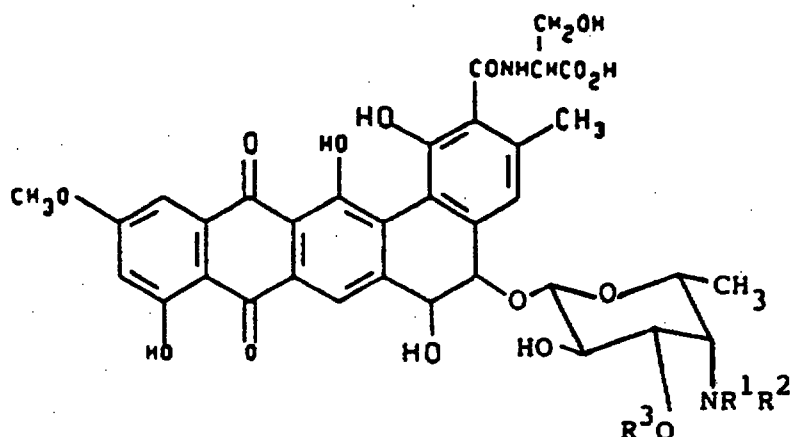


III

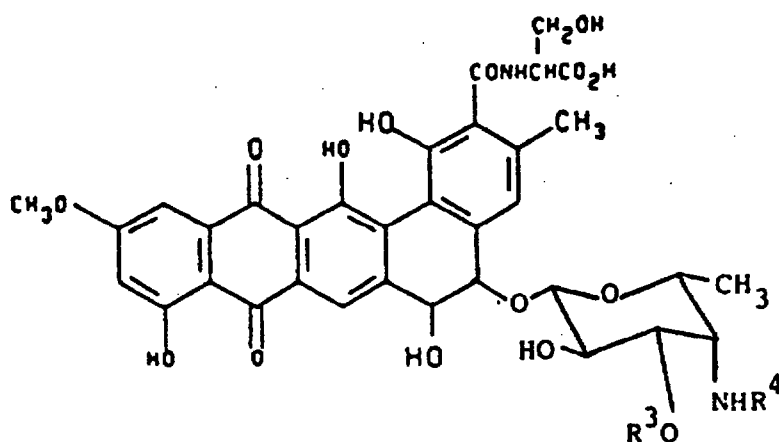
wherein R^1 is H or methyl, which comprises cultivating a strain of *Actinomadura hibisca* capable of producing said antibiotic in a medium containing assimilable sources of carbon and nitrogen, and D- or DL-serine under aerobic condition, and recovering said antibiotic from the cultured broth.



15. A process for preparing a compound having the formula



wherein R^1 and R^2 are independently C_{1-6} alkyl; or R^3 is H or β -D-xylosyl; or a pharmaceutically acceptable salt thereof, which comprises the steps of
(a) reacting a compound of formula



wherein R^3 is H or β -D-xylosyl and R^4 is H or methyl, or a pharmaceutically acceptable salt thereof with an aldehyde or a ketone having 1 to 6 carbon atoms to form an imine; and (b) treating the imine of (a) with a reducing agent.

16. A process for preparing a pharmaceutical composition which comprises incorporating at least one compound of claim 1 into a pharmaceutically acceptable carrier.

17. The use of at least one compound of claim 1 for preparing a pharmaceutical composition for treating fungal infections in a mammalian host.

18. A biologically pure culture of the microorganism *Actinomadura hibisca* having the identifying characteristics of ATCC 53815 and which is capable of producing an antibiotic of formula III upon cultivation in an aqueous medium containing an assimilable source of carbon, nitrogen and D-serine.

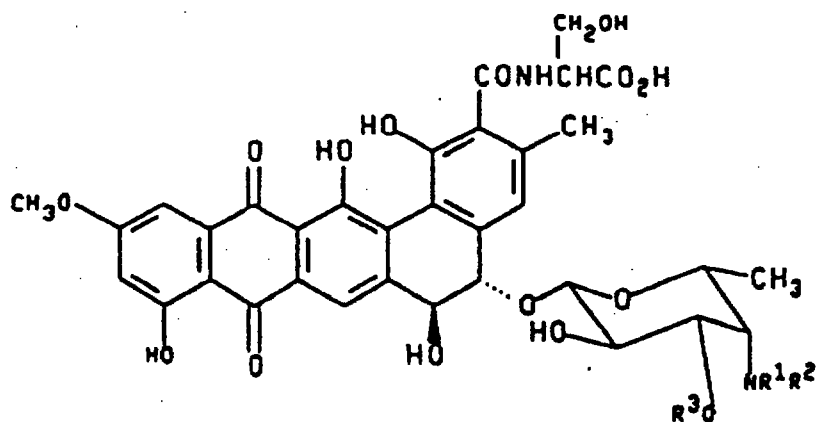
19. A biologically pure culture of the microorganism *Actinomadura hibisca* having the identifying characteristics of ATCC 53816 and which is capable of producing an antibiotic of formula III upon cultivation in an aqueous medium containing an assimilable source of carbon, nitrogen and D-serine.



Patentansprüche

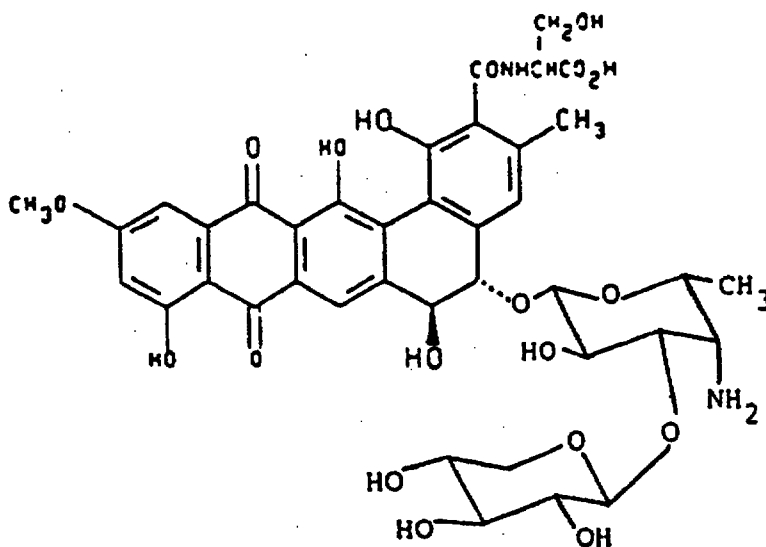
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Verbindungen der Formel II



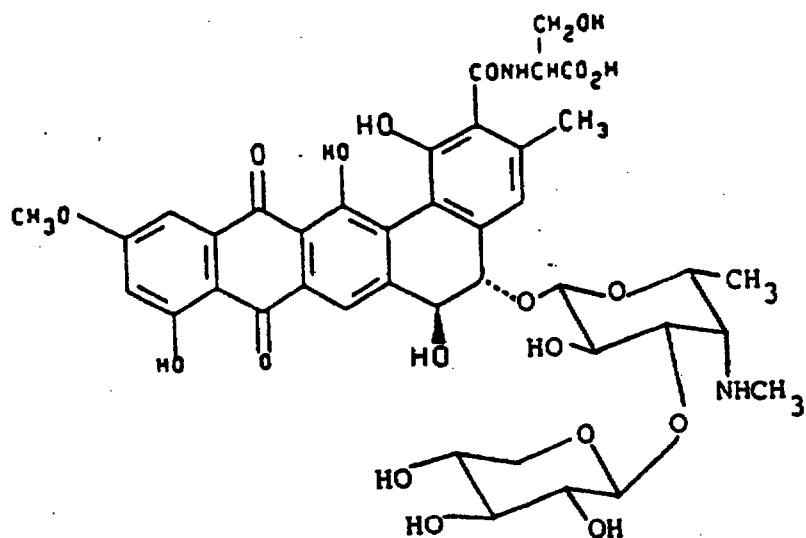
worin es sich beim Serin um D-Serin handelt; R^1 und R^2 unabhängig voneinander für H oder C_{1-6} -Alkyl stehen; und R^3 für H oder β -D-Xylosyl steht; oder die pharmazeutisch verträglichen Salze davon.

2. Verbindungen nach Anspruch 1, worin R^3 für β -D-Xylosyl steht, oder die pharmazeutisch verträglichen Salze davon.
3. Verbindungen nach Anspruch 1, worin R^3 für H steht, oder die pharmazeutisch verträglichen Salze davon.
4. Verbindungen nach Anspruch 1, worin R^1 für H oder Methyl steht und R^2 für H oder Methyl steht, oder die pharmazeutisch verträglichen Salze davon.
5. Verbindungen nach Anspruch 1, worin R^1 und R^2 unabhängig voneinander für C_{2-6} -Alkyl stehen; oder R^1 für H oder Methyl steht und R^2 für C_{2-6} -Alkyl steht, oder die pharmazeutisch verträglichen Salze davon.
6. Verbindung nach Anspruch 1 der Formel



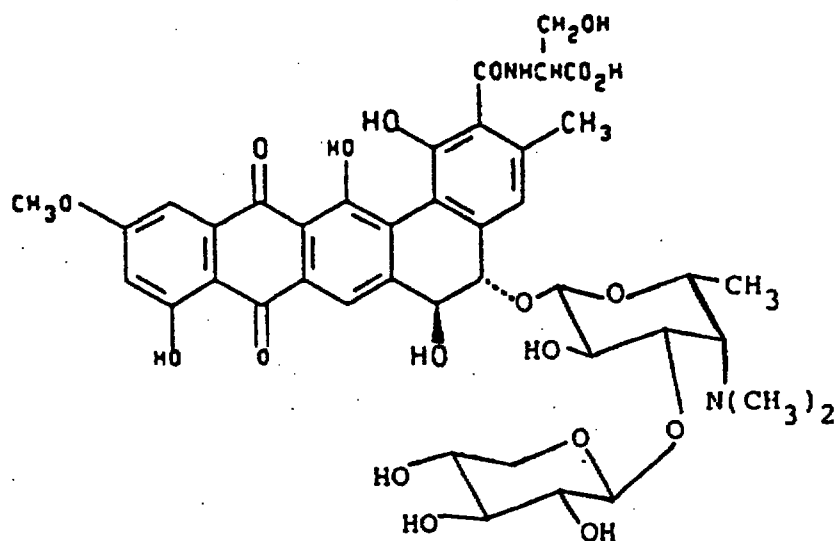
oder ein pharmazeutisch verträgliches Salz davon.

7. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

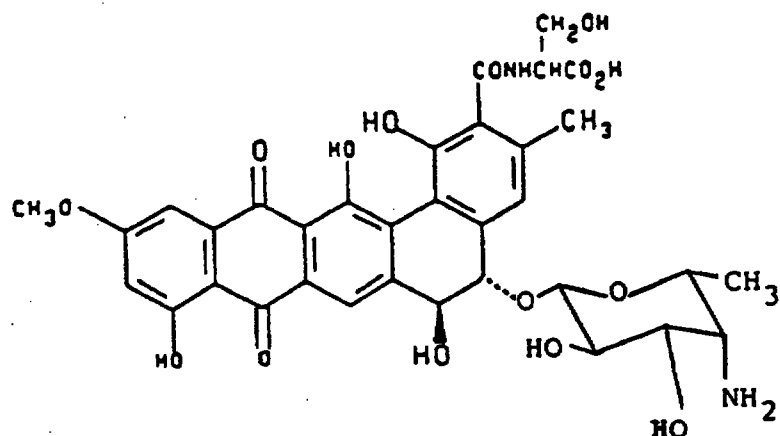
8. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

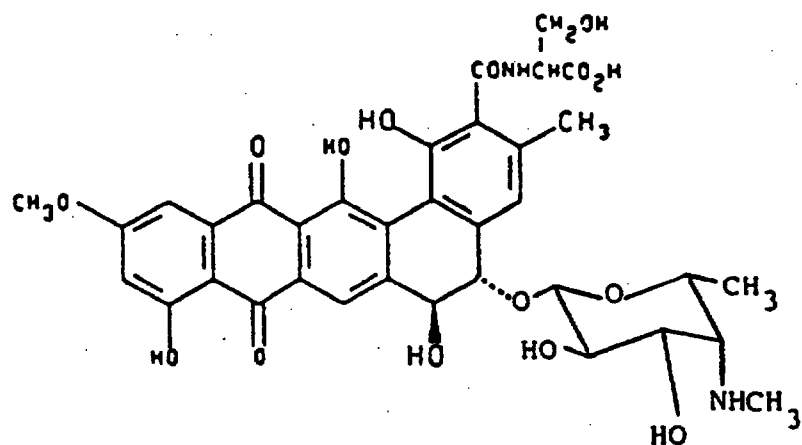


9. Verbindung nach Anspruch 1 der Formel



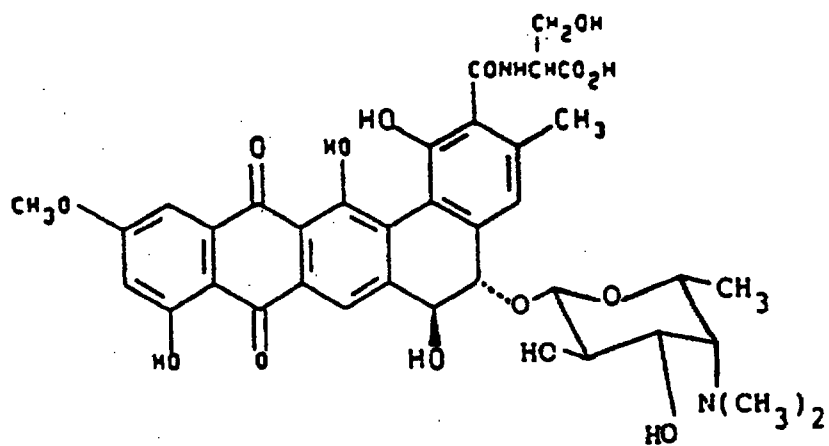
oder ein pharmazeutisch verträgliches Salz davon.

10. Verbindung nach Anspruch 1 der Formel



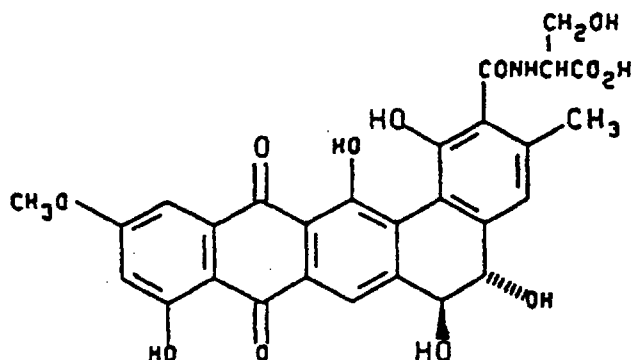
oder ein pharmazeutisch verträgliches Salz davon.

11. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

12. Verbindung der Formel



IV

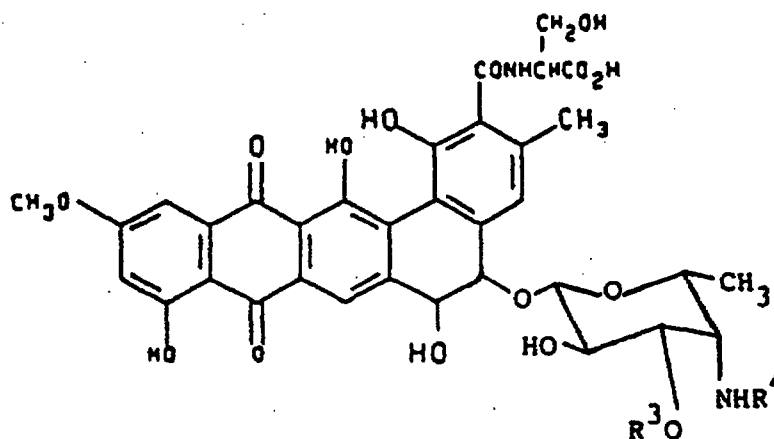
worin es sich bei dem Serin um D-Serin handelt, oder ein Salz davon.

13. Verfahren zur Herstellung der Verbindungen der Formel II nach Anspruch 1, wobei man

A. einen *Actinomadura Hibisca*-Stamm, der in der Lage ist, das Antibiotikum zu produzieren, in einem Medium, das assimilierbare Kohlenstoff- und Stickstoffquellen und D- oder DL-Serin enthält, unter aeroben Bedingungen kultiviert und das Antibiotikum aus der Kulturbrühe gewinnt, wobei man eine Verbindung der Formel II erhält, worin R¹ für H oder Methyl steht, R² für H und R³ für β -D-Xylosyl steht;

B. gewünschtenfalls eine Verbindung der Formel III in einem sauren Medium erhitzt, wobei man das entsprechende Desxylosylderivat erhält,

C. gewünschtenfalls eine gemäß den Stufen A oder B erhaltene Verbindung der Formel V



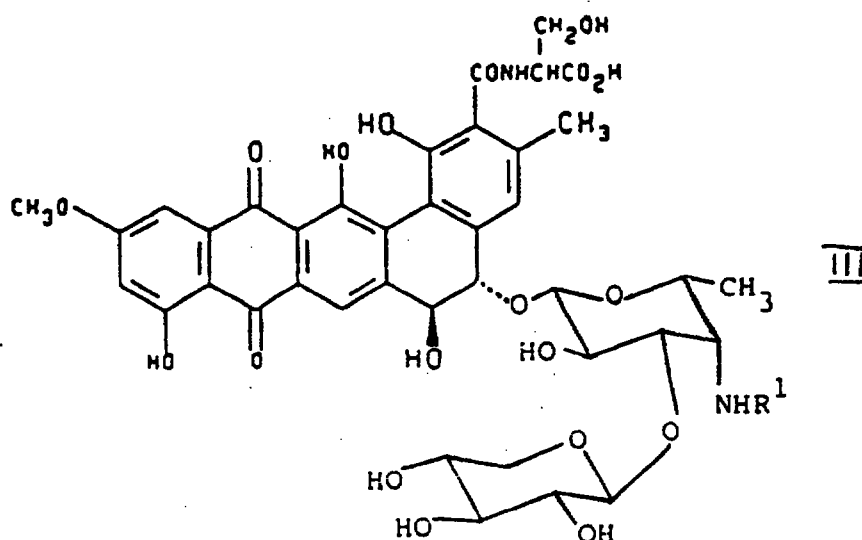
V

worin R³ für H oder β -D-Xylosyl steht und R⁴ für H oder Methyl steht oder ein pharmazeutisch verträgliches Salz davon mit einem Aldehyd oder einem Keton mit 1 bis 6 Kohlenstoffatomen zu einem Imin umgesetzt und das Imin mit einem Reduktionsmittel behandelt, wobei man eine Verbindung der Formel II,

worin R¹ und R² unabhängig voneinander für C₁₋₆-Alkyl stehen und R³ für H oder β -D-Xylosyl steht, oder ein pharmazeutisch verträgliches Salz davon erhält.

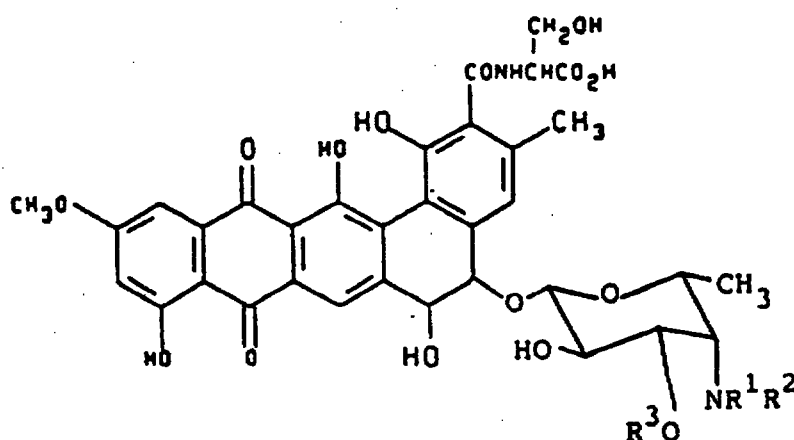


14. Verfahren zur Herstellung eines Antibiotikums der Formel III



worin R¹ für H oder Methyl steht, wobei man einen Actinomadura hibisca-Stamm, der in der Lage ist, das Antibiotikum zu produzieren, in einem Medium, das assimilierbare Kohlenstoff- und Stickstoffquellen und D- oder DL-Serin enthält, unter aeroben Bedingungen kultiviert und das Antibiotikum aus der Kulturbrühe gewinnt.

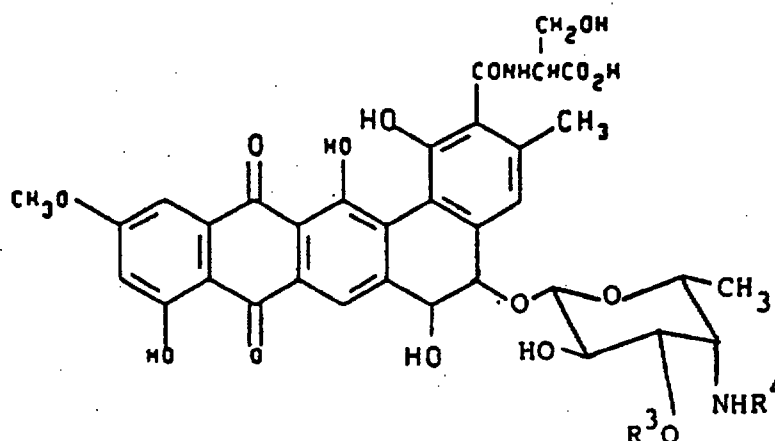
15. Verfahren zur Herstellung einer Verbindung der Formel



worin R¹ und R² unabhängig voneinander für C₁-₆-Alkyl stehen; und R³ für H oder β -D-Xylosyl steht; oder eines pharmazeutisch verträglichen Salzes davon, wobei man



(a) eine Verbindung der Formel



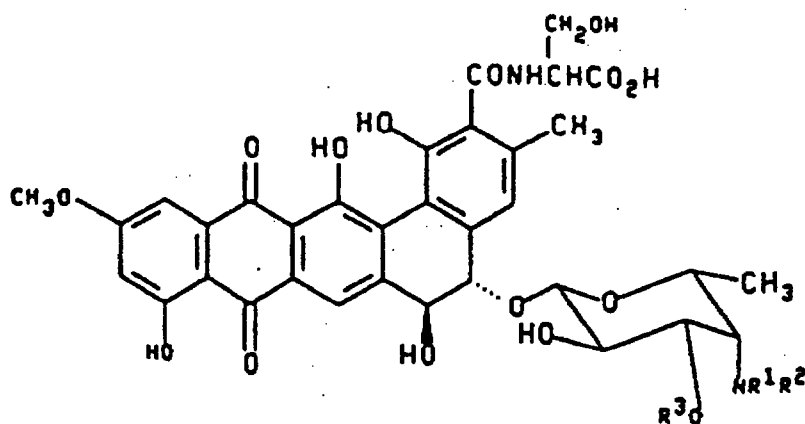
worin R^3 für H oder β -D-Xylosyl steht und R^4 für H oder Methyl steht oder ein pharmazeutisch verträgliches Salz davon mit einem Aldehyd oder einem Keton mit 1 bis 6 Kohlenstoffatomen zu einem Imin umsetzt; und
(b) das Imin der Stufe (a) mit einem Reduktionsmittel behandelt.

16. Pharmazeutisches Mittel, umfassend wenigstens eine Verbindung nach Anspruch 1 und einen pharmazeutisch verträglichen Träger.
17. Verfahren zur Herstellung des Mittels nach Anspruch 16, wobei man wenigstens eine Verbindung nach Anspruch 1 einem pharmazeutisch verträglichen Träger einverleibt.
18. Verwendung wenigstens einer Verbindung nach Anspruch 1 zur Herstellung eines pharmazeutischen Mittels zur Behandlung von Pilzinfektionen bei Säugetieren.
19. Biologisch reine Kultur des Mikroorganismus Actinomadura hibisca mit den kennzeichnenden Eigenschaften von ATCC 53815, der in der Lage ist, beim Kultivieren in einem wäßrigen Medium, das eine assimilierbare Kohlenstoff- und Stickstoffquelle und D-Serin enthält, ein Antibiotikum der Formel III zu produzieren.
20. Biologisch reine Kultur des Mikroorganismus Actinomadura hibisca mit den kennzeichnenden Eigenschaften von ATCC 53816, der in der Lage ist, beim Kultivieren in einem wäßrigen Medium, das eine assimilierbare Kohlenstoff- und Stickstoffquelle und D-Serin enthält, ein Antibiotikum der Formel III zu produzieren.



Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung von Verbindungen der Formel II

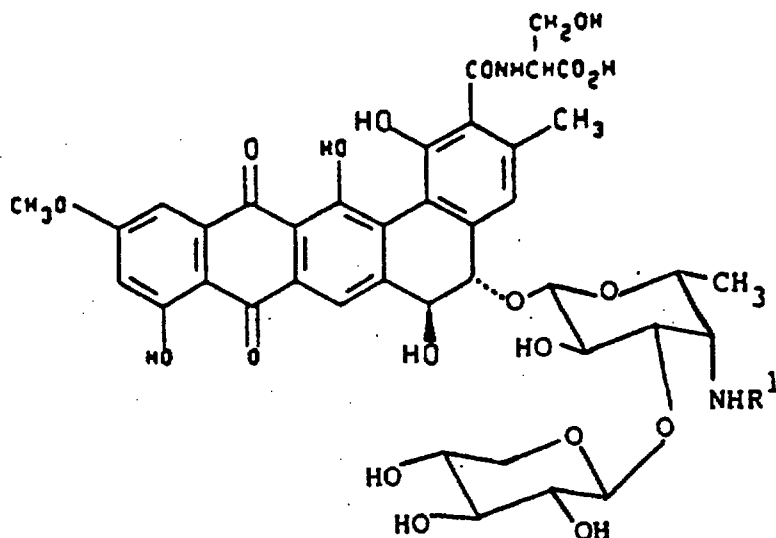


II

worin es sich beim Serin um D-Serin handelt; R¹ und R² unabhängig voneinander für H oder C₁₋₆-Alkyl stehen; und R³ für H oder β -D-Xylosyl steht; oder der pharmazeutisch verträglichen Salze davon, wobei man

A. einen Actinomadura Hibisca-Stamm, der in der Lage ist, das Antibiotikum zu produzieren, in einem Medium, das assimilierbare Kohlenstoff- und Stickstoffquellen und D- oder DL-Serin enthält, unter aeroben Bedingungen kultiviert und das Antibiotikum aus der Kulturbrühe gewinnt, wobei man eine Verbindung der Formel II erhält, worin R¹ für H oder Methyl steht, R² für H und R³ für β -D-Xylosyl steht;

B. gewünschtenfalls eine Verbindung der Formel III

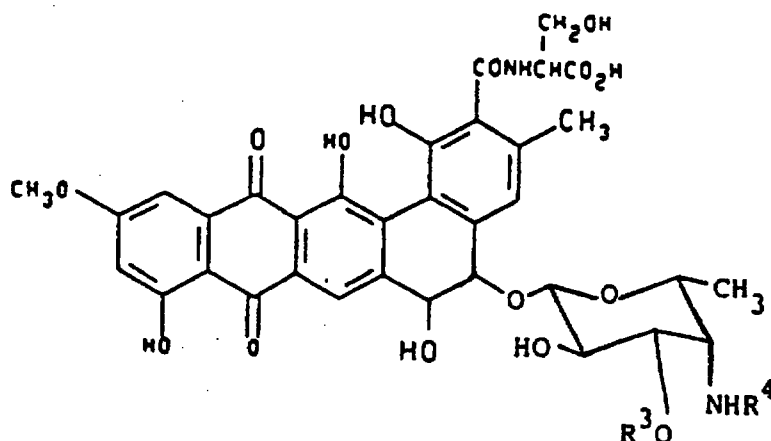


III

worin R¹ für H oder Methyl steht, in einem sauren Medium erhitzt, wobei man das entsprechende Desxylosylderivat erhält,



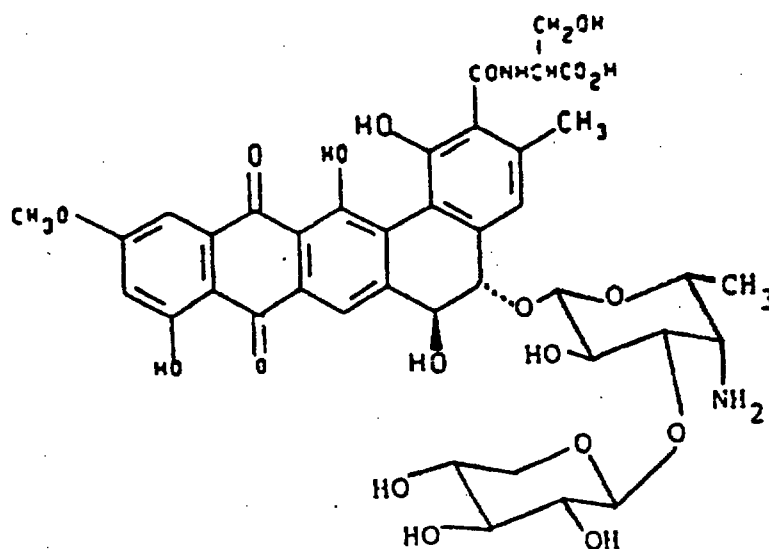
C. gewünschtenfalls eine gemäß den Stufen A oder B erhaltene Verbindung der Formel V



worin R³ für H oder β -D-Xylosyl steht und R⁴ für H oder Methyl steht oder ein pharmazeutisch verträgliches Salz davon mit einem Aldehyd oder einem Keton mit 1 bis 6 Kohlenstoffatomen zu einem Imin umgesetzt und das Imin mit einem Reduktionsmittel behandelt, wobei man eine Verbindung der Formel II,

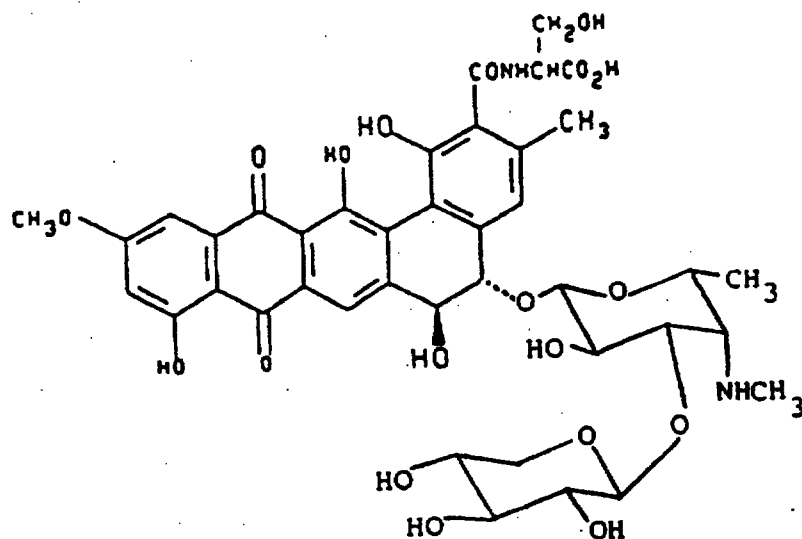
worin R¹ und R² unabhängig voneinander für C₁₋₆-Alkyl stehen und R³ für H oder β -D-Xylosyl steht, oder ein pharmazeutisch verträgliches Salz davon erhält.

2. Verfahren nach Anspruch 1, worin R³ für β -D-Xylosyl steht oder die pharmazeutisch verträglichen Salze davon.
3. Verfahren nach Anspruch 1, worin R³ für H steht oder die pharmazeutisch verträglichen Salze davon.
4. Verfahren nach Anspruch 1, worin R¹ für H oder Methyl steht und R² für H oder Methyl steht oder die pharmazeutisch verträglichen Salze davon.
5. Verfahren nach Anspruch 1, worin R¹ und R² unabhängig voneinander für C₂₋₆-Alkyl stehen; oder R¹ für H oder Methyl steht und R² für C₂₋₆-Alkyl steht, oder die pharmazeutisch verträglichen Salze davon.
6. Verfahren nach Anspruch 1, wobei man eine Verbindung der Formel



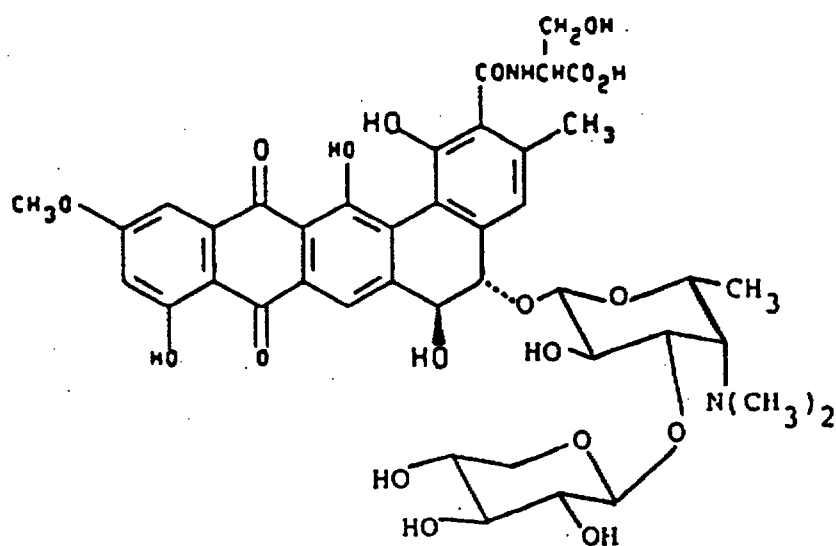
oder ein pharmazeutisch verträgliches Salz davon erhält.

7. Verfahren nach Anspruch 1, wobei man eine Verbindung der Formel



oder ein pharmazeutisch verträgliches Salz davon erhält.

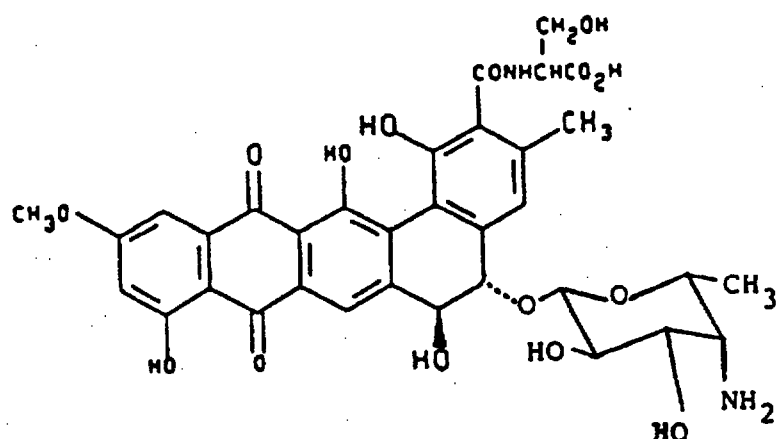
8. Verfahren nach Anspruch 1, wobei man eine Verbindung der Formel



oder ein pharmazeutisch verträgliches Salz davon erhält.

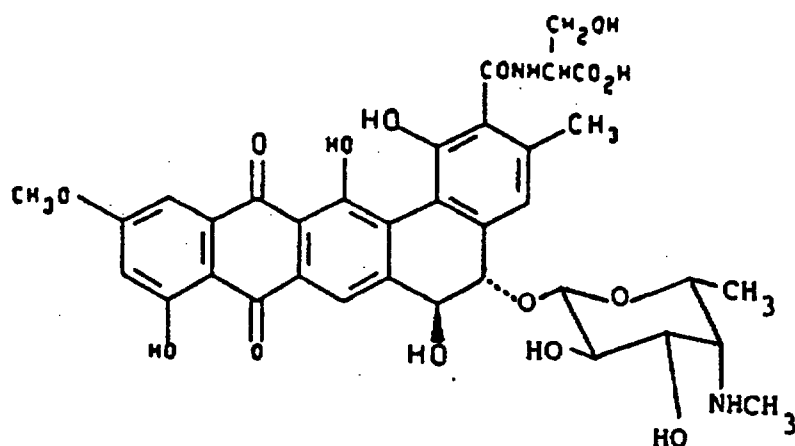


9. Verfahren nach Anspruch 1, wobei man eine Verbindung der Formel



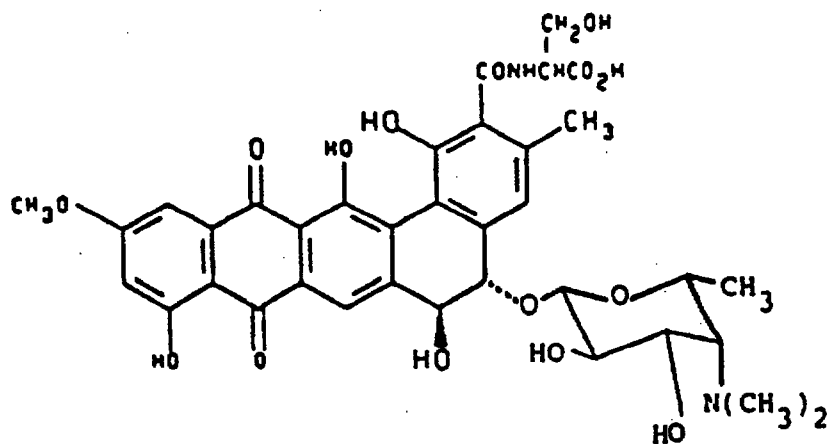
oder ein pharmazeutisch verträgliches Salz davon erhält.

10. Verfahren nach Anspruch 1, wobei man eine Verbindung der Formel



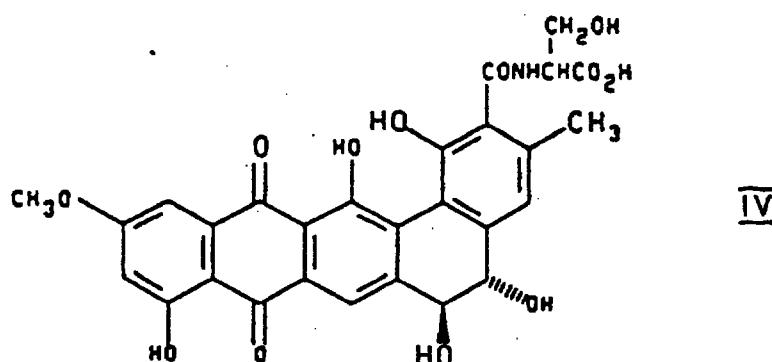
oder ein pharmazeutisch verträgliches Salz davon erhält.

11. Verfahren nach Anspruch 1, wobei man eine Verbindung der Formel



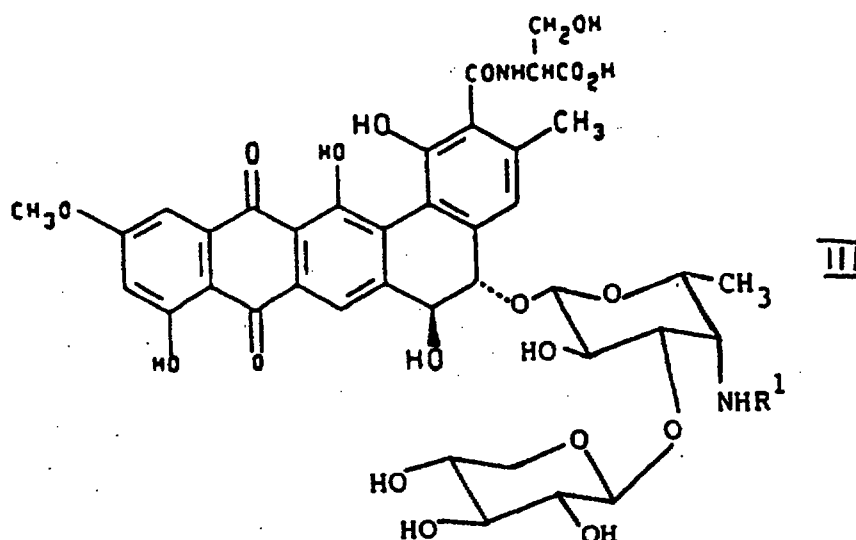
oder ein pharmazeutisch verträgliches Salz davon erhält.

12. Verfahren zur Herstellung von Verbindungen der Formel



worin es sich bei dem Serin um D-Serin handelt oder eines Salzes davon, das die saure Hydrolyse der Verbindung der Formel II, worin R³ für β -D-Xyloxyl steht und R¹ und R² für Methyl stehen, umfaßt.

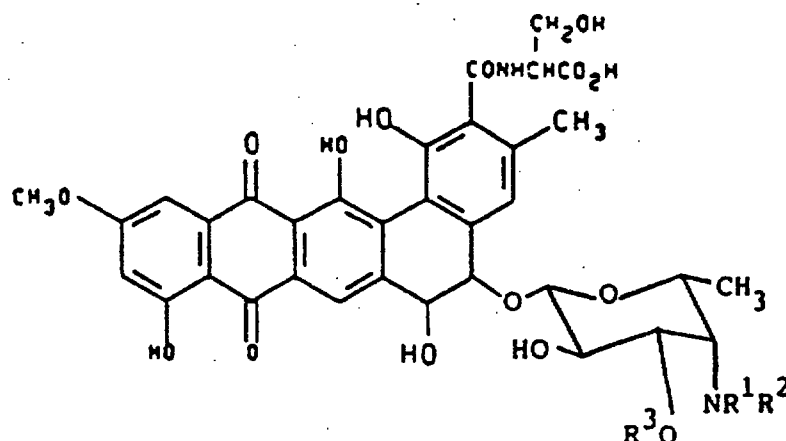
13. Verfahren zur Herstellung eines Antibiotikums der Formel III



worin R¹ für H oder Methyl steht, wobei man einen *Actinomadura hibisca*-Stamm, der in der Lage ist, das Antibiotikum zu produzieren, in einem Medium, das assimilierbare Kohlenstoff- und Stickstoffquellen und D- oder DL-Serin enthält, unter aeroben Bedingungen kultiviert und das Antibiotikum aus der Kulturbrühe gewinnt.



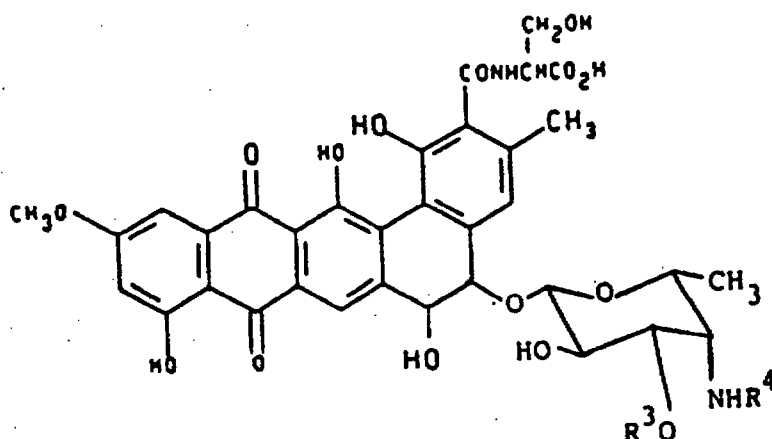
14. Verfahren zur Herstellung von Verbindungen mit der Formel



II

worin R^1 und R^2 unabhängig voneinander für C_{1-6} -Alkyl stehen;
oder R^3 für H oder β -D-Xyloxy stehen, oder eines pharmazeutisch akzeptablen Salzes davon, wobei man

(a) eine Verbindung der Formel



worin R^3 für H oder β -D-Xylosyl steht und R^4 für H oder Methyl steht oder ein pharmazeutisch verträgliches Salz davon mit einem Aldehyde oder einem Keton mit 1 bis 6 Kohlenstoffatomen zu einem Imin umsetzt; und

(b) das Imin der Stufe (a) mit einem Reduktionsmittel behandelt.

15. Verfahren zur Herstellung des pharmazeutischen Mittels wobei man wenigstens eine Verbindung nach Anspruch 1 einem pharmazeutisch verträglichen Träger einverleibt.

16. Verwendung wenigstens einer Verbindung nach Anspruch 1 zur Herstellung eines pharmazeutischen Mittels zur Behandlung von Pilzinfektionen bei Säugetieren.

17. Biologisch reine Kultur des Mikroorganismus *Actinomadura hibisca* mit den kennzeichnenden Eigenschaften von ATCC 53815, der in der Lage ist, beim Kultivieren in einem wäßrigen Medium, das eine assimilierbare Kohlenstoff- und Stickstoffquelle und D-Serin enthält, ein Antibiotikum der Formel III zu produzieren.

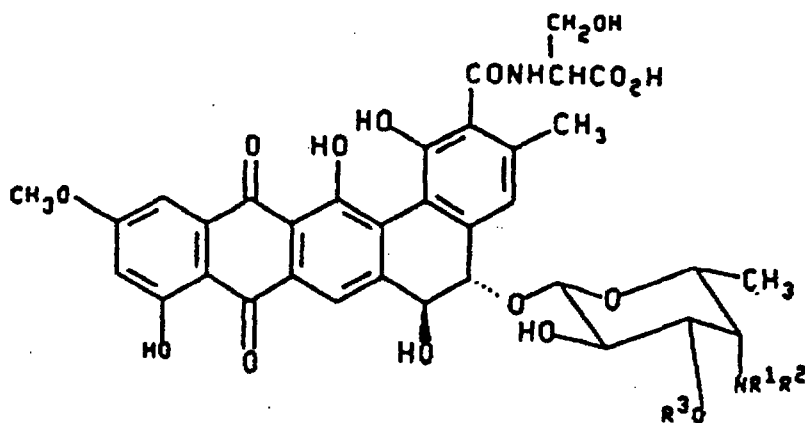
18. Biologisch reine Kultur des Mikroorganismus *Actinomadura hibisca* mit den kennzeichnenden Eigenschaften von ATCC 53816, der in der Lage ist, beim Kultivieren in einem wäßrigen Medium, das eine



assimilierbare Kohlenstoff- und Stickstoffquelle und D-Serin enthält, ein Antibiotikum der Formel III zu kultivieren.

Patentansprüche für folgenden Vertragsstaat : GR

1. Verbindungen der Formel II



worin es sich beim Serin um D-Serin handelt; R¹ und R² unabhängig voneinander für H oder C₁₋₆-Alkyl stehen; und R³ für H oder β -D-Xylosyl steht; oder die pharmazeutisch verträglichen Salze davon.

2. Verbindungen nach Anspruch 1, worin R³ für β -D-Xylosyl steht, oder die pharmazeutisch verträglichen Salze davon.

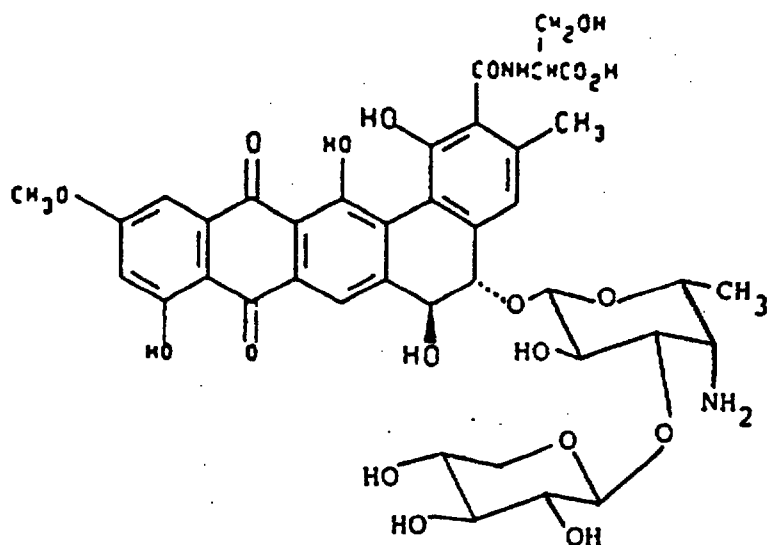
3. Verbindungen nach Anspruch 1, worin R³ für H steht, oder die pharmazeutisch verträglichen Salze davon.

4. Verbindungen nach Anspruch 1, worin R¹ für H oder Methyl steht und R² für H oder Methyl steht, oder die pharmazeutisch verträglichen Salze davon.

5. Verbindungen nach Anspruch 1, worin R¹ und R² unabhängig voneinander für C₂₋₆-Alkyl stehen; oder R¹ für H oder Methyl steht und R² für C₂₋₆-Alkyl steht, oder die pharmazeutisch verträglichen Salze davon.

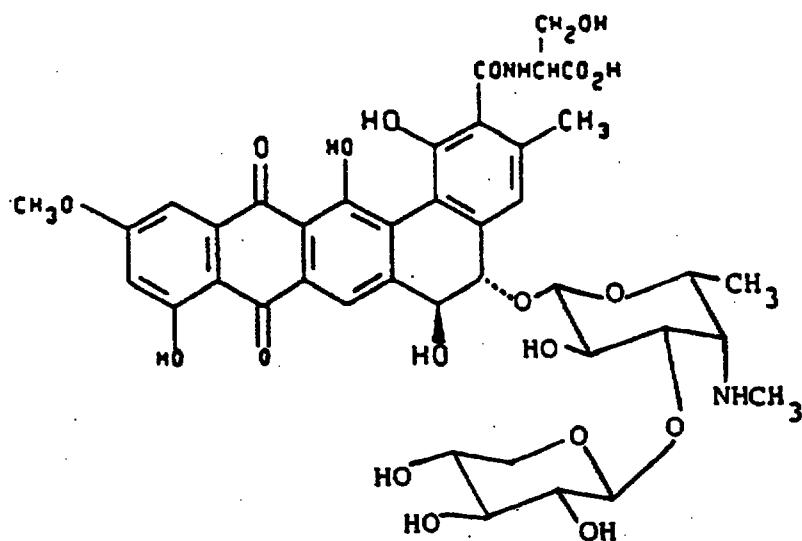


6. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

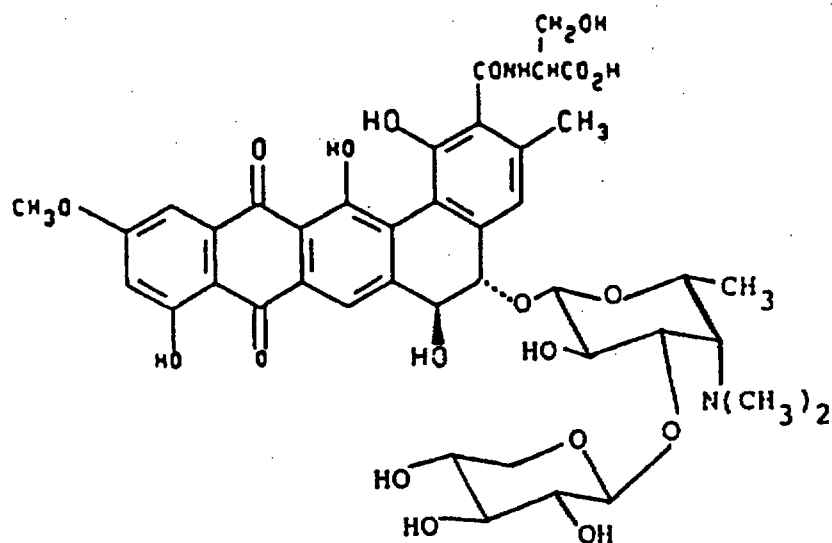
7. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

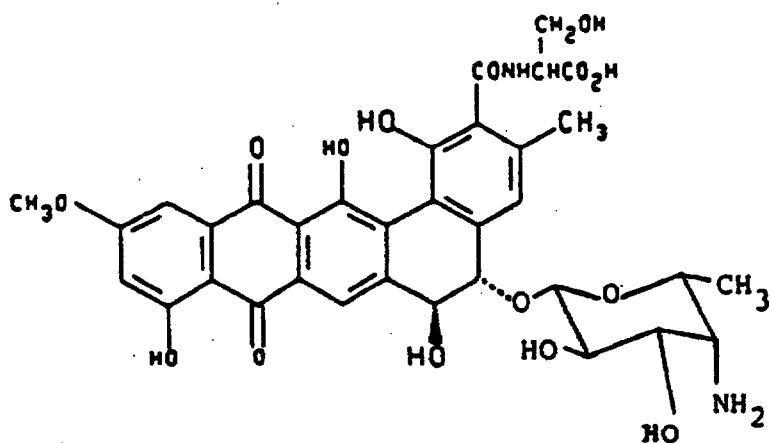


8. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

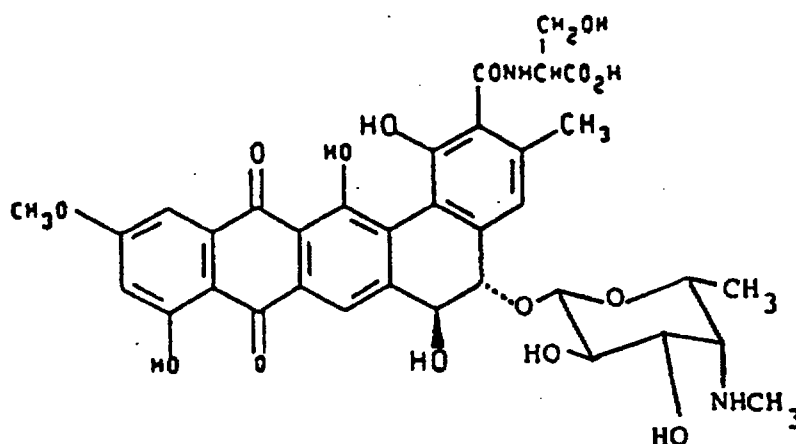
9. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

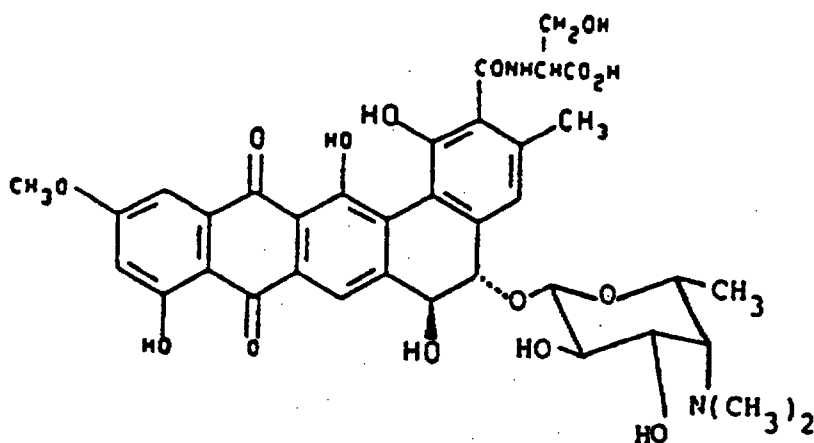


10. Verbindung nach Anspruch 1 der Formel



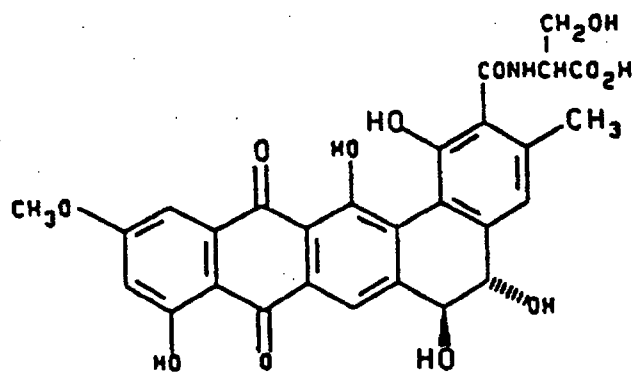
oder ein pharmazeutisch verträgliches Salz davon.

11. Verbindung nach Anspruch 1 der Formel



oder ein pharmazeutisch verträgliches Salz davon.

12. Verbindung der Formel



IV

worin es sich bei dem Serin um D-Serin handelt, oder ein Salz davon.

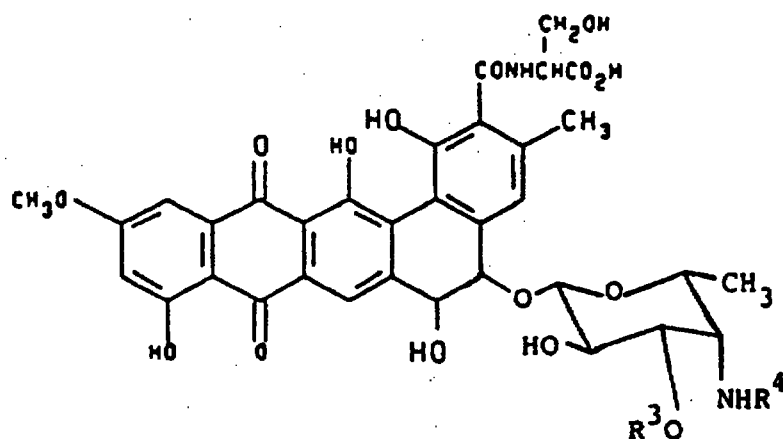


13. Verfahren zur Herstellung der Verbindungen der Formel II nach Anspruch 1, wobei man

A. einen *Actinomadura Hibisca*-Stamm, der in der Lage ist, das Antibiotikum zu produzieren, in einem Medium, das assimilierbare Kohlenstoff- und Stickstoffquellen und D- oder DL-Serin enthält, unter aeroben Bedingungen kultiviert und das Antibiotikum aus der Kulturbrühe gewinnt, wobei man eine Verbindung der Formel II erhält, worin R¹ für H oder Methyl steht, R² für H und R³ für β -D-Xylosyl steht;

B. gewünschtenfalls eine Verbindung der Formel III in einem sauren Medium erhitzt, wobei man das entsprechende Desxylosylderivat erhält,

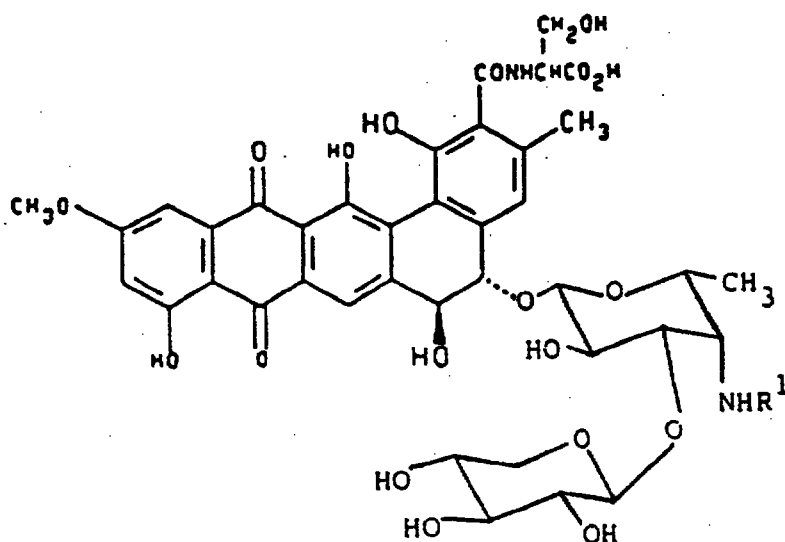
C. gewünschtenfalls eine gemäß den Stufen A oder B erhaltene Verbindung der Formel V



worin R³ für H oder β -D-Xylosyl steht und R⁴ für H oder Methyl steht oder ein pharmazeutisch verträgliches Salz davon mit einem Aldehyd oder einem Keton mit 1 bis 6 Kohlenstoffatomen zu einem Imin umgesetzt und das Imin mit einem Reduktionsmittel behandelt, wobei man eine Verbindung der Formel II,

worin R¹ und R² unabhängig voneinander für C₁₋₅-Alkyl stehen und R³ für H oder β -D-Xylosyl steht, oder ein pharmazeutisch verträgliches Salz davon erhält.

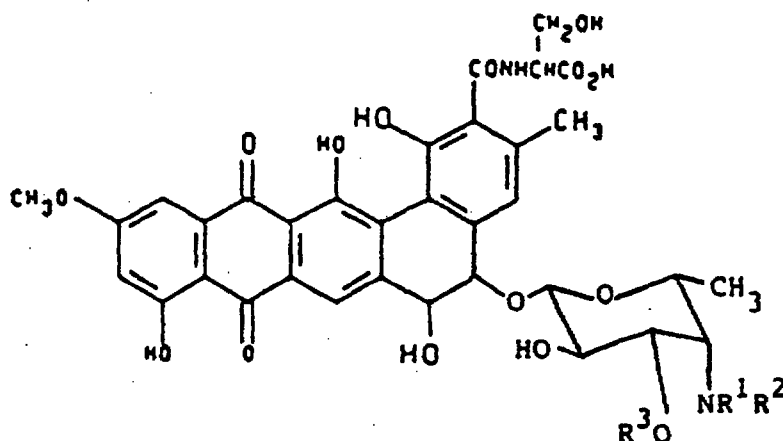
14. Verfahren zur Herstellung eines Antibiotikums der Formel III



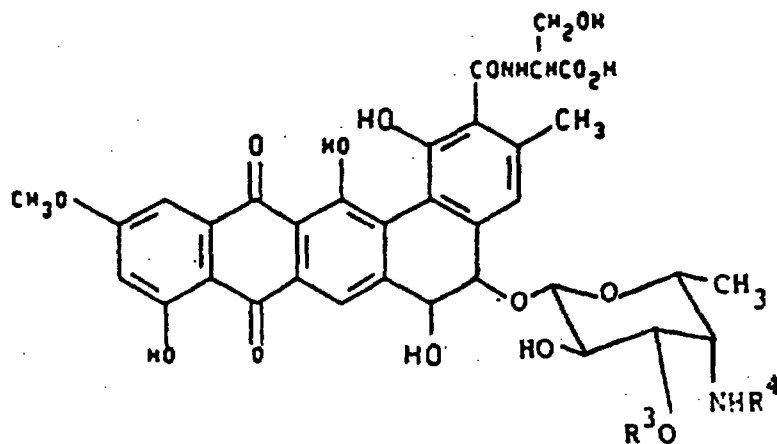
worin R¹ für H oder Methyl steht, wobei man einen *Actinomadura hibisca*-Stamm, der in der Lage ist, das Antibiotikum zu produzieren, in einem Medium, das assimilierbare Kohlenstoff- und Stickstoffquellen und D- oder DL-Serin enthält, unter aeroben Bedingungen kultiviert und das Antibiotikum aus der Kulturbrühe gewinnt.



15. Verfahren zur Herstellung einer Verbindung der Formel



worin R^1 und R^2 unabhängig voneinander für C_{1-6} -Alkyl stehen; und R^3 für H oder β -D-Xylosyl steht; oder eines pharmazeutisch verträglichen Salzes davon, wobei man
(a) eine Verbindung der Formel



worin R^3 für H oder β -D-Xylosyl steht und R^4 für H oder Methyl steht oder ein pharmazeutisch verträgliches Salz davon mit einem Aldehyd oder einem Keton mit 1 bis 6 Kohlenstoffatomen zu einem Imin umsetzt; und
(b) das Imin der Stufe (a) mit einem Reduktionsmittel behandelt.

16. Verfahren zur Herstellung eines pharmazeutischen Mittels, wobei man wenigstens einen Verbindung nach Anspruch 1 einem pharmazeutisch verträglichen Träger einverleibt.

17. Verwendung wenigstens einer Verbindung nach Anspruch 1 zur Herstellung eines pharmazeutischen Mittels zur Behandlung von Pilzinfektionen bei Säugetieren.

18. Biologisch reine Kultur des Mikroorganismus *Actinomadura hibisca* mit den kennzeichnenden Eigenschaften von ATCC 53815, der in der Lage ist, beim Kultivieren in einem wässrigen Medium, das eine assimilierbare Kohlenstoff- und Stickstoffquelle und D-Serin enthält, ein Antibiotikum der Formel III zu produzieren.

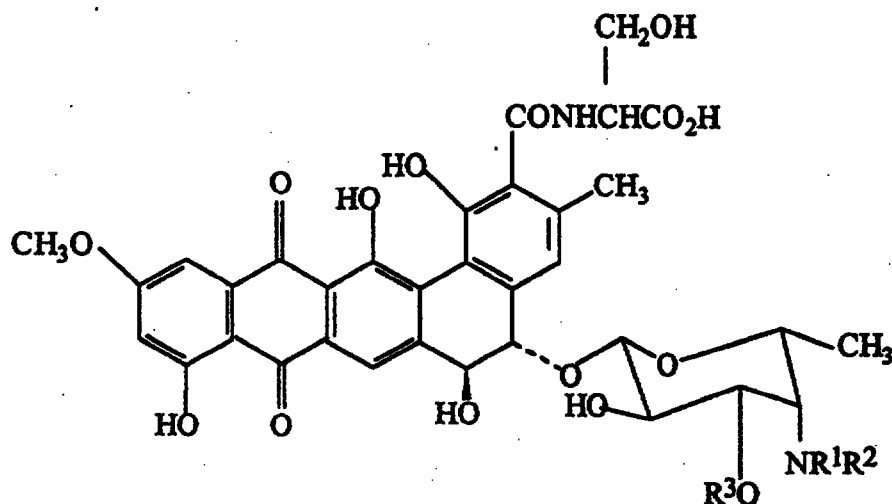
19. Biologisch reine Kultur des Mikroorganismus *Actinomadura hibisca* mit den kennzeichnenden Eigenschaften von ATCC 53816, der in der Lage ist, beim Kultivieren in einem wässrigen Medium, das eine assimilierbare Kohlenstoff- und Stickstoffquelle und D-Serin enthält, ein Antibiotikum der Formel III zu kultivieren.



Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

- 1. Composé ayant la formule II :**

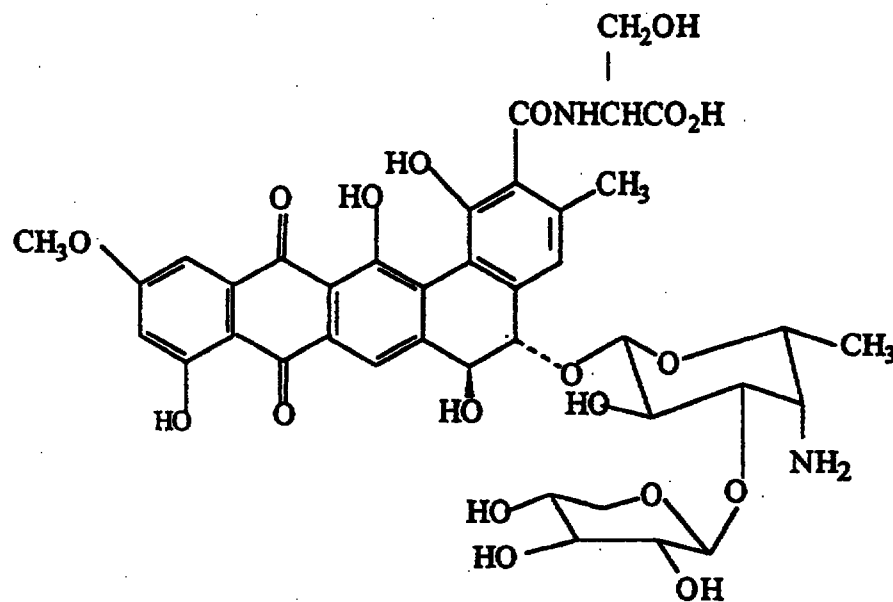


où la sérine est la D-sérine ; R¹ et R² sont indépendamment H ou alkyle C₁₋₆ ; et R³ est H ou β-D-xylosyle ; ou bien un sel acceptable en pharmacie.

2. Composé de la revendication 1 où R³ est β -D-xylosyle, ou un sel acceptable en pharmacie.
3. Composé de la revendication 1 où R³ est H, ou un sel acceptable en pharmacie.
4. Composé de la revendication 1 où R¹ est H ou méthyle et R² est H ou méthyle ou bien un sel acceptable en pharmacie.
5. Composé de la revendication 1 où R¹ et R² sont indépendamment alkyle C₂₋₆ ; ou bien R¹ est H ou méthyle et R² est alkyle C₂₋₆ ou bien un sel acceptable en pharmacie.

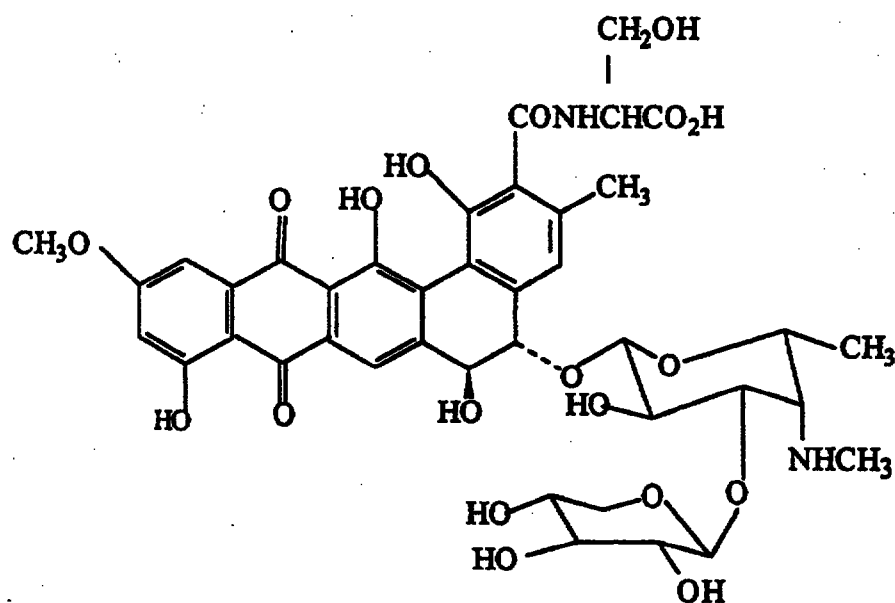


6. Composé de la revendication 1 ayant pour formule :



ou son sel acceptable en pharmacie.

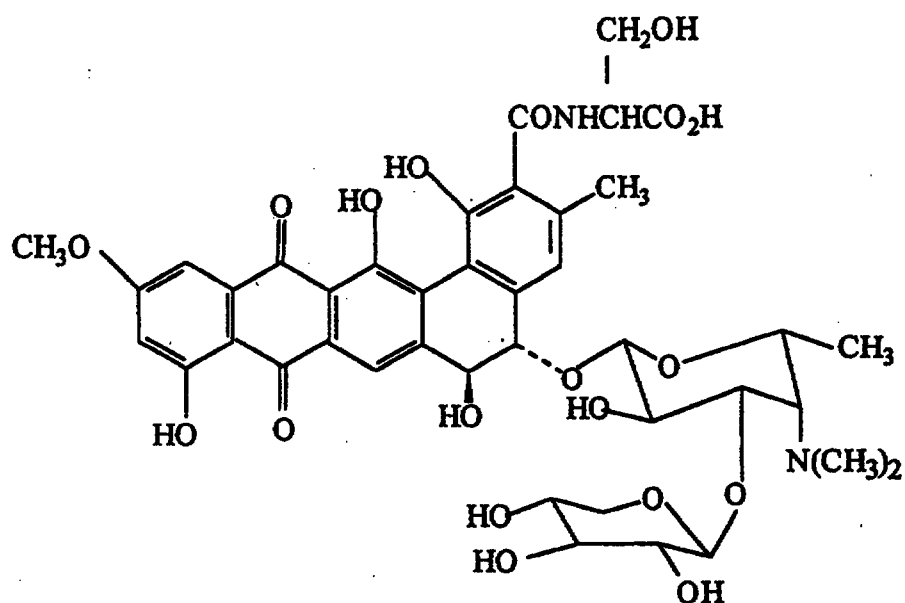
7. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.

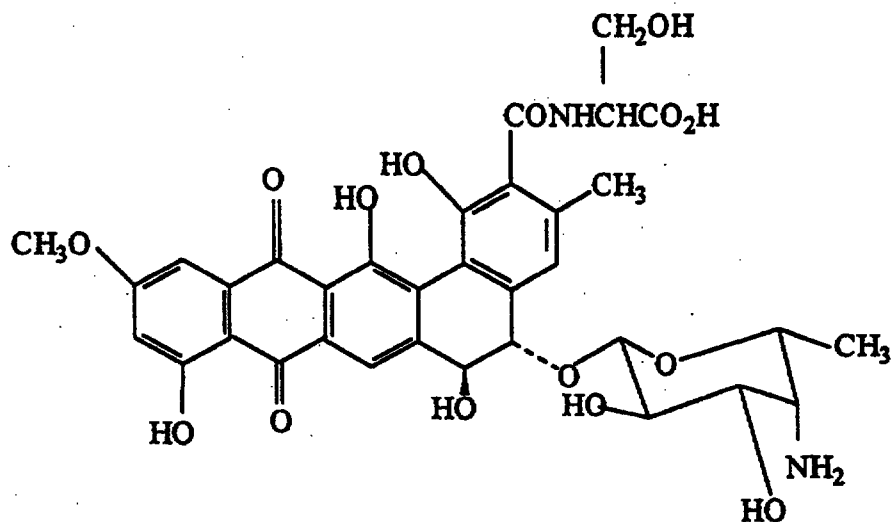


8. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.

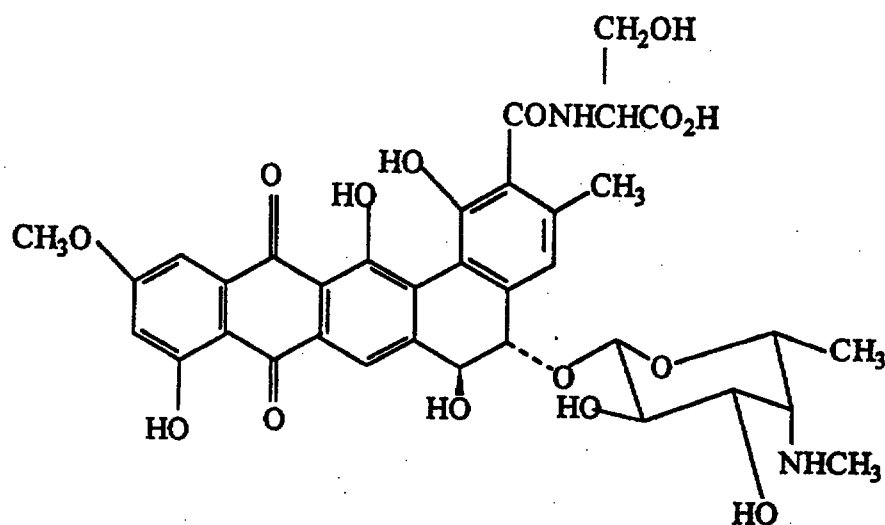
9. Composé de la revendication 1, ayant pour formule:



ou son sel acceptable en pharmacie.

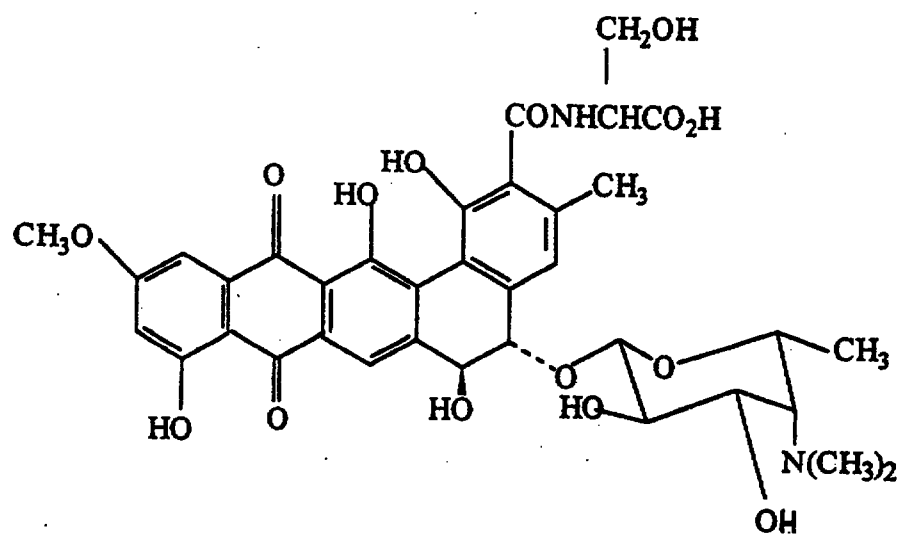


10. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.

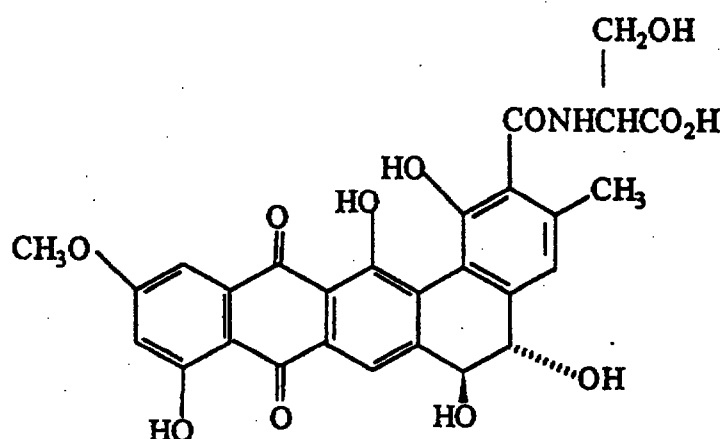
11. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.



12. Composé de la formule :



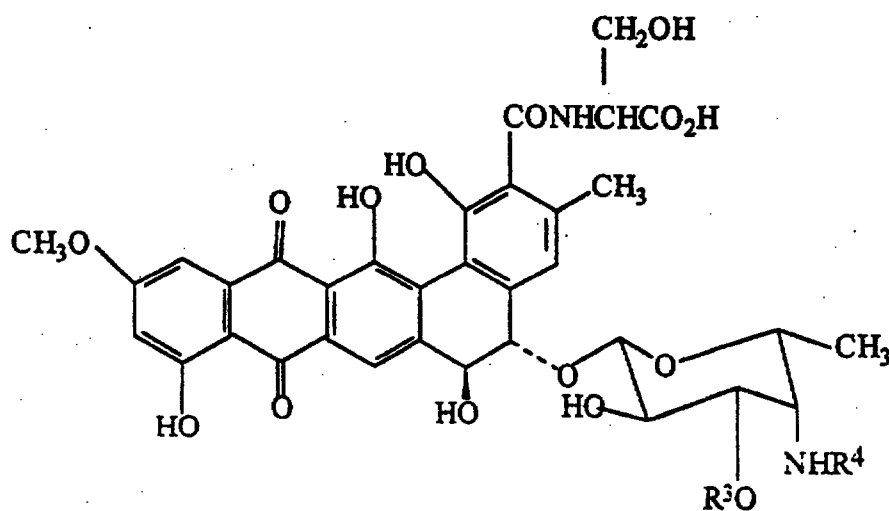
où la sérine est la D-sérine; ou son sel.

13. Procédé de préparation d'un composé de formule II selon la revendication 1, qui comprend :

A. La mise en culture d'une souche d'*Actinomadura Hibisca* capable de produire ledit antibiotique dans un milieu contenant des sources assimilables de carbone et d'azote et la D- ou DL-sérine en conditions aérobies, et la récupération dudit antibiotique du bouillon de culture afin d'obtenir un composé de formule II où R¹ est H ou méthyle, R² est H et R³ est β -D-xylosyle.

B. Si on le souhaite, le chauffage d'un composé de formule III dans un milieu acide pour obtenir le dérivé de D-xylosyle correspondant.

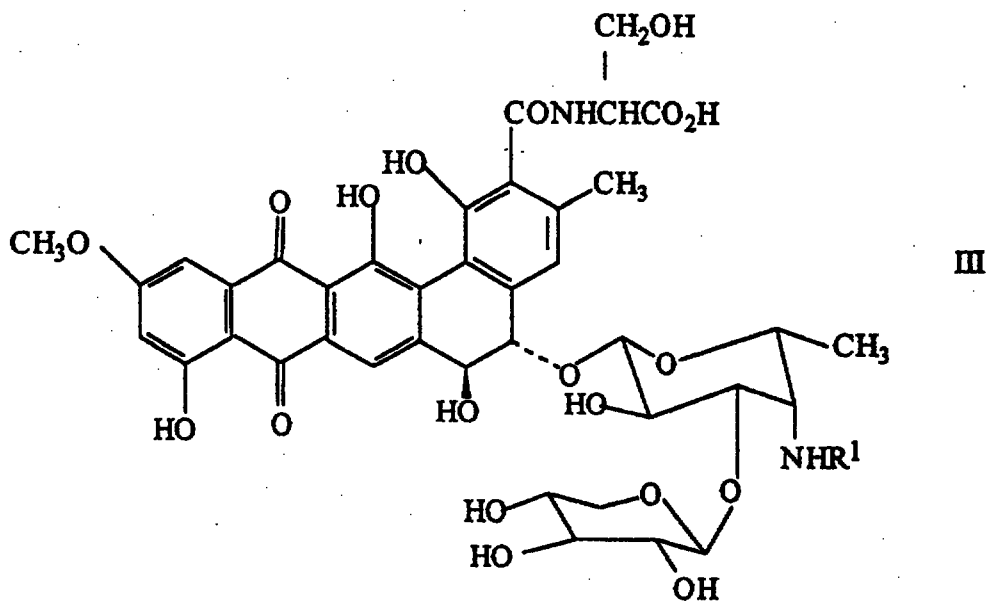
C. Si on le souhaite, la réaction d'un composé obtenu selon l'étape A ou B, qui correspond à la formule V :



où R³ est H ou β -D-xylosyle et R⁴ est H ou méthyle, ou son sel acceptable en pharmacie, avec un aldéhyde ou une cétone ayant 1 à 6 atomes de carbone pour former une imine et le traitement de l'imine avec un agent réducteur pour obtenir un composé de formule II où R¹ et R² sont indépendamment alkyle C₁₋₆ et R³ est H ou β -D-xylosyle ou bien un sel acceptable en pharmacie.

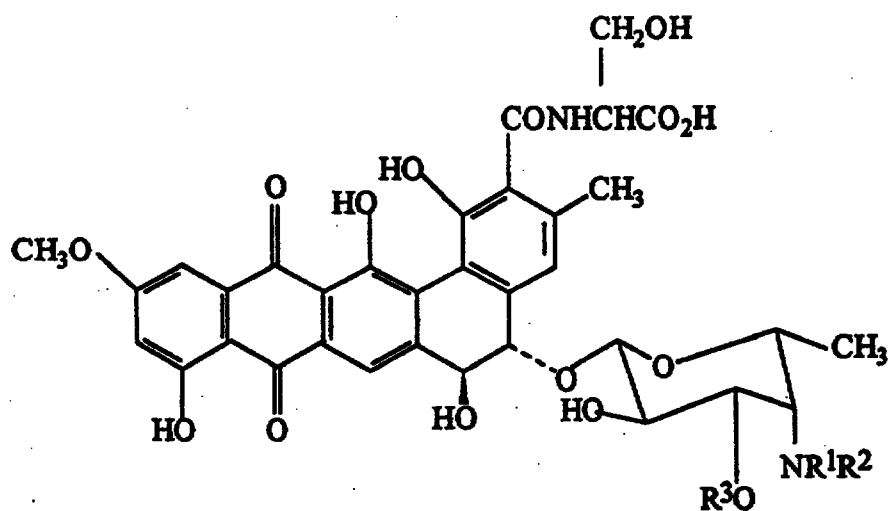


14. Procédé pour la préparation d'un antibiotique de formule III



où R¹ est H ou méthyle, qui comprend la mise en culture d'une souche d'*Actinomadura Hibisca* capable de produire ledit antibiotique dans un milieu contenant des sources assimilables de carbone et d'azote et la D- ou DL-sérine en conditions aérobies et la récupération dudit antibiotique, du bouillon de culture.

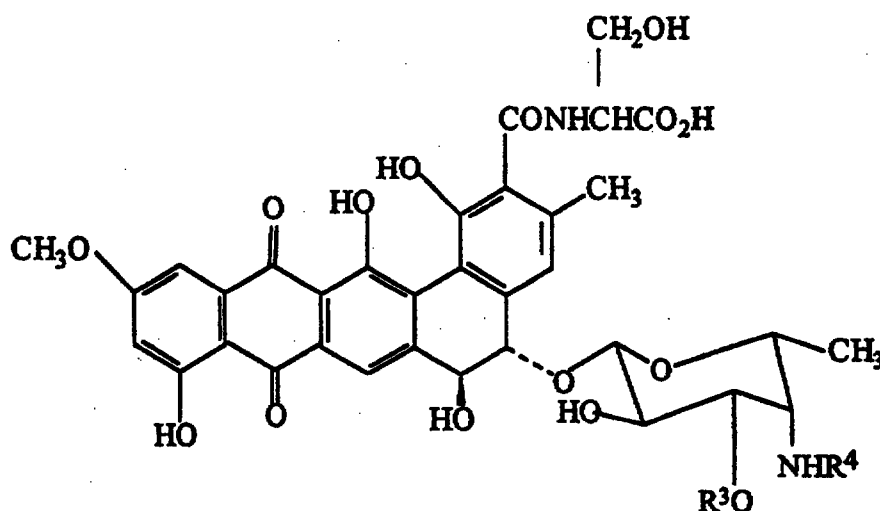
15. Procédé de préparation d'un composé ayant pour formule :



où R¹ et R² sont indépendamment alkyle C₁₋₆ ; ou bien R³ est H ou β-D-xylosyle ; ou bien un sel acceptable en pharmacie, qui comprend les étapes de :

(a) faire réagir un composé de formule :





où R^3 est H ou β -D-xylosyle et R^4 est H ou méthyle ou bien son sel acceptable en pharmacie, avec un aldéhyde ou une cétone ayant 1 à 6 atomes de carbone pour former une imine ; et (b) le traitement de l'imine de (a) avec un agent réducteur.

16. Composition pharmaceutique qui comprend au moins un composé de la revendication 1 et un support acceptable en pharmacie.

17. Procédé de préparation de la composition de la revendication 16 qui comprend l'incorporation d'au moins un composé de la revendication 1 dans un support acceptable en pharmacie.

18. Utilisation d'au moins un composé de la revendication 1 pour la préparation d'une composition pharmaceutique pour le traitement d'infections fongiques chez un hôte mammifère.

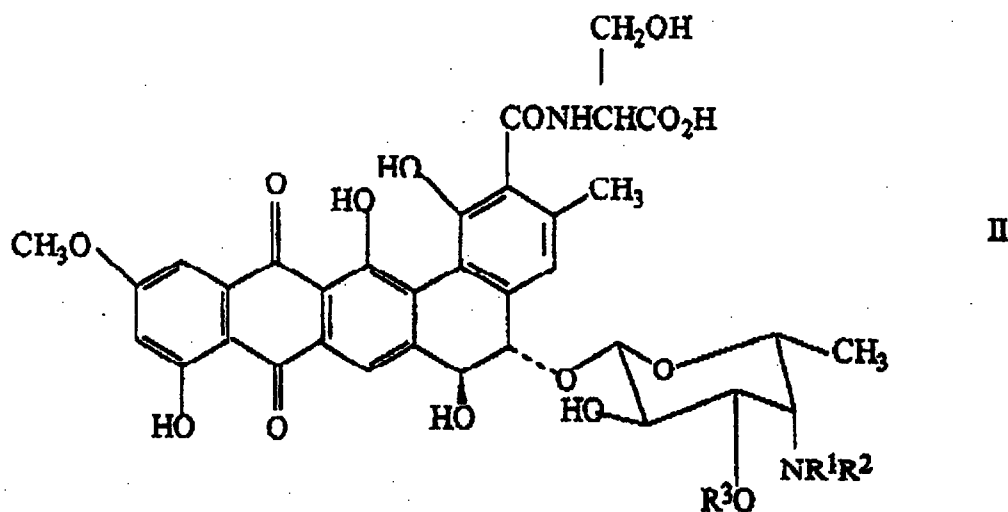
19. Culture biologiquement pure du microorganisme Actinomadura Hibisca ayant les caractéristiques d'identification ATCC 53815 et qui est capable de produire un antibiotique de la formule III lors d'une mise en culture dans un milieu aqueux contenant une source assimilable de carbone, d'azote et de la D-sérine.

20. Culture biologiquement pure du microorganisme Actinomadura Hibisca ayant les caractéristiques d'identification ATCC 53816 et qui est capable de produire un antibiotique de la formule III lors d'une mise en culture dans un milieu aqueux contenant une source assimilable de carbone, d'azote et de la D-sérine.



Revendications pour l'Etat contractant suivant : ES

1. Procédé de préparation d'un composé ayant la formule II :

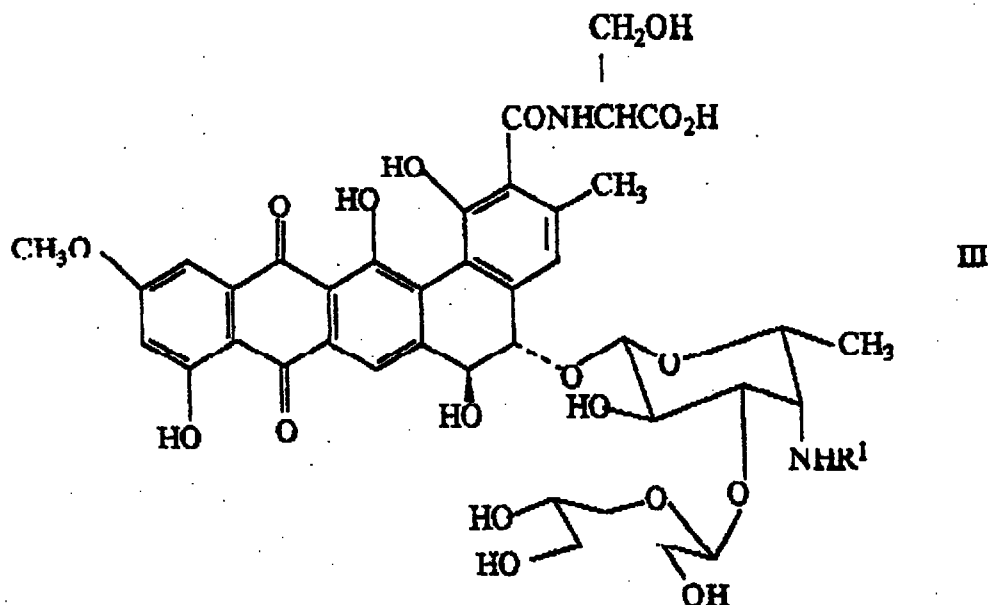


où la sérine est la D-sérine ; R^1 et R^2 sont indépendamment H ou alkyle $C_1 - 6$; et R^3 est H ou β -D-xylosyle ; ou bien un sel acceptable en pharmacie.

qui comprend :

A. La mise en culture d'une souche d'*Actinomadura Hibisca* capable de produire ledit antibiotique dans un milieu contenant des sources assimilables de carbone et d'azote et la D- ou DL-sérine en conditions aérobies, et la récupération dudit antibiotique du bouillon de culture pour obtenir un composé de formule II où R^1 est H ou méthyle, R^2 est H et R^3 est β -D-xylosyle ;

B. Si on le souhaite, le chauffage d'un composé de formule III

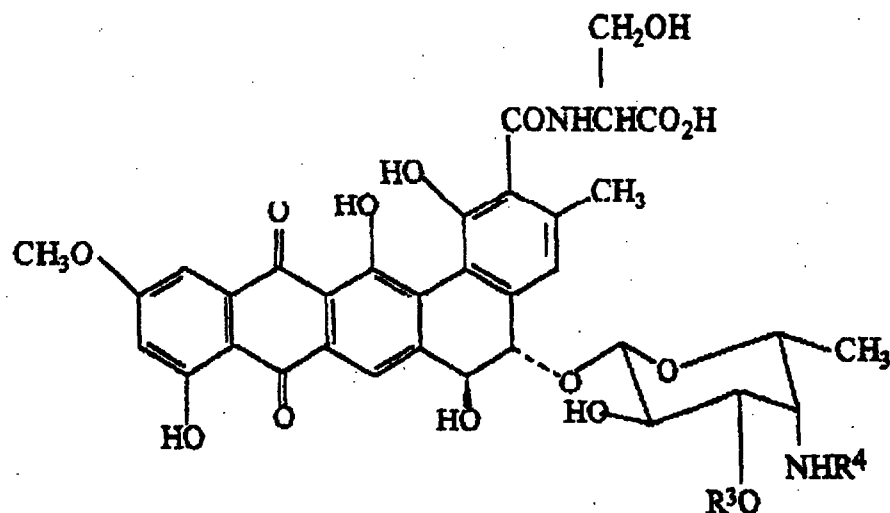


où R^1 est H ou méthyle,

dans un milieu acide pour obtenir le dérivé de D-xylosyle correspondant.

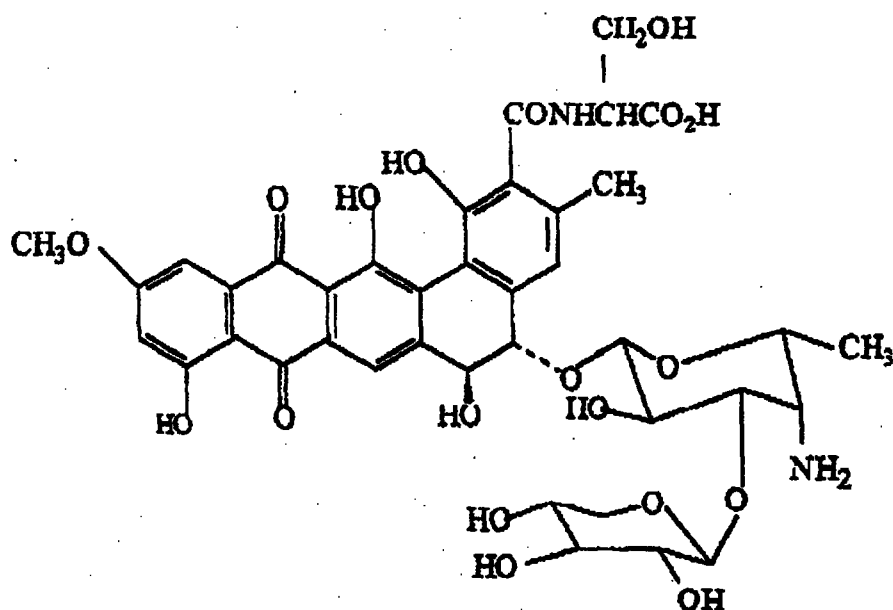
C. Si on le souhaite, la réaction d'un composé obtenu selon l'étape A ou B, qui correspond à la formule V





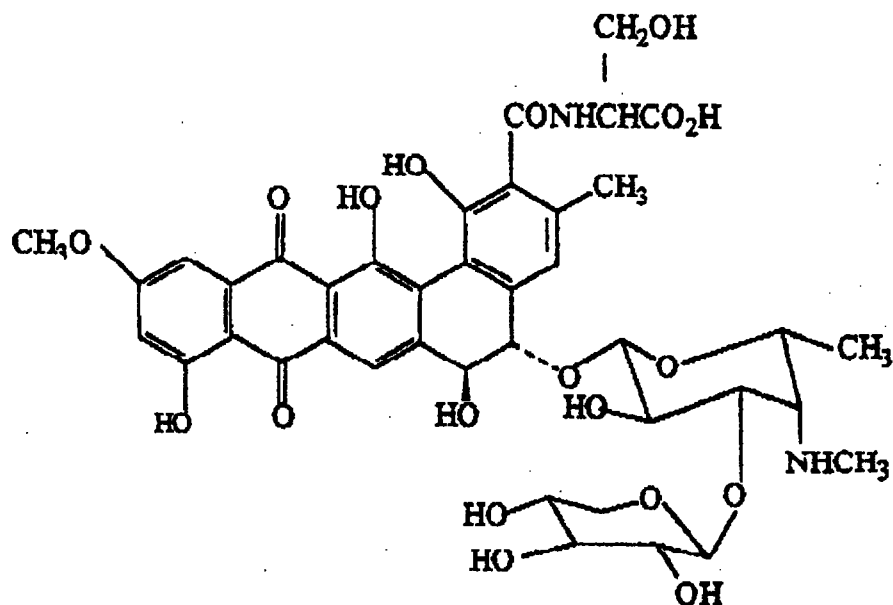
20 où R^3 est H ou β -D-xylosyle et R^4 est H ou méthyle, ou son sel acceptable en pharmacie, avec un aldéhyde ou une cétone ayant 1 à 6 atomes de carbone pour former une imine et le traitement de l'imine avec un agent réducteur pour obtenir un composé de formule II, où R^1 et R^2 sont indépendamment alkyle C_{1-6} et R^3 est H ou β -D-xylosyle ou un sel acceptable en pharmacie.

- 25 2. Procédé selon la revendication 1 où R^3 est β -D-xylosyle, ou un sel acceptable en pharmacie.
3. Procédé selon la revendication 1 où R^3 est H, ou un sel acceptable en pharmacie.
- 30 4. Procédé selon la revendication 1 où R^1 est H ou méthyle et R^2 est H ou méthyle ou bien un sel acceptable en pharmacie.
5. Procédé selon la revendication 1 où R^1 et R^2 sont indépendamment alkyle C_{2-6} ; ou bien R^1 est H ou méthyle et R^2 est alkyle C_{2-6} ou bien un sel acceptable en pharmacie.
- 35 6. Procédé selon la revendication 1 où on obtient un composé de la formule :



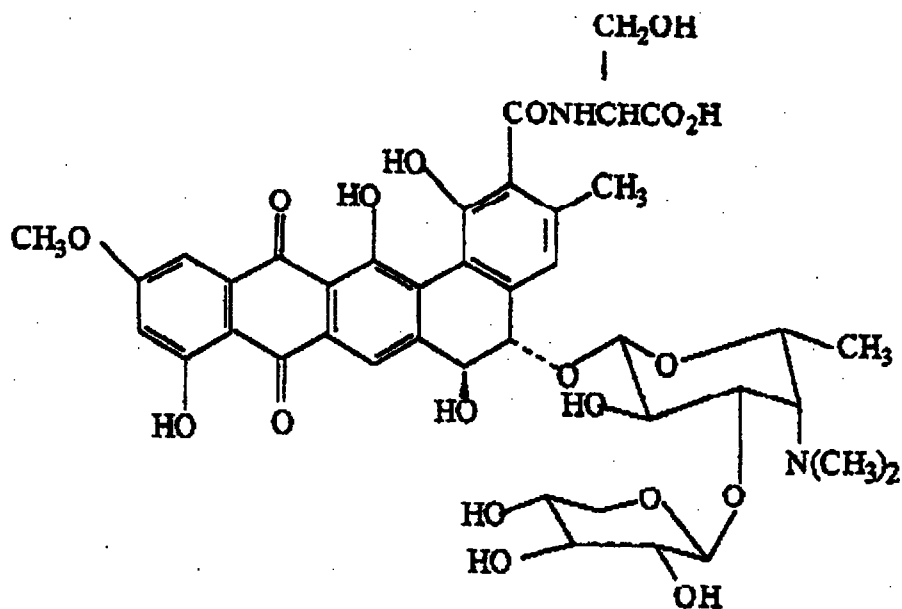
ou son sel acceptable en pharmacie.

7. Procédé selon la revendication 1, où on obtient un composé de la formule:



ou son sel acceptable en pharmacie.

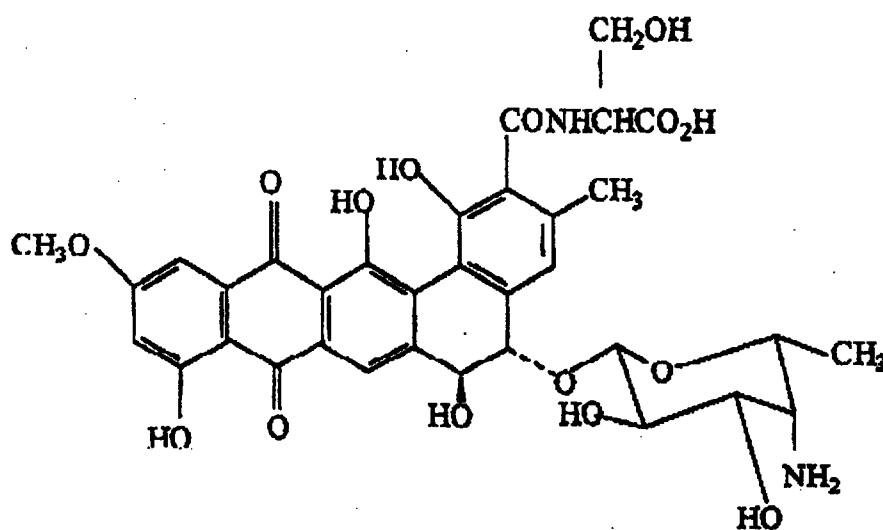
8. Procédé selon la revendication 1, où on obtient un composé de la formule:



ou son sel acceptable en pharmacie.

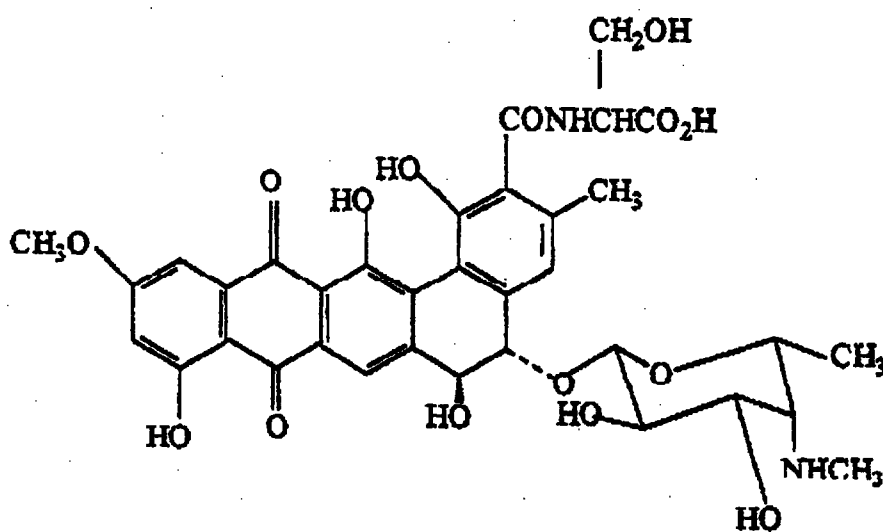


9. Procédé selon la revendication 1, où on obtient un composé de la formule:



ou son sel acceptable en pharmacie.

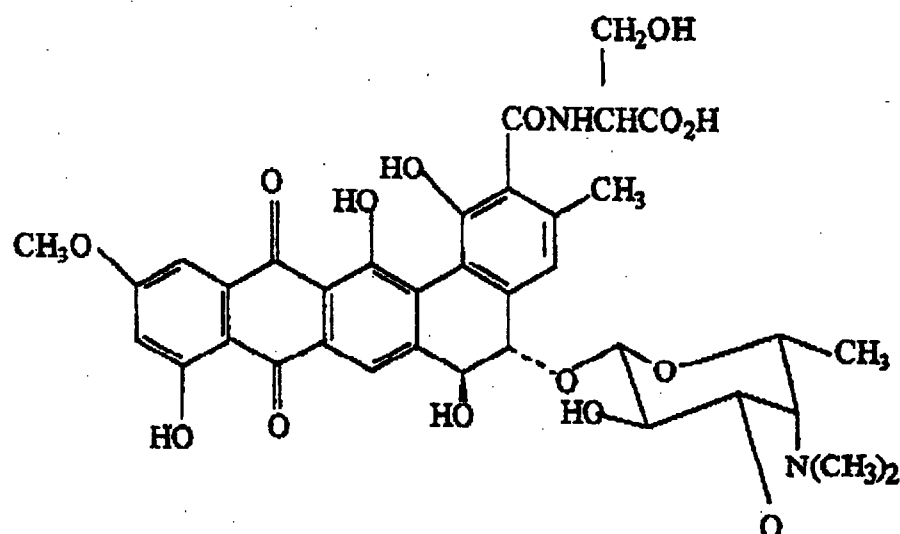
10. Procédé selon la revendication 1, où on obtient un composé de la formule :



ou son sel acceptable en pharmacie.

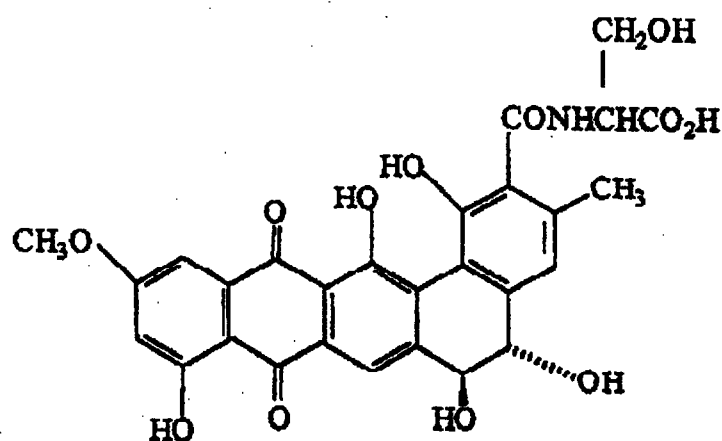


11. Procédé selon la revendication 1, où on obtient un composé de la formule:



ou son sel acceptable en pharmacie.

12. Procédé de préparation d'un composé de la formule :

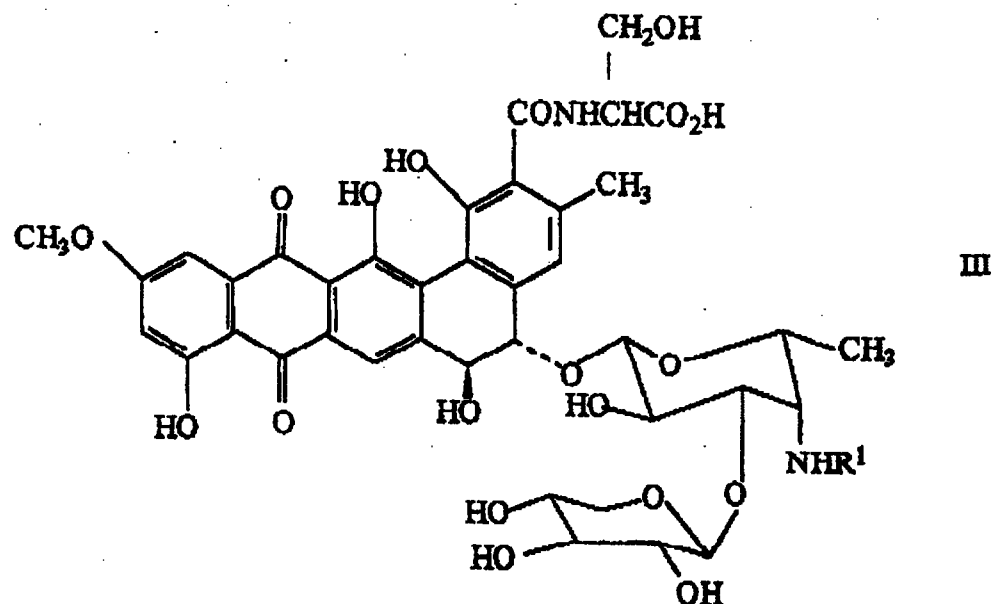


IV

où la sérine est la D-sérine ; ou son sel, qui comprend l'hydrolyse acide du composé de formule II
où R³ est β -D-xylosyle et R¹ et R² sont méthyle.

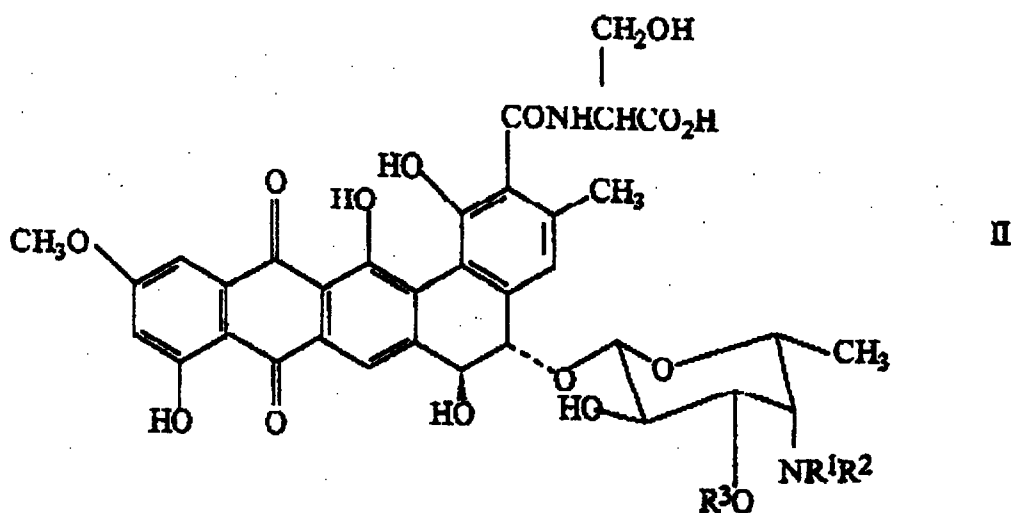


13. Procédé de préparation d'un antibiotique de formule III



25 où R¹ est H ou méthyle, qui comprend la mise en culture d'une souche d'*Actinomadura Hibisca* capable de produire ledit antibiotique dans un milieu contenant des sources assimilables de carbone et d'azote et la S- ou DL-sérine en conditions aérobies, et la récupération dudit antibiotique du bouillon de culture.

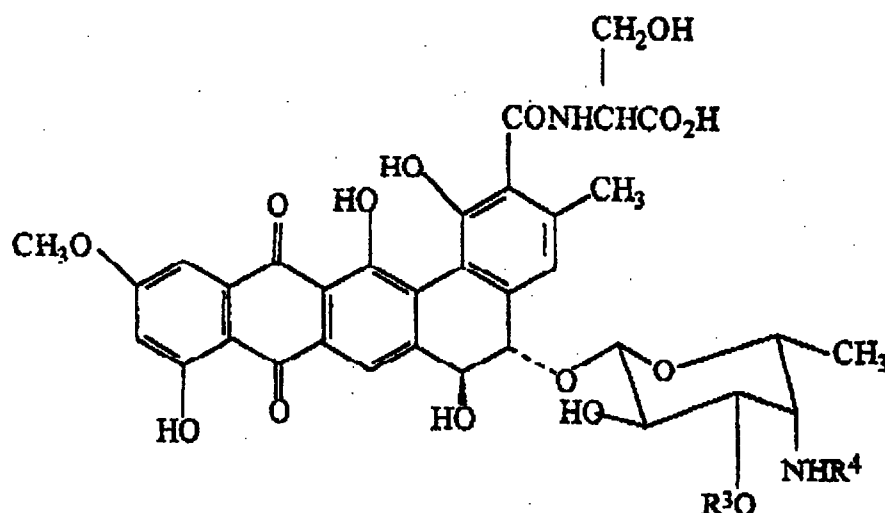
14. Procédé de préparation d'un composé ayant pour formule :



55 où R¹ et R² sont indépendamment alkyle C₁₋₆ ; ou bien R³ est H ou β-D-xylosyle ; ou bien un sel acceptable en pharmacie qui comprend les étapes de:

(a) Faire réagir un composé de formule :





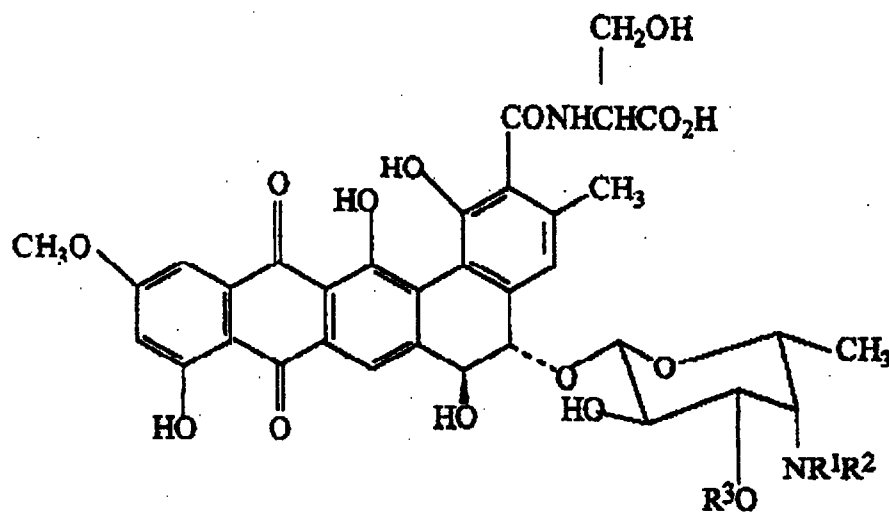
où R^3 est H ou β -D-xylosyle et R^4 est H ou méthyle ou bien un sel acceptable en pharmacie, avec un aldéhyde ou une cétone ayant 1 à 6 atomes de carbone pour former une imine ; et (b) le traitement de l'imine de (a) avec un agent réducteur.

15. Procédé de préparation d'une composition pharmaceutique qui comprend l'incorporation d'au moins un composé tel que défini à la revendication 1 dans un support acceptable en pharmacie.
16. Utilisation d'au moins un composé de la revendication 1 pour la préparation d'une composition pharmaceutique pour le traitement d'infections fongiques chez un hôte mammifère.
17. Culture biologiquement pure du microorganisme Actinomadura Hibisca ayant les caractéristiques d'identification ATCC 53815 et qui est capable de produire un antibiotique de la formule III lors d'une mise en culture dans un milieu aqueux contenant une source assimilable de carbone, d'azote et de la D-sérine.
18. Culture biologiquement pure du microorganisme Actinomadura Hibisca ayant les caractéristiques d'identification ATCC 53816 et qui est capable de produire un antibiotique de la formule III lors d'une mise en culture dans un milieu aqueux contenant une source assimilable de carbone, d'azote et de la D-sérine.



Revendications pour l'Etat contractant suivant : GR

1. Composé ayant la formule II :

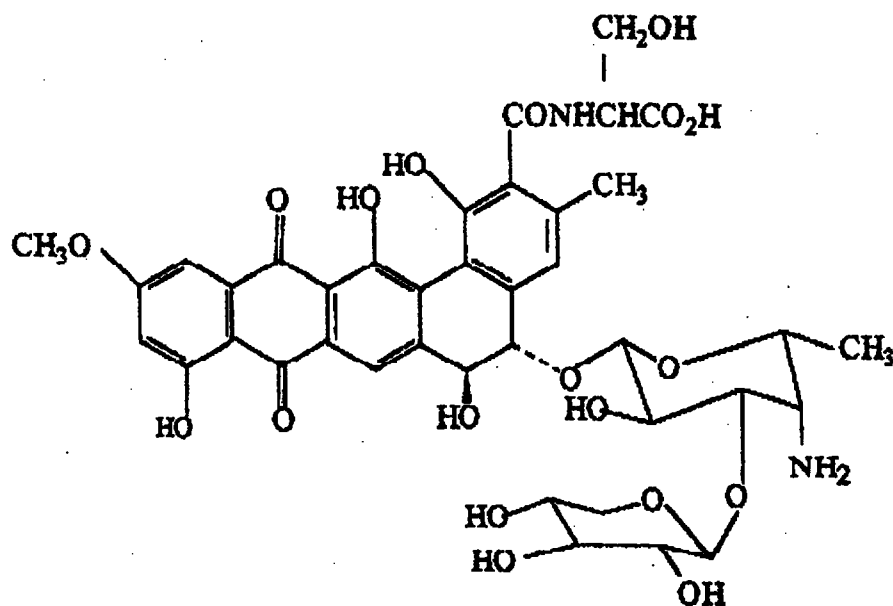


où la sérine est la D-sérine ; R^1 et R^2 sont indépendamment H ou alkyle C_{1-6} ; et R^3 est H ou β -D-xylosyle ; ou bien un sel acceptable en pharmacie.

2. Composé de la revendication 1 où R^3 est β -D-xylosyle, ou un sel acceptable en pharmacie.
3. Composé de la revendication 1 où R^3 est H, ou un sel acceptable en pharmacie.
4. Composé de la revendication 1 où R^1 est H ou méthyle et R^2 est H ou méthyle ou bien un sel acceptable en pharmacie.
5. Composé de la revendication 1 où R^1 et R^2 sont indépendamment alkyle C_{2-6} ; ou bien R^1 est H ou méthyle et R^2 est alkyle C_{2-6} ou bien un sel acceptable en pharmacie.

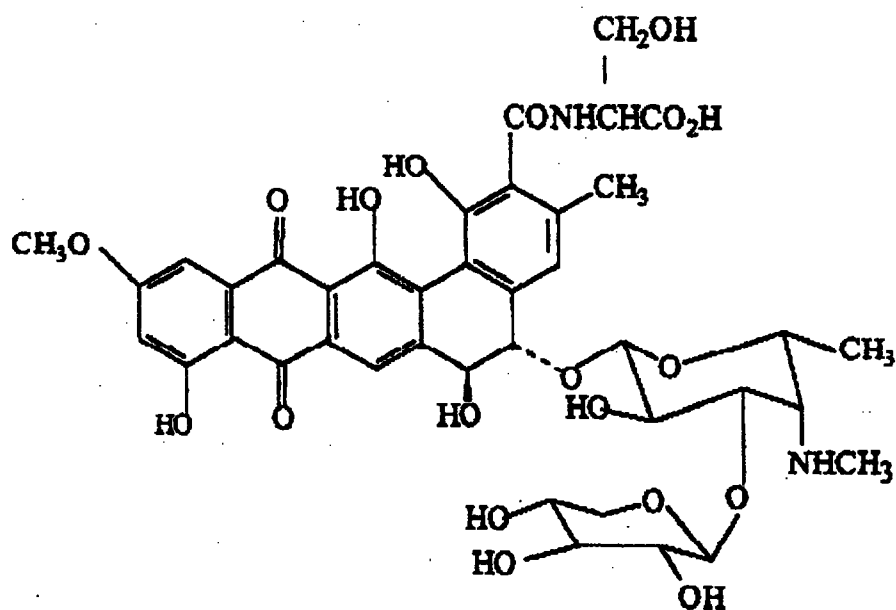


6. Composé de la revendication 1 ayant pour formule :



ou son sel acceptable en pharmacie.

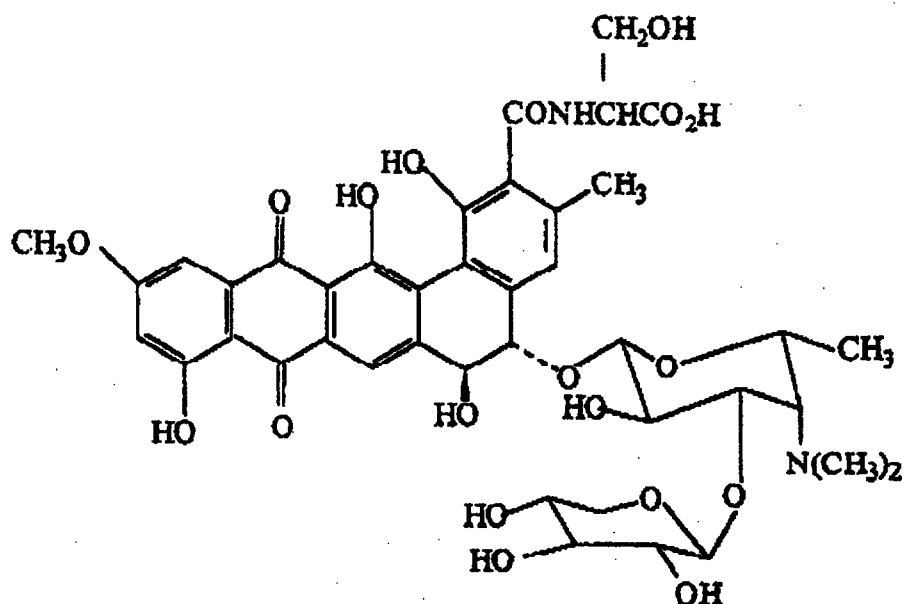
7. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.

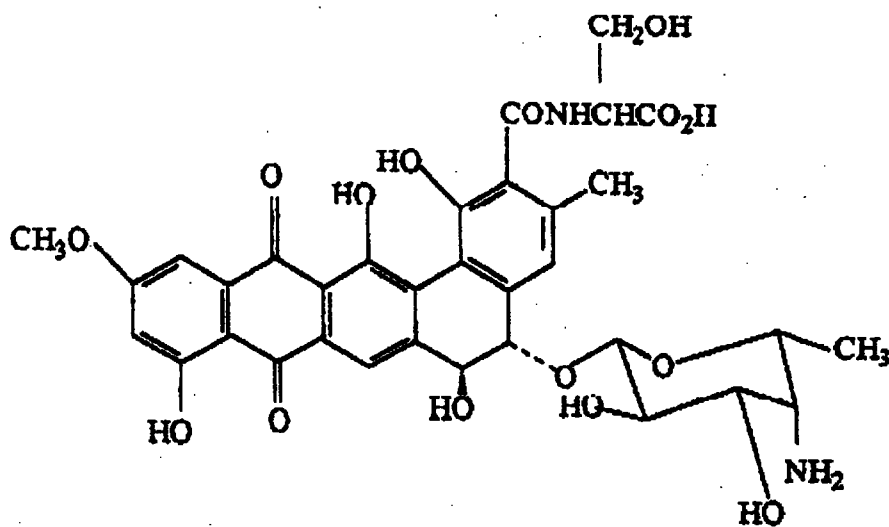


8. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.

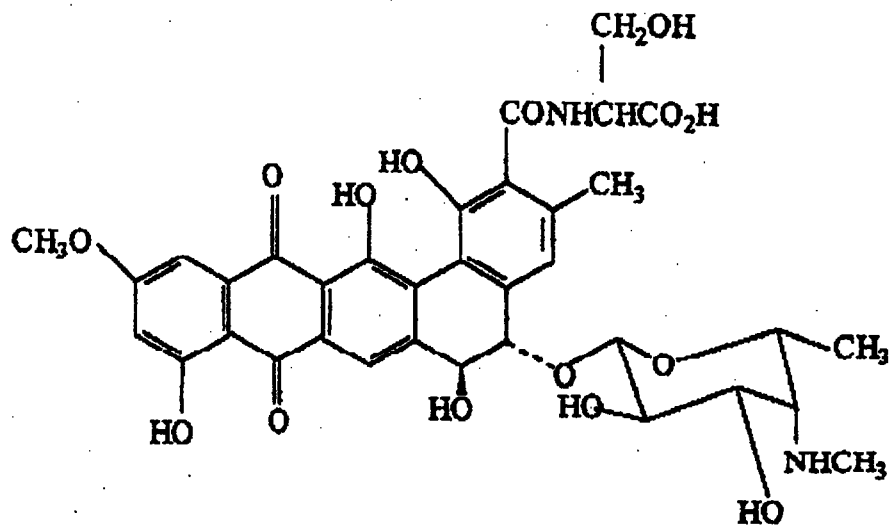
9. Composé de la revendication 1, ayant pour formule:



ou son sel acceptable en pharmacie.

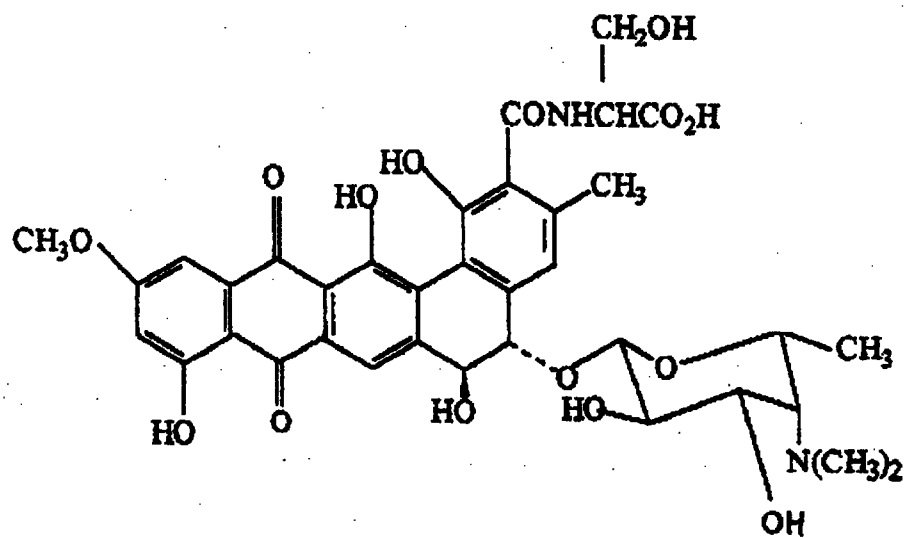


10. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.

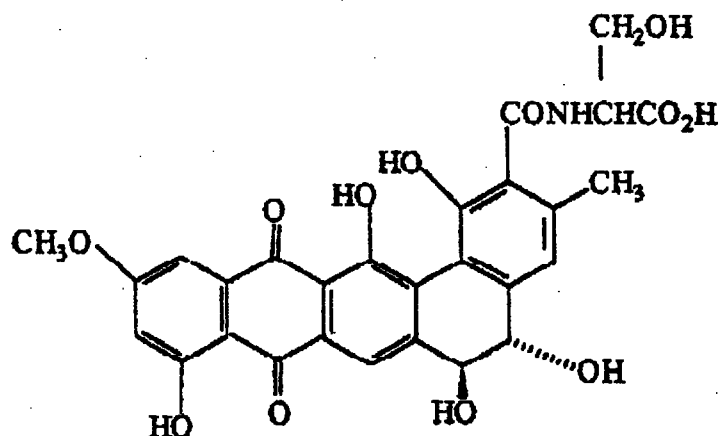
11. Composé de la revendication 1, ayant pour formule :



ou son sel acceptable en pharmacie.



12. Composé de la formule :



IV

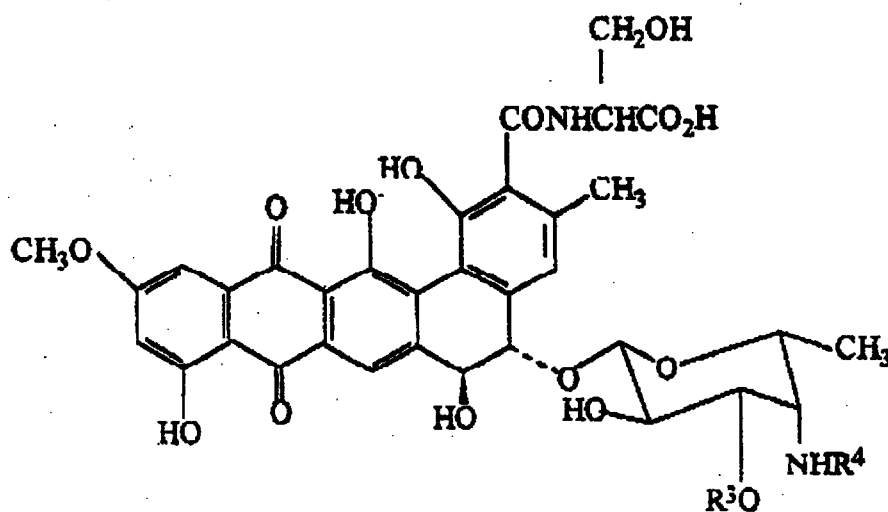
où la sérine est la D-sérine ; ou son sel.

13. Procédé de préparation d'un composé de formule II selon la revendication 1, qui comprend :

A. La mise en culture d'une souche d'*Actinomadura Hibisca* capable de produire ledit antibiotique dans un milieu contenant des sources assimilables de carbone et d'azote et la D- ou DL-sérine en conditions aérobies, et la récupération dudit antibiotique du bouillon de culture afin d'obtenir un composé de formule II où R¹ est H ou méthyle, R² est H et R³ est β-D-xylosyle.

B. Si on le souhaite, le chauffage d'un composé de formule III dans un milieu acide pour obtenir le dérivé de D-xylosyle correspondant.

C. Si on le souhaite, la réaction d'un composé obtenu selon l'étape A ou B, qui correspond à la formule V :

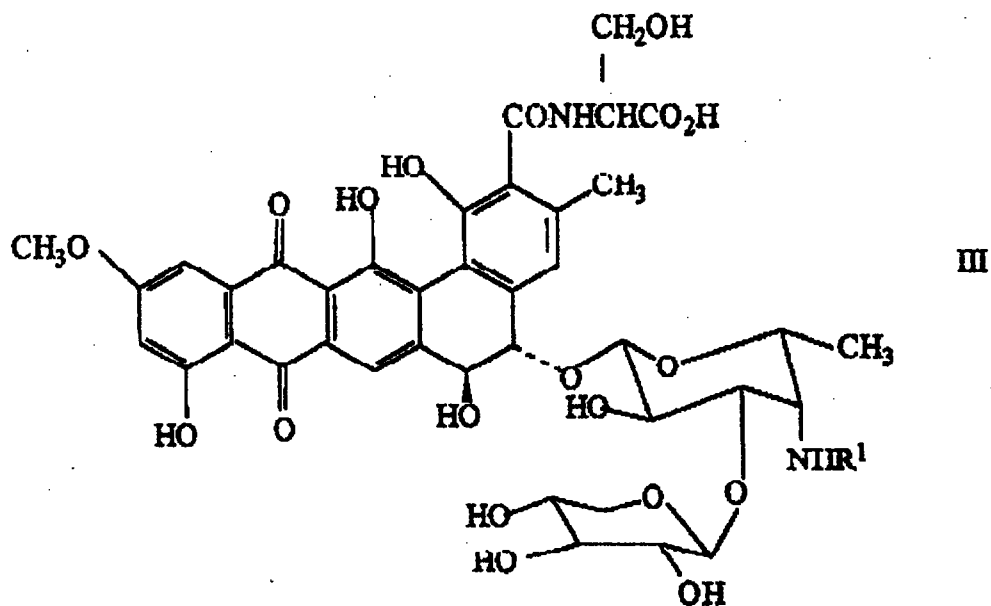


V

où R³ est H ou β-D-xylosyle et R⁴ est H ou méthyle, ou son sel acceptable en pharmacie, avec un aldéhyde ou une cétone ayant 1 à 6 atomes de carbone pour former une imine et le traitement de l'imine avec un agent réducteur pour obtenir un composé de formule II où R¹ et R² sont indépendamment alkyle C₁₋₆ et R³ est H ou β-D-xylosyle ou bien un sel acceptable en pharmacie.

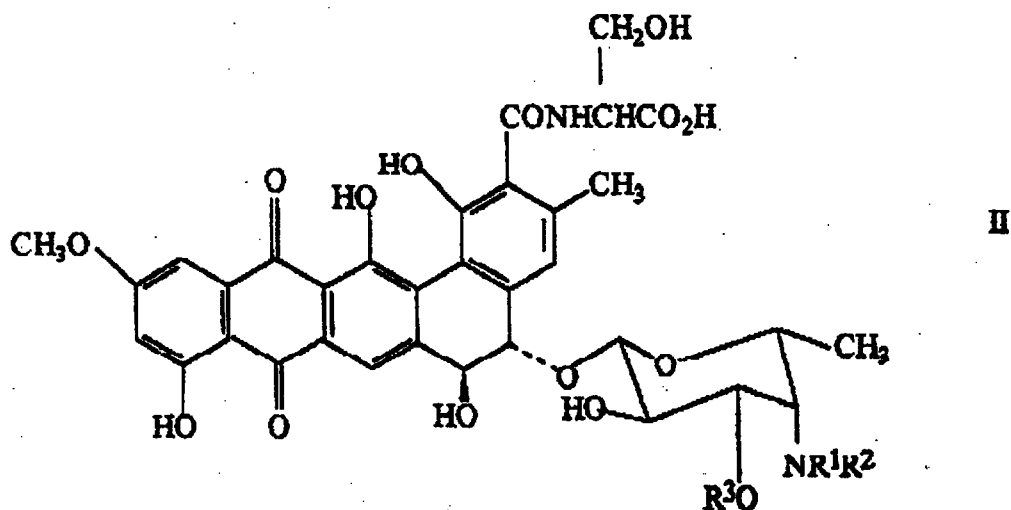


14. Procédé pour la préparation d'un antibiotique de formule III



où R¹ est H ou méthyle, qui comprend la mise en culture d'une souche d'*Actinomadura Hibisca* capable de produire ledit antibiotique dans un milieu contenant des sources assimilables de carbone et d'azote et la D- ou DL-sérine en conditions aérobies et la récupération dudit antibiotique, du bouillon de culture.

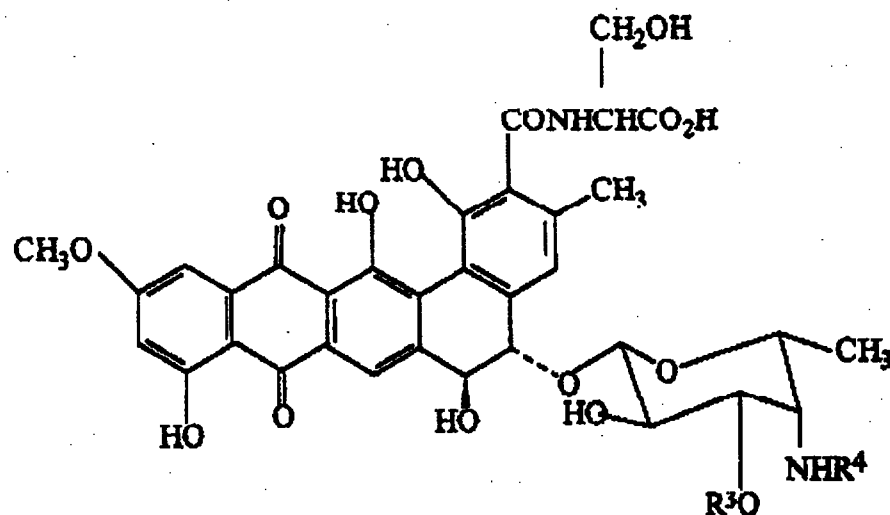
15. Procédé de préparation d'un composé ayant pour formule :



où R¹ et R² sont indépendamment alkyle C₁₋₆ ; ou bien R³ est H ou β-D-xylosyle ; ou bien un sel acceptable en pharmacie, qui comprend les étapes de:

(a) faire réagir un composé de formule :





où R^3 est H ou β -D-xylosyle et R^4 est H ou méthyle ou bien son sel acceptable en pharmacie avec un aldéhyde ou une cétone ayant 1 à 6 atomes de carbone pour former une imine ; et (b) le traitement de l'imine de (a) avec un agent réducteur.

16. Procédé de préparation d'une composition pharmaceutique qui comprend l'incorporation d'au moins un composé de la revendication 1 dans un support acceptable en pharmacie.
17. Utilisation d'au moins un composé de la revendication 1 pour la préparation d'une composition pharmaceutique pour le traitement des infections fongiques chez un hôte mammifère.
18. Culture biologiquement pure du microorganisme Actinomadura Hibisca ayant les caractéristiques d'identification ATCC 53815 et qui est capable de produire un antibiotique de la formule III lors d'une mise en culture dans un milieu aqueux contenant une source assimilable de carbone, d'azote et de la D-sérine.
19. Culture biologiquement pure du microorganisme Actinomadura Hibisca ayant les caractéristiques d'identification ATCC 53816 et qui est capable de produire un antibiotique de la formule III lors d'une mise en culture dans un milieu aqueux contenant une source assimilable de carbone, d'azote et de la D-sérine.