SULTRIECIN†, A NEW ANTIFUNGAL AND ANTITUMOR ANTIBIOTIC FROM *Streptomyces roseiscleroticus*

PRODUCTION, ISOLATION, STRUCTURE AND BIOLOGICAL ACTIVITY

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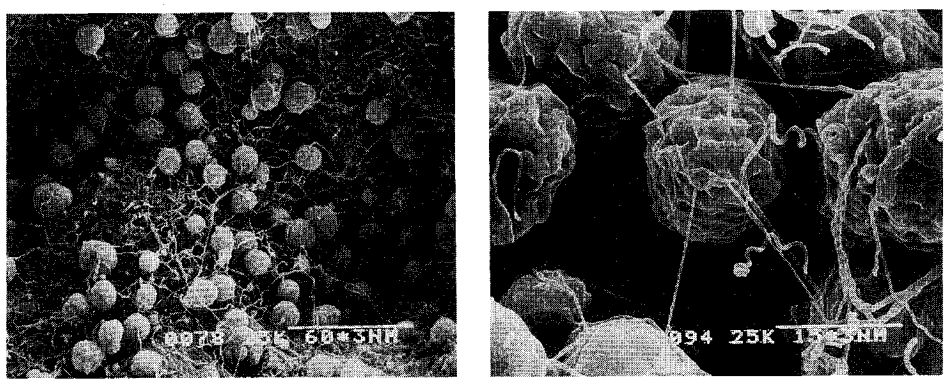
Streptomyces roseiscleroticus L827-7 (ATCC 53903) produced a novel antifungal and antitumor antibiotic, sultriecin. It exhibited *in vitro* antifungal activity and potent *in vivo* antitumor activity against P388 and L1210 leukemias, and B16 melanoma. Sultriecin is composed of several unique structural units; a conjugated triene, an α,β-unsaturated δ-lactone, and a sulfate functionality.

In our continuing search for new microbial metabolites with antitumor activity, *Streptomyces roseiscleroticus* No. L827-7, isolated from a soil sample of Gujarat State in India, was found to produce a novel antibiotic with potent *in vivo* activity against P388 and L1210 leukemias, and B16 melanoma. The active principle, sultriecin[1,2] was recovered from the fermentation broth with *n*-butanol extraction and purified by chromatography. Pentalenolactone[3,4], aburamycin[5,6] and chromomycins[7,8] were isolated as co-products from the crude extract. Structural studies by spectroscopic analysis of sultriecin and its desulfated derivative, revealed that sultriecin was 5,6-dihydro-5-hydroxy-6-(6-hydroxy-5-methyl-4-hydroxysulfonyloxyheptadec-1,7,9,11-tetraenyl)-2*H*-pyran-2-one (monosodium salt). In this paper, we describe the production, isolation, physico-chemical properties, structural determination and biological properties of sultriecin.

Fig. 1. Sclerotia of strain L827-7 grown on yeast extract-malt extract agar, 28°C for 20 days.

A B



† Sultriecin was originally called BU-3285T.

Taxonomy of the Producing Organism

Morphology: The aerial mycelium was scantily or not formed on agar media, and when formed, it bore compact or open spiral spore chains (20~50 spores per chain). The spores were oval or oblong (0.6~0.8×1.2 μm), and had a smooth surface (Fig. 2).

The substrate mycelium was well branched and not fragmented. In the substrate mycelium, a balloon-like, semi-transparent body (3~10 μm) with a phorous hypha was numerously born and became a sclerotic globular body (10~30 μm). Scanning electron microscopy showed that the globular surface waved and had no membrane (Fig. 1, A and B). Thin section micrography indicated the globules envelop irregularly coiled and partially swollen hyphae which were cemented with an extracellular metabolite.

Cultural and physiological characteristics: The aerial mycelium was not formed on most agar media, but was seen scantily on ISP media Nos. 4 and 7. The reverse color of the substrate mycelium was deep orange to dark reddish brown. Melanin was not produced. Growth occurred between 18°C and 45°C (Tables 1 and 2).

Cell chemistry: The amino acids, sugars and phospholipids in the whole cell hydrolysate were analyzed by the methods of LECHEVALIER[9,10]. The whole cell hydrolysate was found to contain LL-2,6-diaminopimelic acid and mannose which indicated the cell wall-type I. The phospholipids detected were phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol placing the strain in pattern P-II. The hydrolysate of globular bodies contained 2,3-diaminopropionic acid[11].

Taxonomic position: The globular bodies are identified as a sclerotium which is described in *Chainia*[11,12]. The morphology and cell chemistry

Fig. 2. Spore chain of strain L827-7 grown on tyrosine agar, 28°C for 20 days.

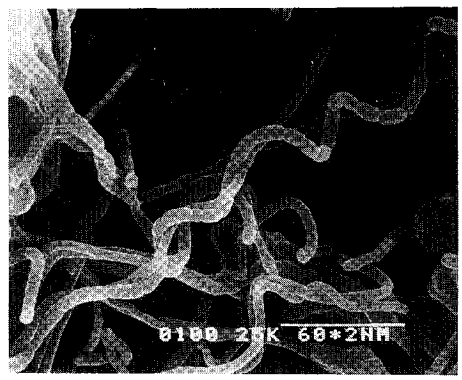


Table 1. Cultural characteristics of strain L827-7.

Medium	Growth	Aerial mycelium	Reverse color	Diffusible pigment
Sucrose-nitrate agar (Czapek-Dox agar)	Good	None	Moderate reddish-brown (43)	None
Tryptone-yeast extract broth (ISP No. 1)	Moderate, not turbid	None	Colorless	None
Yeast extract-malt extract agar (ISP No. 2)	Good	None	Deep orange (51)	Dark orange yellow (72)
Oatmeal agar (ISP No. 3)	Moderate	None	Moderate yellow (87)	Grayish yellow (90)
Inorganic salts-starch agar (ISP No. 4)	Moderate	Poor; white	Brownish orange (54)	None
Glycerol-asparagine agar (ISP No. 5)	Moderate	None	Dark reddish brown (44)	Light reddish brown (42)
Peptone-yeast extract-iron agar (ISP No. 6)	Poor	None	Colorless	None
Tyrosine agar (ISP No. 7)	Moderate	Scant; white	Dark brown (59)	Moderate brown (58)
Glucose-asparagine agar	Poor	None	Strong yellow (84)	None

Observation after incubation at 28°C for 3 weeks.
Color name in parentheses used: ISCC-NBS color name charts.

Table 2. Physiological characteristics of strain L827-7.

Hydrolysis of:		Lactose	+
Gelatin	+	Cellobiose	+
Starch	+	Melibiose	−
Casein	+	Trehalose	+
Production of:		Raffinose	−
Nitrate reductase	−	D-Melezitose	−
Tyrosinase	−	Soluble starch	+
Urease	+	Dulcitol	−
Tolerance to:		*i*-Inositol	+
Lysozyme 0.01% (w/v)	−	D-Mannitol	+
NaCl 0~7% (w/v)	+	D-Sorbitol	−
8% (w/v)	−	Salicin	+
pH 5.0	+	Benzoate	−
Temperature:		Oxalate	+
Growth range	18°C~45°C	Tartrate	−
Optimal growth	35°C~40°C	Acid from:	
No growth	14°C & 48°C	Adonitol	−
Utilization of:		Dulcitol	−
Glycerol	+	*i*-Erythritol	−
D-Arabinose	−	*i*-Inositol	+
L-Arabinose	+	Lactose	+
D-Xylose	+	D-Mannitol	+
D-Ribose	+	D-Melibiose	+
D-Rhamnose	+	α-Methylglucoside	+
D-Glucose	+	Raffinose	−
D-Galactose	+	L-Rhamnose	+
D-Fructose	+	D-Sorbitol	−
D-Mannose	+	Sucrose	−
L-Sorbose	−	D-Xylose	+
Sucrose	+		

Basal medium: PRIDHAM-GOTTLIEB's inorganic medium (ISP medium No. 9).

revealed that strain L827-7 belongs to the sclerotium-forming species of genus *Streptomyces*. Its cultural and physiological characteristics (Tables 1 and 2) are similar to those of *Streptomyces flaviscleroticus*[12,13], *Streptomyces minutiscleroticus*[12,13], *Streptomyces roseiscleroticus*[12,13] and *Streptomyces ruber*[12,13]. Physiological comparisons of strain L827-7 with the type strain of these species showed that it is accordant with *S. roseiscleroticus* Pridham IFO 13363^T (ISP 5303^T), although the latter forms aerial mycelium and reddish brown pigment more abundantly. Thus, strain L827-7 was identified with *Streptomyces roseiscleroticus*, and has been deposited with the American Type Culture Collection, Rockville, Maryland under the accession number ATCC 53903.

Antibiotic Production

A well grown agar slant of *S. roseiscleroticus* strain L827-7 was used to inoculate a vegetative medium (100 ml in a 500-ml flask) consisting of malt extract (Oriental) 2%, corn steep liquor (Oji Corn Starch Co.) 2%, NaCl 0.5%, K_2HPO_4 0.02% and $CaCO_3$ 0.2%, the pH being adjusted to 7.0 before sterilization. The medium was incubated at 28°C for 3 days on a rotary shaker (200 rpm) and 5 ml of the growth was transferred into a 500-ml Erlenmeyer flask containing 100 ml of the production medium having the same composition as the vegetative medium. The fermentation was carried out at 28°C for 4 days with shaking on a rotary shaker. The antibiotic production in the fermentation broth was determined by the paper disc-agar diffusion method using *Candida albicans* A9540 and *Cryptococcus neoformans* IAM 4514 as the

test organisms. The fermentation was also carried out in a tank fermenter. A 2-liter portion of the vegetative culture prepared by the flask fermentation was transferred into a 200-liter tank fermenter containing 120 liters of the production medium. Fermentation was run at 28°C with agitation at 250 rpm and aeration rate of 120 liters per minute. The antibiotic production reached a maximum of 30 μg/ml after about 70 hours fermentation.

Isolation and Purification

Sultriecin was extracted from the fermentation broth with *n*-butanol and precipitated from the concentrated extract by *n*-hexane. The crude solid was purified by column chromatography on Diaion HP-20, silica gel, reversed phase silica gel and Sephadex LH-20 using paper disc assay with *C. albicans* A9540 and TLC (SiO_2, *n*-BuOH - AcOH - H_2O (4 : 1 : 1), detection by I_2). The detailed purification and isolation procedures are described in the Experimental section.

Physico-chemical Properties

The physico-chemical properties of sultriecin are summarized in Table 3 together with those of its desulfated derivative. Both compounds are soluble in water, methanol, ethanol and dimethyl sulfoxide, slightly soluble in acetone and ethyl acetate, but practically insoluble in chloroform and *n*-hexane. They showed positive response to iodine vapor and sulfuric acid, while they were negative to ninhydrin and anthrone tests. The molecular formula of sultriecin was established as $C_{23}H_{33}O_8SNa$ by HRFAB-MS spectrum. The FAB-MS spectrum of the desulfated derivative showed the corresponding *quasi*-molecular ions at *m/z* 413 $(M+Na)^+$ and 429 $(M+K)^+$. The two antibiotics exhibited characteristic UV absorption at 260, 269 and 279 nm in methanol which did not shift in acidic and alkaline solution. The IR spectrum of sultriecin is shown in Fig. 3. The ^1H NMR spectrum (Table 4) in DMSO-d_6 exhibited two methyl, five methylene (including one nonequivalent methylene at δ 2.17 and 2.38), five methine and ten olefinic protons. The ^{13}C NMR spectrum of sultriecin (Table 4) showed 23 carbon signals which were assigned to two methyl, five methylene, five methine, ten sp^2 and one carbonyl carbon.

Structural Studies

The UV absorption maxima of sultriecin (**1**) indicated the presence of a triene group. Its IR spectrum displayed an α,β-unsaturated δ-lactone at 1720 cm^{-1} and a sulfate absorption at 1260 cm^{-1}. These spectra

Table 3. Physico-chemical properties of sultriecin and its desulfated derivative.

	Sultriecin	Desulfated sultriecin
Appearance	White amorphous powder	White amorphous powder
MP (dec)	123~125°C	223~226°C
$[\alpha]_D^{24}$ (*c* 1.0, MeOH)	+23°	—
UV λ_{max} nm (ε) in MeOH	260 (25,600), 269 (31,600), 279 (24,400)	260 (25,300), 270 (31,500), 280 (24,600)
Molecular formula	$C_{23}H_{33}O_8SNa$	$C_{23}H_{34}O_5$
FAB-MS (*m/z*)	493 $(M+H)^+$, 515 $(M+Na)^+$, 531 $(M+K)^+$	413 $(M+Na)^+$, 429 $(M+K)^+$
HRFAB-MS:	515.1692 $(M+Na)^+$ (Calcd for $C_{23}H_{33}O_8SNa_2$ *m/z* 515.1714)	
TLC, SiO_2 (*n*-BuOH - AcOH - H_2O = 4 : 1 : 1 v/v)	Rf 0.42	Rf 0.70

Fig. 3. IR spectrum of sultriecin (KBr).

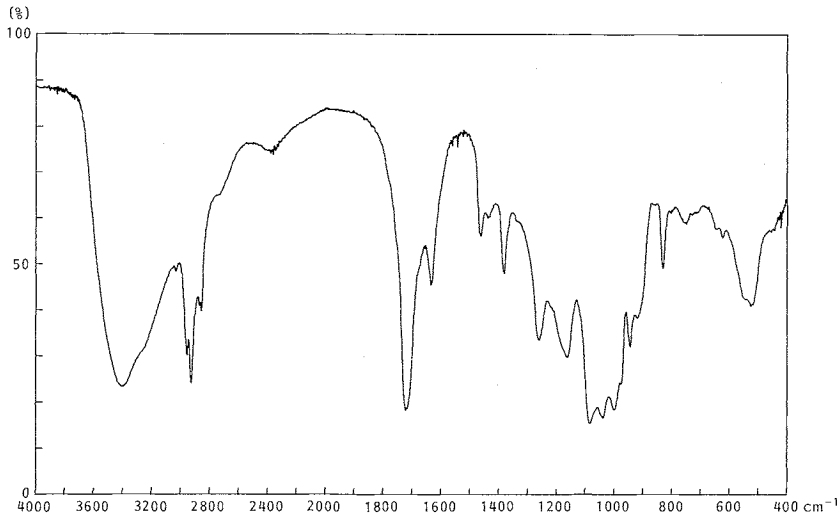


Table 4. ^{1}H and ^{13}C NMR spectra of sultriecin (400 and 100 MHz, respectively in DMSO-d_6).

No.	δ_H	δ_C	No.	δ_H	δ_C
1		163.4 (s)*	13	6.14 (1H, t, J=11.3 Hz)	123.0 (d)
2	5.95 (1H, d, J=9.7 Hz)	120.9 (d)	14	6.47 (1H, t, J=11.3 Hz)	123.5 (d)
3	6.99 (1H, dd, J=9.7, 5.4 Hz)	146.9 (d)	15	6.00 (1H, t, J=11.3 Hz)	129.7 (d)
4	4.09 (1H, dd, J=5.4, 2.9 Hz)	61.6 (d)	16	6.54 (1H, dd, J=15.1, 11.3 Hz)	125.6 (d)
5	4.79 (1H, dd, J=7.3, 2.9 Hz)	81.4 (d)	17	5.74 (1H, m, J=15.1, 7.3 Hz)	136.5 (d)
6	5.67 (1H, dd, J=15.7, 7.3 Hz)	126.8 (d)	18	2.10 (2H, m)	32.4 (t)
7	5.79 (1H, m)	131.9 (d)	19	1.36 (2H, m)	28.4 (t)
8	2.17, 2.38 (2H, m)	36.8 (t)	20	1.23~1.29 (4H, m)	30.8 (t)
9	4.46 (1H, m)	71.0 (d)	21		21.9 (t)
10	1.23~1.29 (1H, m)	40.1 (d)	22	0.87 (3H, t, J=6.8 Hz)	13.9 (q)
11	4.29 (1H, t, J=10.2 Hz)	66.8 (d)	23	0.64 (3H, d, J=6.8 Hz)	8.7 (q)
12	5.35 (1H, dd, J=11.3, 10.2 Hz)	135.2 (d)			

* Multiplicity in off-resonance spectrum.

resemble to those of fostriecin (CI-920)[14,15] which has a triene, a pyrone ring and a phosphate ester. However, **1** is clearly different from the latter in the MS and NMR spectra, and in the absence of phosphate ester as shown by negative Hanes reaction. The negative ion FAB-MS of **1** exhibited a fragment ion at m/z 389 $(M-SO_3Na)^-$, together with the *quasi*-molecular ion at m/z 491 ($(M-H)^-$, $C_{23}H_{32}O_8SNa$) suggesting the presence of a monosodium sulfate ester. This was evidenced by the fact that incubation of **1** with a sulfatase (Sigma Chem.) in 0.1 M Tris-HCl buffer (pH 7.5) afforded a desulfated derivative (**2**, $C_{23}H_{34}O_5$). The IR and UV spectra of **2** are very similar to those of **1** except for the absence of IR absorption at 1260 cm^{-1} assigned as the sulfate group.

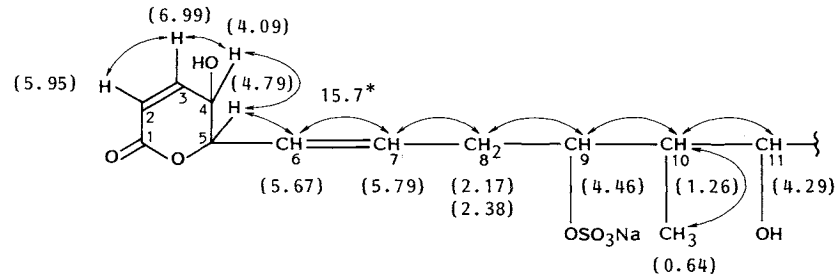
The ^{1}H NMR spectrum of **1** (Table 4) revealed ten olefinic protons, with two of them at δ 5.95 and 6.99 being assigned to the α and β-protons, respectively, of the α,β-unsaturated δ-lactone. The ^{1}H-^{1}H COSY spectrum revealed the connectivity of nine methine and one methylene protons from 2-H to 11-H and established the partial structure of unit A in Fig. 4-1. The ^{1}H NMR spectrum of **2** closely resembled

Fig. 4. Partial structure of sultriecin.

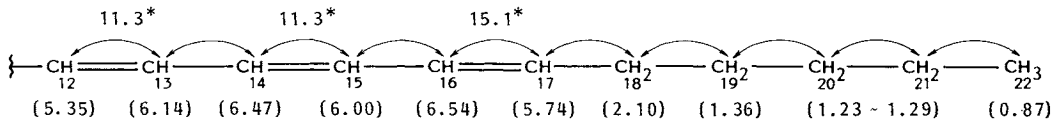
(1) ^{1}H-^{1}H COSY experiment and partial structures of sultriecin.

( ); δ_H in DMSO-d_6, *; J in Hz.

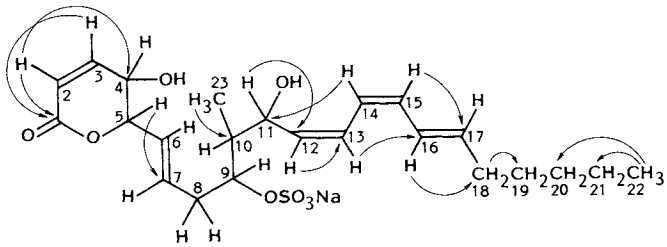
Unit A



Unit B



(2) Long range ^{13}C-^{1}H COSY experiment of sultriecin



that of **1** except that a methine proton at δ 4.46 (9-H) observed in **1** moved to the upfield at δ 3.94 in **2**, indicating that **2** has a hydroxyl group in place of the sulfate group at C-9 of **1**. The low field methine carbon at δ 71.0 in the ^{13}C NMR spectrum (Table 4) of **1**, was assigned to the carbon (C-9) substituted by a sulfate ester. Acetylation of **1** in pyridine afforded a diacetate (**3**) which showed downfield shift of two oxymethine signals at δ 4.09 (4-H) and 4.29 (11-H) of **1** to δ 5.24 and 5.28, respectively, supporting the unit A structure.

The remaining structure ($C_{11}H_{17}$, unit B) of **1** was determined as follows. The presence of a conjugated triene fragment in **1** has been deduced by the UV and NMR spectra (Table 4). The additional one methyl and four methylenes (Table 4) were determined to be $CH_2CH_2CH_2CH_2CH_3$ by the ^{1}H-^{1}H COSY experiment (Fig. 4-1). The terminal methylene (18-H_2) of this *n*-pentyl group exhibited a clear connectivity with a methine proton (16-H, δ 5.74) of the triene moiety establishing unit B structure.

The connection of the units A and B was accomplished by long range ^{13}C-^{1}H COSY experiment (Fig. 4-2). Although proton-proton coupling was not observed between 11-H (δ 4.29) and 12-H (δ 5.35), 11-H coupled to the terminal sp^2 carbon (C-12, δ 135.2) of the triene moiety in the long range ^{13}C-^{1}H COSY. The stereochemistries of the four double bonds in the side chain were established as *E* (6~7), *Z* (12~13),

Z (14~15), E (16~17) on the basis of their coupling constants (11.3 Hz for Z, 15.1 Hz for E) assisted by the ^1H-^1H COSY experiment. Thus, the total structures of **1** and **2** have been determined as Fig. 5.

Antifungal Activity

In vitro antifungal activity was determined against various fungi by the serial 2-fold agar dilution method using SABOURAUD's dextrose agar (pH 7.0). MIC values of **1** and the reference compounds,

Table 5. Antifungal spectrum of sultriecin by an agar dilution method.

Test organism	MIC (μg/ml)		
	Sultriecin	Amphotericin B	Ketoconazole
Candida albicans IAM 4888	25	1.6	50
C. albicans A9540	25	1.6	50
Cryptococcus neoformans D49	1.6	0.8	0.4
C. neoformans IAM 4514	1.6	0.8	0.4
Aspergillus fumigatus IAM 2530	25	3.1	3.1
A. fumigatus IAM 2034	>100	3.1	6.3
Aspergillus flavus FA21436	50	1.6	0.4
Fusarium moniliforme A2284	25	3.1	6.3
Piricularia oryzae D91	50	12.5	3.1
Trichophyton mentagrophytes D155	25	6.3	1.6
T. mentagrophytes No. 4329	12.5	6.3	1.6
Blastomyces dermatidis D40	12.5	12.5	0.4
Sporothrix schenckii IFO 8158	100	>100	6.3
Petriellidium boydii IFO 8078	0.8	>100	1.6
Mucor spinosus IFO 5317	25	6.3	100

Table 6. *In vitro* cytotoxicity.

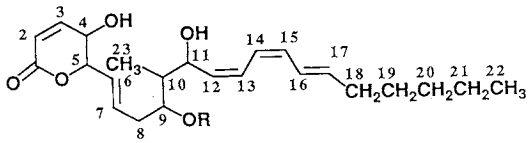
Compound	IC_{50} (μg/ml)								
	B16-F10	HCT-116	Moser	P388	P388/VCR	A549	A549/VP29	K562	K562/ADM
Sultriecin	37.7	1.92	0.85	3.80	4.40	4.14	17.0	0.67	0.37
Desulfated sultriecin	14.2	5.50	ND	ND	ND	12.7	14.7	6.7	11.10
Mitomycin C	0.50	0.80	1.20	ND	ND	ND	ND	ND	ND
Doxorubicin	0.03	0.30	ND	ND	ND	ND	ND		
VP16	0.21	5.3	ND	ND	ND	1.2	40.0	ND	ND

ND: Not tested.

Table 7. Inhibition of macromolecule biosynthesis in L1210 leukemia cells.

Compound	IC_{50} (μg/ml)		
	DNA	RNA	Protein
Sultriecin	>100	5.8	2.4
Desulfated sultriecin	>100	6.1	8.1
Mitomycin C	1.7	>100	>100

Fig. 5. Structures of sultriecin and desulfated sultriecin.



Sultriecin (**1**): R = SO_3Na
Desulfated sultriecin (**2**): R = H

Table 8. Antitumor activity of sultriecin against P388 leukemia (ip).

Compound	Dose (mg/kg/day)	Treatment schedule (ip)	MST (day)	T/C (%)	Average weight change on day 4 (g)
Sultriecin	10	Q1D×3	16.0	160	0.0
	3	Q1D×3	15.0	150	+1.5
	1	Q1D×3	14.0	140	+1.0
	0.3	Q1D×3	13.0	130	+1.0
	0.1	Q1D×3	12.0	120	+1.5
Mitomycin C	3	Q1D×3	20.0	200	−1.0
	1	Q1D×3	13.5	135	+0.5
	0.3	Q1D×3	14.0	140	+1.0
	0.1	Q1D×3	11.5	115	+1.8
Vehicle	—	Q1D×3	10.0	—	+1.3

Table 9. Antitumor activity of sultriecin against B16 melanoma (ip).

Compound	Dose (mg/kg/day)	Treatment schedule (ip)	MST (day)	T/C (%)	Average weight change on day 5 (g)
Sultriecin	10	Q3D×3	21.5	126	+0.5
	3	Q3D×3	26.5	156	0.0
	1	Q3D×3	22.5	132	+0.5
	0.3	Q3D×3	20.0	118	+0.3
	0.1	Q3D×3	18.5	109	+0.8
Mitomycin C	2	Q3D×3	34.5	203	0.0
	1	Q3D×3	23.0	135	+1.0
	0.5	Q3D×3	22.0	129	+0.3
	0.25	Q3D×3	20.0	118	+0.3
Vehicle	—	Q3D×3	17.0	—	+0.8

amphotericin B and ketoconazole against 15 different fungi are shown in Table 5. **1** exhibited moderate and broad spectrum antifungal activity, but it is not active against *Aspergillus fumigatus* IAM 2034 and *Sporothrix schenckii* IFO 8158. The desulfated derivative **2** lacks antifungal activity.

Antitumor Activity

Both **1** and **2** inhibited growth of the murine and human tumor cell lines with the IC_{50} values of 0.85~37.7 μg/ml and 5.5~14.7 μg/ml, respectively (Table 6). Among six cell lines tested, **1** exhibited the most potent cytotoxic activity against Moser (human colorectal carcinoma) and K562 (human myelogenous leukemia) cells. Interestingly, **1** and **2** showed almost the comparable cytotoxicity against the resistant (P388/VCR, A548/VP29 and K562/ADM) and their parent cell lines. The inhibitory effects of **1** and **2** on macromolecule biosynthesis were determined in cultured L1210 murine leukemia cells. As shown in Table 7, **1** and **2** primarily inhibited RNA and protein synthesis with the IC_{50} values around 2.4~8.1 μg/ml, and did not inhibit DNA synthesis at 100 μg/ml.

The *in vivo* antitumor activity was determined in tumor-bearing mice. **1** demonstrated significant prolongation of the survival time of mice inoculated with P388 (Table 8) and B16 melanoma (Table 9). The activity of **1** was comparable to mitomycin C against P388 leukemia but somewhat weaker against B16 melanoma in terms of minimum effective dose. **2** was devoid of *in vivo* antitumor activity against P388 leukemia.

Discussion

Sultriecin is a structurally unique antibiotic containing an α,β-unsaturated δ-lactone, a conjugated triene and a sulfate ester. It is partly related to fostriecin and its analogs[14,15] in having an α,β-unsaturated δ-lactone and a conjugated triene system, but is distinctly different from them in possessing a sulfate group. Phospholine[16], phosphazomycins[17] and phoslactomycin[18] also contain an α,β-unsaturated δ-lactone and a phosphate group and were reported to show antifungal and/or antitumor activity.

Fostriecin showed antitumor activities against P388 leukemia but no activity against solid tumors. Although sultriecin had relatively weak *in vitro* cytotoxicity, it exhibited good *in vivo* antitumor activities against P388 leukemia and B16 melanoma. It is interesting to note that the desulfated derivative exhibited *in vitro* cytotoxicity and inhibition of macromolecule biosynthesis comparable to those of sultriecin, but it did not show *in vitro* antifungal activity and *in vivo* anti-P388 effect.

Experimental

General

MP's were determined using a Yanagimoto micro-melting point apparatus and uncorrected. TLC was performed on precoated silica gel plate (Kieselgel $60F_{254}$, Merck.) The IR and UV spectra were recorded on a Jasco IR-810 IR spectrophotometer and a Jasco UVIDEC-610C spectrophotometer, respectively. The ^1H and ^{13}C NMR spectra were recorded on a Jeol JMN-GX400 spectrometer operated in the Fourier transform mode using DMSO-d_6 (δ_C 39.5, δ_H 2.50) as the internal standard. The FAB-MS spectra were measured on JMS-AX 505H mass spectrometer, and the high-resolution FAB-MS (HRFAB-MS) on a VG 70SE spectrometer. Optical rotations were determined with a Jasco Model DIP-140.

Isolation and Purification of Sultriecin (1)

The fermentation broth (58 liters, pH 7.5) was stirred with *n*-BuOH (40 liters) for one hour. The organic layer (30 liters) separated was concentrated *in vacuo* to one liter which was added dropwise into *n*-hexane (10 liters) to deposit a crude solid of the antibiotic complex (32.2 g). This was dissolved in 50% aqueous MeOH (2.8 liters) and applied on a column of Diaion HP-20 (8.0 i.d. × 20 cm) which was developed successively with 50% and 80% aqueous MeOH and 80% aqueous Me_2CO. The eluate was collected in fractions, and examined by paper disc assay using *C. albicans* A9540. The combined active fractions were concentrated *in vacuo* and the concentrate was chromatographed on a silica gel column (Wako gel C-200, 4.0 i.d. × 70 cm). The eluate was monitored on the basis of the bioassay and TLC (SiO_2, *n*-BuOH - AcOH - H_2O, 4 : 1 : 1). After elution of co-produced pentalenolactone, aburamycin and chromomycins with *n*-hexane - Me_2CO (1 : 1), sultriecin was eluted with Me_2CO. The appropriate fractions were concentrated and purified on a reversed phase silica gel column (YMC-GEL ODS-60, 4.0 i.d. × 32 cm) using acetonitrile - 1/15 M phosphate buffer, pH 7.0, (2 : 8) and then (3 : 7). The active fractions were concentrated and desalted by Diaion HP-20 to afford a semi-pure solid of sultriecin (2.2 g). The solid was dissolved in 10% aqueous MeOH (pH 9.0, 100 ml) containing EDTA (100 mg) and stirred for 3 hours. The solution was passed through a column of Diaion HP-20, and the adsorbed antibiotic was recovered by MeOH elution. The eluate was concentrated and chromatographed on a column of Sephadex LH-20 (4.0 i.d. × 40 cm) with MeOH elution. The fractions containing sultriecin were concentrated *in vacuo* to yield 753 mg of pure sultriecin as a white amorphous powder.

Enzymatic Hydrolysis of Sultriecin (1) with Sulfatase

To a solution of **1** (45 mg) in 0.1 M Tris-HCl buffer (pH 7.5, 10 ml), was added sulfatase (28 mg, S-3009 type H-5, Sigma Chemical Co.) and the mixture was incubated at 37°C for 18 hours. The reaction mixture was poured into water (40 ml) and extracted with two 50 ml portions of EtOAc. The combined extracts were evaporated to dryness *in vacuo* and the residue was purified by preparative TLC (SiO_2, CH_2Cl_2 - MeOH, 5 : 1) followed by Sephadex LH-20 chromatography using MeOH as a developing solvent to afford the desulfated sultriecin (**2**, 8 mg). **2**: White amorphous solid; IR ν_{max} (KBr) cm^{-1} 3410, 2930, 1715, 1670, 1635 and 1085; ^1H NMR (400 MHz, DMSO-d_6) δ 0.67 (3H, d, J=6.8 Hz), 0.86 (3H, t, J=6.5 Hz), 1.23~1.26 (5H, m), 1.36 (2H, m), 2.11 (2H, m), 2.11 and 2.20 (2H, m), 3.94 (1H, m), 4.05

(1H, m), 4.33 (1H, m), 4.81 (1H, dd, J=7.7 and 2.5 Hz), 5.38 (1H, m), 5.67 (1H, m), 5.71 (1H, m), 5.84 (1H, m), 5.94 (1H, d, J=9.8 Hz), 6.00 (1H, m), 6.15 (1H, t, J=11.1 Hz), 6.48 (1H, t, J=11.1 Hz), 6.52 (1H, dd, J=15.2 and 11.1 Hz) and 7.01 (1H, dd, J=9.8 and 5.5 Hz).

Acetylation of **1**

1 (20 mg) was stirred with acetic anhydride (0.3 ml) and pyridine (1.0 ml) for 18 hours at room temperature. The solution was diluted with EtOAc (20 ml), and washed successively with dil HCl (20 ml) and water (20 ml). The organic solution was evaporated to a residue which was purified by preparative TLC (SiO_2, CH_2Cl_2-MeOH, 10:1) followed by Sephadex LH-20 chromatography to afford di-*O*-acetylsultriecin (**3**, 12 mg). **3**: White amorphous solid, SI-MS m/z 577 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1} 3440, 2960, 1735 and 1635; ^1H NMR (400 MHz, DMSO-d_6) δ 0.75 (3H, d, J=7.3 Hz), 0.84~0.87 (3H, m), 1.23~1.26 (4H, m), 1.36 (2H, m), 1.47 and 1.70 (2H, m), 2.00~2.08 (2H, m), 2.04 (6H, s×2), 2.29 and 2.65 (2H, m), 4.23 (1H, m), 5.15 (1H, m), 5.24 (1H, t, J=10.2 Hz), 5.28 (1H, m), 5.40 (1H, t, J=9.6 Hz), 5.59 (1H, m), 5.81~5.86 (2H, m), 6.09 (1H, t, J=10.4 Hz), 6.20 (1H, d, J=9.8 Hz), 6.27 (1H, t, J=12.0 Hz), 6.53 (1H, t, J=15.3 Hz), 6.58 (1H, dd, J=12.0 and 10.2 Hz) and 7.04 (1H, dd, J=9.8 and 5.5 Hz).

In Vitro Cytotoxicity and Inhibition of Macromolecule Biosynthesis

B16-F10 (murine melanoma) and Moser cells were grown in EAGLE's minimum essential medium (Nissui) supplemented with fetal calf serum (FCS, 10%) and kanamycin (60 μg/ml), A549 (human lung carcinoma), A549/VP29 (an etoposide-resistant subline of A549), and HCT-116 (human colon carcinoma) cells were grown in McCOY's 5A Medium (Gibco) supplemented with FCS (10%), penicillin (100 U/ml) and streptomycin (100 μg/ml), and K562 and K562/ADM (an doxorubicin-resistant subline of K562), P388 (murine leukemia) and P388/VCR (a vincristine-resistant subline of P388) were in RPMI 1640 medium supplemented with FCS (10%), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C under humidified atmosphere in a CO_2 incubator. The exponentially growing B16-F10, Moser, A549 (A549/VP29), K562 (K562/ADM), HCT-116 and P388 (P388/VCR) cells were harvested, counted and suspended in the culture media at 1.5×10^4, 2.5×10^5, 3×10^4, 3×10^4, 3×10^4 and 3×10^4 cells/ml, respectively. The test materials were planted into the wells of 96- or 24-well tissue culture plate and incubated for 72 hours. The cytotoxic activities were colorimetrically determined at 540 nm after staining viable cells with neutral red solution.

The inhibition of macromolecule biosynthesis was assessed using L1210 murine leukemia cells. The cells (5×10^5 cells/ml) were first incubated with the test materials at 37°C for 15 minutes and after the addition of the labeled precursor, [^3H]thymidine, [^{14}C]uridine or [^3H]leucine into the cultured mixture, further incubated for 60 minutes. After washing the cells with chilled 5% TCA solution, the radioactivity incorporated into the acid-insoluble fraction of the tumor cells was determined with a liquid scintillation counter.

In Vivo Antitumor Activity

B16 melanoma was intraperitoneally inoculated with 0.5 ml of 10% brei per male BDF_1 mouse, and P388 leukemia intraperitoneally inoculated with 10^6 cells per female CDF_1 mouse. The test materials were intraperitoneally administered to the tumor-bearing mice by the following treatment schedules: Once daily on days 1 to 3 (Q1D×3), once a day on days 1, 4 and 7 (Q3D×3).

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