DEACETYLRAVIDOMYCIN *N*-OXIDE†, A NEW ANTIBIOTIC

TAXONOMY AND FERMENTATION OF THE PRODUCING ORGANISM AND ISOLATION, STRUCTURE AND BIOLOGICAL PROPERTIES OF THE ANTIBIOTIC

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(Received for publication September 12, 1988)

Streptomyces ravidus S50905 was found to produce a new antibiotic, deacetylravidomycin *N*-oxide, together with ravidomycin and deacetylravidomycin in a culture medium containing sodium anthraquinone-β-sulfonate. The structure of this new compound was determined from NMR and mass spectrometric data, and further confirmed by chemical synthesis from deacetylravidomycin.

Deacetylravidomycin *N*-oxide was antitumor active against P388 leukemia and Meth A fibrosarcoma in a wide range of doses, and considerably less toxic than deacetylravidomycin. Its antibacterial activity was less potent than deacetylravidomycin. Ravidomycin *N*-oxide was also synthesized from ravidomycin and its biological properties were tested.

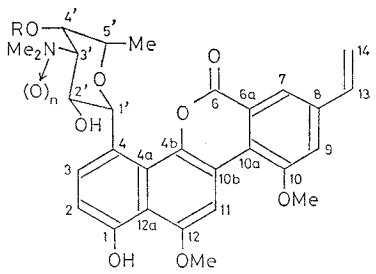
In the course of our screening program for new antibiotics, a streptomycete, strain S50905, designated as *Streptomyces ravidus* S50905 was found to produce a new antibiotic, deacetylravidomycin *N*-oxide (**1**), along with ravidomycin (**4**)[1~4] and deacetylravidomycin (**2**)[4]. The present paper describes the taxonomy and fermentation of the producing strain and the isolation, physico-chemical properties, structure assignment and biological properties of deacetylravidomycin *N*-oxide.

Taxonomy

Strain S50905 was isolated from a soil sample collected at Atsugi-city, Kanagawa Prefecture, Japan. The culture was characterized following the methods outlined by the International Streptomyces Project (ISP)[5]. The color notations were from the ISCC-NBS Centroid Color Charts.

The culture characteristics of strain S50905 on various media are shown in Table 1. The aerial mass color was in the gray color series. The reverse color was pale yellow to olive brown. Soluble pigments were faint yellow or yellowish brown.

Fig. 1. Structures of ravidomycins.



Deacetylravidomycin *N*-oxide (**1**)	R=H	n=1
Deacetylravidomycin (**2**)	R=H	n=0
Ravidomycin *N*-oxide (**3**)	R=Ac	n=1
Ravidomycin (**4**)	R=Ac	n=0

† This antibiotic was originally designated as SS50905A (Jpn. Kokai 181275 ('87), Aug. 8, 1987).

Table 1. Cultural characteristics of strain S50905.

Sucrose - nitrate agar	G:	Poor
	R:	Pale yellow
	Am:	Thin; 63.1.br Gy
	Sp:	None
Glucose - asparagine agar	G:	Moderate
	R:	Pale yellow
	Am:	Moderate; 93.y gray
	Sp:	None
Glycerol - asparagine agar	G:	Moderate
	R:	88.d.Y
	Am:	Thin; 264.1.gray~93.y gray
	Sp:	Faint yellow
Inorganic salts - starch agar	G:	Good
	R:	91.d.gy.Y
	Am:	Good; 265.med.Gy~112.1.Ol Gy
	Sp:	Faint yellowish brown
Tyrosine agar	G:	Moderate
	R:	107.m.Ol
	Am:	Thin; 93.y gray
	Sp:	Faint yellow
Nutrient agar	G:	Good
	R:	91.d.gy.Y
	Am:	Thin; 263.white
	Sp:	Faint yellowish brown
Yeast extract - malt extract agar	G:	Good
	R:	95.m.Ol Br
	Am:	Good; 265.med.Gy
	Sp:	Faint yellowish brown
Oatmeal agar	G:	Poor
	R:	Pale yellow
	Am:	Thin; 92.y white~93.y gray
	Sp:	None
Glycerol - nitrate agar	G:	Poor
	R:	Pale yellow
	Am:	Thin; 93.y gray
	Sp:	None
Calcium - malate agar	G:	Poor
	R:	94.1.Ol Br~100.deep g Y
	Am:	Thin; 263.white~264.1.gray
	Sp:	Faint yellowish brown

G: Growth, R: reverse, Am: aerial mycelium, Sp: soluble pigment.

Morphological observations were made of the cultures grown at 28°C for 14 days on sucrose - nitrate agar or yeast extract - malt extract agar. Mature spore chains had 10 or more spores in the form of open loops, hooks or very loose spirals. The spores were oval or cylindrical and $0.5\sim0.7\times 0.7\sim1.2$ μm in size with a smooth surface under an electron microscope. Therefore, strain S50905 characteristically belongs to PRIDHAM's[6] group *Retinaculum Apertum.*

The physiological characteristics of strain S50905 are presented in Table 2. The utilization of carbon sources by strain S50905 was observed on ISP medium 9. The pattern of carbon utilization by strain S50905 is shown in Table 3.

Hydrolyzed whole cells contained LL-diaminopimelic acid.

Table 2. Physiological properties of strain S50905.

Temperature range for growth	14~39°C
Optimum temperature	27~33°C
Nitrate reduction	Positive
Starch hydrolysis	Positive
Milk coagulation	Positive
Milk peptonization	Positive
Melanin production	Negative
Gelatin liquefaction	Positive

Table 3. Carbon utilization of strain S50905.

D-Glucose	+
L-Arabinose	−
Sucrose	−
D-Xylose	+
i-Inositol	+
D-Mannitol	−
D-Fructose	±
L-Rhamnose	−
Raffinose	−
D-Galactose	+
Salicin	+

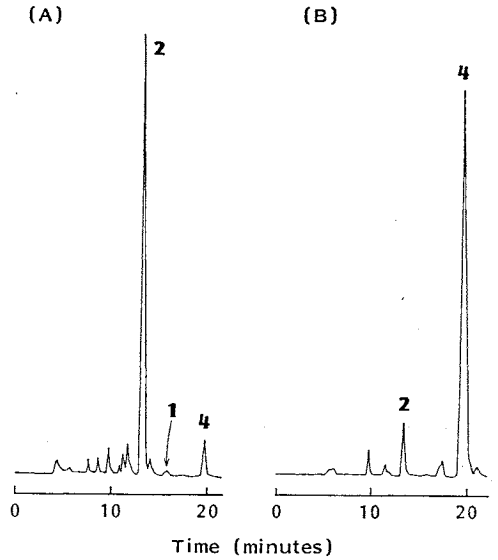
+: Utilized, ±: weakly utilized, −: not utilized.

Microscopic studies and whole cell analysis of strain S50905 indicated that strain S50905 was classified in the genus *Streptomyces*. Strain S50905 also produced ravidomycin. Accordingly, the properties of strain S50905 was compared with those of *S. ravidus* AY B-1198[1] which produces ravidomycin, and good agreement was obtained except for utilization of D-galactose and salicin. Therefore, strain S50905 was designated as *S. ravidus* S50905. Strain S50905 has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, where it has been assigned accession No. FERM P-8424.

Fermentation

The stock culture was grown on yeast - starch agar at 28°C for 14 days. A loopful of cells from the stock culture was inoculated into 500-ml flasks each containing 120 ml of a medium composed of glucose 2%, soluble starch 3%, Polypeptone 0.5%, Polypepton S (Daigo Nutritive Chemicals Ltd.) 1%, corn steep liquor 1%, NaCl 0.3%, $CaCO_3$ 0.3%. The pH of the medium was adjusted to pH 7.0 before sterilization. The flasks were cultured at 28°C for 2 days on reciprocal shaker.

Fig. 2. HPLC analysis of fermentation broths.
(A) With Anq, (B) without Anq.

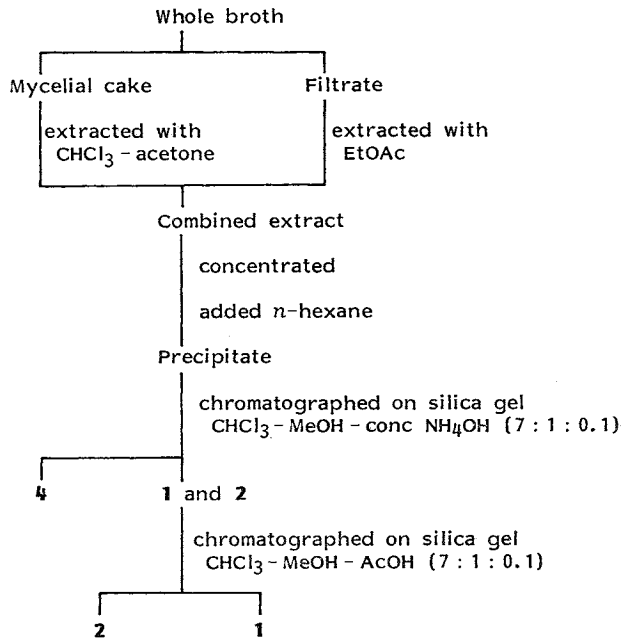


Column: Nucleosil 5 C_{18} (4.6×250 mm). Mobil phase: 0.05 M tartrate buffer (pH 3) - CH_3CN (1 : 1) containing 0.1% sodium lauryl sulfate. Flow rate: 0.7 ml/minute. Monitored at 280 nm. Chart speed: 5 mm/minute.
Anq: Anthraquinone-β-sulfonate

A 30-liter jar fermentor was charged with 16 liters of the above medium containing sodium anthraquinone-β-sulfonate (Anq) (0.5%) and inoculated with 200 ml of the seed culture. The fermentation was carried out at 33°C for 5 days with aeration of 16 liters/minute and agitation of 400 rpm.

HPLC analysis of the fermentation broth showed that Anq in the medium affected the production pattern of the antibiotic complex. Typical chromatograms of fermentation broths with and without Anq are shown in Fig. 2. In the case of the fermentation run in the presence of Anq (0.5%), the major product was **2**, and **1** was detected as a minor constituent. In the absence of Anq, the

Fig. 3. Procedure for the isolation of **1**, **2** and **4**.



major product was **4**, and **1** was not detected.

Isolation and Purification

The isolation procedure for **1**, **2** and **4** is shown in Fig. 3. After the fermentation was terminated, the culture broth was centrifuged to separate the mycelium from the broth. The mycelial cake and the filtrate were extracted with $CHCl_3$ - acetone and EtOAc, respectively, and the extracts were combined. The combined extract was concentrated and *n*-hexane was added to give the crude antibiotic complex as a precipitate. This was purified by column chromatography on silica gel ($CHCl_3$ - MeOH - conc NH_4OH, 7 : 1 : 0.1) to give **4** as yellow crystals (0.45 g) and a crude mixture of **1** and **2**. The latter was further purified by column chromatography on silica gel ($CHCl_3$ - MeOH - AcOH, 7 : 1 : 0.1) to separate **1** and **2**, and each fraction was washed with dil NH_4OH solution and concentrated to give **1** (0.15 g) and **2** (3.5 g), respectively, as yellow crystals.

Physico-chemical Properties

The spectral data of **2** and **4** were identical with literature data[1~4]. The physico-chemical properties of **1** are summarized in Table 4. It is soluble in $CHCl_3$ and MeOH, but insoluble in water and *n*-hexane. The UV spectrum (Fig. 4) was similar to those of gilvocarcin V[7], chrysomycin A[8] and ravidomycin. The IR spectrum is shown in Fig. 5. The molecular formula was determined to be $C_{29}H_{31}NO_9$ by fast atom bambardment (FAB)-MS and elemental analysis. The ^1H and ^{13}C NMR data are presented in Tables 5 and 6. The physico-chemical properties of ravidomycin *N*-oxide (**3**, synthesized from **4**) are also shown in Table 4.

Structure Determination

The physico-chemical properties of **1** were quite similar to those of **2** ($C_{29}H_{31}NO_8$). Comparison of the molecular formulae showed that **1** had one more oxygen atom than **2**.

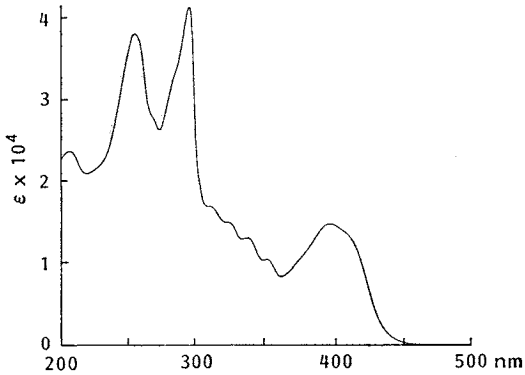
Table 4. Physico-chemical properties of **1** and **3**.

	1	**3**
Appearance	Yellow crystals	Yellow crystals
MP (dec, °C)	237	200~205
Molecular formula	$C_{29}H_{31}NO_9$	$C_{31}H_{33}NO_{10}$
Anal Found:	C 64.35, H 5.93, N 2.78	C 64.40, H 5.71, N 2.39
Calcd:	C 64.80, H 5.81, N 2.61	C 64.24, H 5.74, N 2.42
FAB-MS *m/z* $(M+H)^+$	538	580
UV λ_{max}^{MeOH} nm (ε)	248 (38,100), 262 (sh), 278 (sh), 287 (41,400), 307 (17,200), 321 (15,200), 334 (13,300), 349 (10,600), 392 (15,000)	247 (31,200), 277 (sh), 287 (31,800), 308 (13,000), 321 (12,300), 334 (10,800), 350 (8,500), 390 (12,200)
IR (KBr) cm^{-1}	3380, 1710, 1620, 1605, 1590	3400, 1740, 1710, 1630, 1610, 1590
$[\alpha]_D^{25}$	−35.6° (*c* 0.5, $CHCl_3$ - MeOH, 1 : 1)	−107.6° (*c* 0.5, $CHCl_3$)
Rf value[a] (1)	0.23	0.38
(2)	0.38	0.42

[a] Silica gel plate: Merck Art. No. 5715. Solvents: (1) $CHCl_3$ - MeOH - AcOH, 100 : 20 : 1, (2) $CHCl_3$ - MeOH - conc NH_4OH, 100 : 20 : 1.

Comparison of the ^1H NMR data of **1** and **2** showed that the structures of the sugar moieties were different. The chemical shifts for 3′-H (δ 3.77) and 3′-$N(CH_3)_2$ (δ 3.37 and 3.59) of **1** were observed at lower field compared to those of **2** (3′-H: δ 2.82, 3′-$N(CH_3)_2$: δ 2.60). The chemical shifts for 2′-H and 4′-H of **1** (δ 4.86 and 4.20, respectively) were observed at slightly lower field compared to those of **2** (δ 4.33 and 4.08, respectively). The data for the other protons of **1** and **2** were nearly identical. These data suggested that the additional oxygen atom in **1** was placed on 3′-$N(CH_3)_2$.

Fig. 4. The UV spectrum of **1** (in MeOH).



Similar correlation to that cited above was observed between the carbon chemical shifts of **1** and **2** (C-3′, 3′-$N(CH_3)_2$, C-2′ and C-4′, see Table 6). The data of the other carbons of **1** and **2** were nearly identical. These data also showed that the additional oxygen atom in **1** must be placed on 3′-$N(CH_3)_2$.

In addition, **1** was obtained when **2** was treated with *m*-chloroperbenzoic acid. On the basis of the results cited above, the chemical structure of **1** was determined to be the *N*-oxide derivative of **2**. (Fig. 1)

Biological Properties

Antimicrobial Activity

MICs of **1**, **2**, **3** and **4** against various bacteria incubated in the dark are shown in Table 7. The two *N*-oxides (**1** and **3**) were active against Gram-positive bacteria but inactive against Gram-negative bacteria.

GREENSTEIN *et al.*[9] showed that the antimicrobial and cytotoxic activities of ravidomycin and deacetylravidomycin were enhanced by light. So, the influence of light on the antimicrobial activities

Fig. 5. The IR spectrum of **1** (KBr).

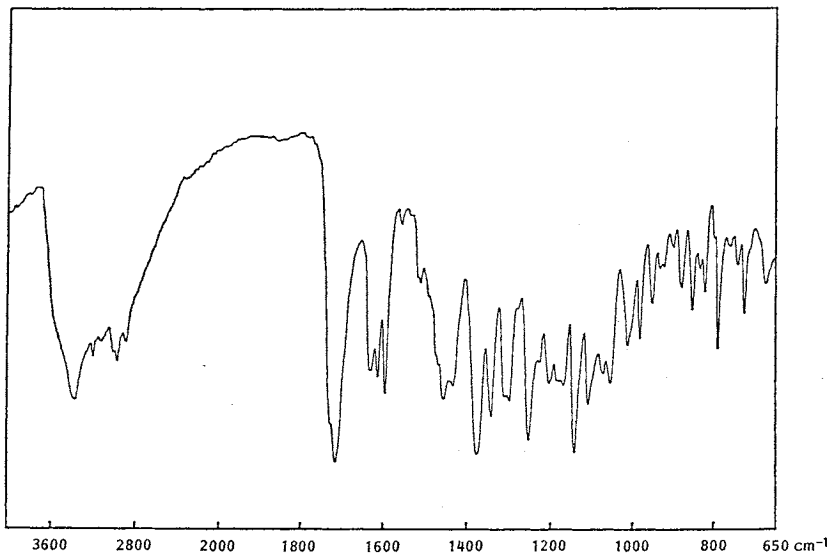


Table 5. ^{1}H NMR data[a] of **1**, **2**, **3** and **4**.

	1[b]	**2**[c]	**3**[b]	**4**[c]
1-OH	—	9.76 br s	—	9.77 s
2-H	7.04 d (8.6)	7.02 d (8.3)	7.07 d (8.9)	7.04 d (8.3)
3-H	7.93 d (8.6)	7.92 d (8.3)	7.94 d (8.9)	7.98 d (8.3)
7-H	8.00 d (1.5)	8.02 d (1.6)	8.04 d (1.6)	8.02 d (1.6)
9-H	7.29 d (1.5)	7.24 d (1.6)	7.35 d (1.6)	7.25 d (1.6)
11-H	8.30 s	8.28 s	8.39 s	8.28 s
13-H	6.77 dd (17.4, 10.8)	6.76 dd (17.5, 11.0)	6.81 dd (17.7, 10.5)	6.76 dd (17.2, 10.5)
14-H_2	5.93 d (17.4)	5.91 d (17.5)	5.96 d (17.7)	5.91 d (17.2)
	5.46 d (10.8)	5.43 d (11.0)	5.48 d (10.5)	5.44 d (10.5)
10-OCH_3	4.04* s	4.00* s	4.06* s	4.00* s
12-OCH_3	4.00* s	4.01* s	4.09* s	4.01* s
1′-H	5.94 d (8.8)	5.83 d (9.1)	5.99 d (8.3)	5.85 d (9.4)
2′-H	4.86 dd (9.9, 8.8)	4.33 dd (9.9, 9.1)	4.89 dd (9.7, 8.3)	4.44 dd (10.2, 9.4)
3′-H	3.77 dd (9.9, 2.4)	2.82 d (9.9)	4.09 dd (9.7, 2.4)	3.08 dd (10.2, 3.0)
4′-H	4.20 d (2.4)	4.08 s	5.72 d (2.4)	5.56 d (3.0)
5′-H	4.44 q (6.4)	4.41 q (6.4)	4.60 q (6.5)	4.49 q (6.4)
3′-$N(CH_3)_2$	3.37 s	2.60 s×2	3.35 s	2.51 s×2
	3.59 s		3.38 s	
4′-OAc	—	—	2.24 s	2.11 s
5′-CH_3	1.25 d (6.4)	1.21 d (6.4)	1.10 d (6.5)	1.05 d (6.4)

[a] J (Hz) in parentheses.
[b] Measured in $CDCl_3$ - CD_3OD (20 : 1) solution.
[c] Measured in $CDCl_3$ solution.
* Assignments in the same column may be interchanged.

of these antibiotics were studied (Table 8). To study the effect of light, the set of plates was irradiated with a fluorescent light (20 W) at a distance of about 20 cm during incubation in our experiment. A dramatic increase in the antibacterial activities of the two *N*-oxides were also observed. The enhanced antibacterial activities of **1** and **3** against Gram-positive bacteria by light were 1,020 to 64-fold and

Table 6. ^{13}C NMR chemical shifts of **1**, **2**, **3** and **4**.

Carbon number	1[a]	2[b]	3[a]	4[b]
C-1	155.1	154.6	154.9	154.7
C-2	112.6	112.3	112.3	112.4
C-3	130.1	129.3	129.6	129.5
C-4	125.9*	125.2	125.4*	125.2
C-4a	124.8*	125.2	123.7*	125.2
C-4b	143.0	142.6	142.5	142.9
C-6	161.4	160.5	160.8	160.6
C-6a	122.5	122.1	122.1	122.2
C-7	119.8	119.6	119.6	119.7
C-8	139.5	138.6	138.9	138.6
C-9	114.7	113.8	114.2	113.8
C-10	157.8	157.0	157.3	157.1
C-10a	123.7	123.2	123.2	123.4
C-10b	114.6	113.8	114.2	113.9
C-11	102.5	101.9	102.1	102.1
C-12	152.4	151.8	152.0	151.8
C-12a	116.4	116.0	116.1	116.3
C-13	135.7	135.1	135.2	135.2
C-14	116.7	116.3	116.6	116.2
10-OCH_3	56.3**	56.0*	56.2**	56.0*
12-OCH_3	56.5**	55.8*	56.0**	55.9*
C-1′	80.4	80.4	80.4	80.5
C-2′	67.7	65.7	67.6	65.4[c]
C-3′	82.3	71.2	80.1	69.6[c]
C-4′	69.8	69.3	70.8	69.4[c]
C-5′	76.2	76.5	74.6	75.0
5′-CH_3	16.8	16.9	16.9	16.8
3′-$N(CH_3)_2$	56.5	42.1	55.3	40.8
	57.8	42.1	58.1	40.8
4′-$COCH_3$			20.9	21.6
			170.5	170.8

[a] Measured in $CDCl_3$ - CD_3OD (20 : 1) solution.
[b] Measured in $CDCl_3$ solution.
[c] Assignments given in ref 3 were revised as shown.
*,** The assignments in the same column may be reversed.

Table 7. Antibacterial activities of **1**, **2**, **3** and **4**.

Organism	MIC (μg/ml)			
	1	2	3	4
Bacillus subtilis ATCC 6633	12.5	1.56	1.56	0.78
Staphylococcus aureus FDA 209P	50	1.56	3.12	1.56
S. aureus Smith	3.12	1.56	3.12	1.56
S. epidermidis ATCC 12228	12.5	1.56	3.12	1.56
Micrococcus luteus ATCC 9341	3.12	0.78	1.56	0.78
M. lysodeikticus IFO 3333	6.25	0.78	1.56	1.56
Enterococcus faecalis IFO 12964	3.12	0.78	0.78	0.78
Escherichia coli O 1	>100	>100	>100	>100
Shigella flexneri 2b	>100	25	>100	50
Pseudomonas aeruginosa IFO 13736	>100	>100	>100	>100
Klebsiella pneumoniae ATCC 10031	>100	>100	>100	100
Proteus vulgaris OXK	>100	>100	>100	>100
Providencia rettgeri	>100	>100	>100	>100
Serratia marcescens NHL	>100	>100	>100	>100

Agar dilution method (Mueller-Hinton agar).

Table 8. Effect of light on antibacterial activities of **1**, **2**, **3** and **4**.

Organism	MIC (μg/ml)			
	1	**2**	**3**	**4**
Bacillus subtilis ATCC 6633	0.098	0.049	0.098	0.024
Staphylococcus aureus FDA 209P	0.049	0.024	0.049	0.024
S. aureus Smith	0.012	0.012	0.012	<0.006
S. epidermidis ATCC 12228	0.098	0.049	0.049	0.049
Micrococcus luteus ATCC 9341	0.049	0.024	0.049	0.012
M. lysodeikticus IFO 3333	0.098	0.049	0.098	0.049
Enterococcus faecalis IFO 12964	0.024	<0.006	<0.006	<0.006
Escherichia coli O 1	>100	3.12	>100	6.25
Shigella flexneri 2b	0.098	0.098	0.195	0.098
Pseudomonas aeruginosa IFO 13736	>100	6.25	>100	12.5
Klebsiella pneumoniae ATCC 10031	0.195	0.098	0.39	0.195
Proteus vulgaris OXK	25	3.12	>100	6.25
Providencia rettgeri	0.78	0.39	0.39	0.39
Serratia marcescens NHL	>100	3.12	>100	6.25

Agar dilution method (Mueller-Hinton agar).

Table 9. Antitumor effects of **1**, **2**, **3** and **4** on P388 leukemia.

Sample	ILS (%)									
	Dose (mg/kg/day)									
	1	2	4	8	16	32	64	90	128	180
1 (Test 1)		23	33	48	53	60	63			
(Test 2)								52	44	52
2 (Test 1)	27	24	0							
(Test 2)			34	58	−5	Toxic				
3 (Test 1)	24	27	33	58						
(Test 2)				44	45	64	Toxic			
4 (Test 1)	22	24	27							
(Test 2)			34	40	50	65				
(Test 3)							5	ND	Toxic	

Each mouse (CDF_1, male) was inoculated intraperitoneally with 1×10^6 cells. The drugs were administered intraperitoneally daily for 10 days from the next day after the inoculation.

ILS: Increase in lifespan.

ND: Not done.

260 to 16-fold, respectively.

Antitumor Activity

The antitumor activities of **1**, **2**, **3** and **4** on P388 leukemia are shown in Table 9. The maximal values in prolongation of lifespan of **1**, **2**, **3** and **4** were similar (increase in lifespan (ILS): 63%, 58%, 58% and 65%, respectively), but the effective dose range of **1** was wider than that of the others.

Table 10 shows the antitumor activities of **1** against Meth A fibrosarcoma and P815 mastocytoma. It exhibited significant prolongation of lifespan in mice against Meth A fibrosarcoma, whereas it was inactive against P815 mastocytoma.

Acute Toxicity

The toxicity of **1** was very low, since no death of mice was observed by ip administration of **1** (1,000 mg/kg) after 14 days. The LD_{50} values in mice of **2**, **3** and **4** by a single ip administration

Table 10. Antitumor effect of **1** on Meth A fibrosarcoma and P815 mastocytoma.

Dose (mg/kg/day)	Meth A		P815	
	MSD	ILS (%)	MSD	ILS (%)
Control	13.1±1.5		10.6±0.5	
4	19.9±3.8	52**	11.4±0.8	8
8	18.6±1.3	42***	12.4±1.3	17
16	22.1±3.1	69***	12.7±2.4	20*
32	29.7±7.3	127**	13.1±3.4	24
64	35.1±8.8	168**	12.9±2.0	22*

Each mouse (CDF_1, male) was inoculated intraperitoneally with 1×10^6 cells. The drug was administered intraperitoneally daily for 10 days from the next day after the inoculation.

ILS: Increase in lifespan.

MSD: Mean survival days (mean±SD).

*,**,*** $P<0.05$, 0.01, 0.001, respectively.

were 50 mg/kg, 150 mg/kg and 150 mg/kg, respectively.

Discussion

Strain S50905 was found to produce a new antibiotic, deacetylravidomycin *N*-oxide, together with large amount of **2** in the culture medium containing Anq. But, in the case of another strain which produced **4** (*Streptomyces* sp. SS50408[10], isolated by us), no effect was observed on the production pattern of antibiotics by the addition of Anq to the fermentation medium (data not shown). Other antibiotics, such as kidamycin[11] and SS21020[12], are also affected by the presence of added Anq in the fermentation medium. Although the mechanism of action of Anq in the fermentation is not known, it is useful to add Anq to the fermentation medium to search for new antibiotics.

Some *N*-oxides, for example, leucinostatins H and K[13], guanine-7-oxide[14], and enteromycin[15], are known microbial metabolites. In this report, we showed that the two *N*-oxides, **1** and **3**, had antitumor activity similar to **2** and **4**. Although the chromophores of **1**, **2**, **3** and **4** are identical, **1** showed particularly low toxicity compared with that of **2**, **3** and **4**. Therefore, the effective dose range of **1** in antitumor activities is very wide. It is interesting that *N*-oxidation of **2** into **1** resulted in low toxicity. Although many glycosides with aminosugars are known, few papers have been published on the *N*-oxide derivatives of these compounds.

Experimental

General

MP's were determined on a Yanagimoto micro melting point apparatus and were not corrected. IR spectra were measured on a Hitachi 285 IR spectrometer. UV spectra were recorded on a Hitachi 200-20 spectrometer. FAB-MS were obtained on a Jeol JMS-DX303 mass spectrometer using 6 KeV xenon atom bombardment. Glycerol was used as the supporting matrix. ^1H and ^{13}C NMR spectra were obtained on a Bruker AM 400 spectrometer. Optical rotations were measured with a Jasco DIP-4 digital polarimeter.

Oxidation of 2 with *m*-Chloroperbenzoic Acid

A solution of **2** (52 mg, 0.1 mmol) and *m*-chloroperbenzoic acid (22 mg, 0.1 mmol) in methylene chloride (20 ml) was stirred at room temperature for 30 minutes. Then, the solution was washed with sodium bicarbonate solution (5%), then with water, and dried over sodium sulfate. The solvent was concentrated and the residue recrystallized from ether to give **1** as yellow crystals (44 mg, 81.9%).

Oxidation of 4 with *m*-Chloroperbenzoic Acid

Using the same procedure as above, **3** was obtained from **4** (70.7%).

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