Isolation of a New Antibiotic Oligomycin G Produced by *Streptomyces* sp. WK-6150

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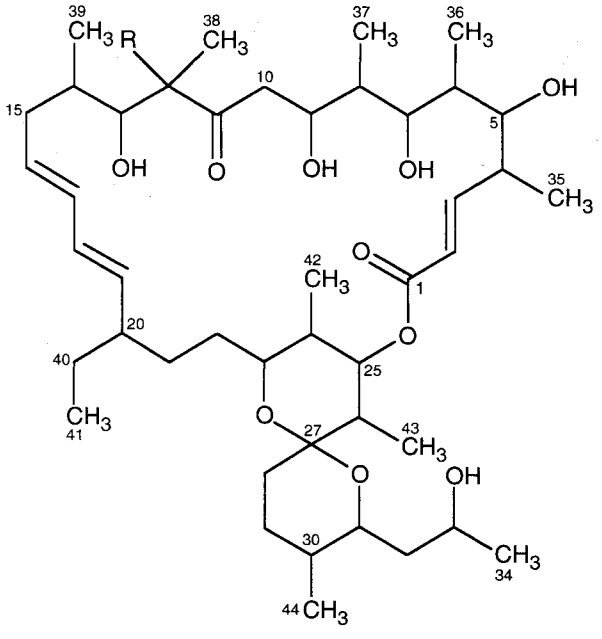
In the course of our screening program for new insecticidal and nematocidal metabolites, a new compound, oligomycin G (**1**, Fig. 1), was isolated from a cultured broth of *Streptomyces* sp. WK-6150. Compound **1** is a new component of oligomycin group. Oligomycins have been isolated as antifungal metabolites[1]. In this report, we describe characteristics of the producing strain and fermentation, isolation, physico-chemical properties, structure elucidation, and biological activities of **1**.

The strain WK-6150 was isolated from a soil sample collected in Washington State, United States. The vegetative mycelia grew abundantly on yeast extract-malt extract agar, oatmeal agar, and other agar media, and did not show fragmentation into coccoid forms or bacillary elements. The aerial mycelia grew abundantly on yeast extract-malt extract agar and oatmeal agar. The spore chains were spiral and each had more than 20 spores per chain. The spores were cylindrical in shape, 1.1×0.9 μm in size and had a smooth surface (Fig. 2). Whirls, sclerotic granules, sporangia, and flagellate spores were not observed. The isomer of DAP in whole-cell hydrolysates of strain WK-6150 was determined to be the LL-form. Major menaquinones were MK-9(H_6), MK-9(H_8) and MK-9(H_4). The cultural characteristics, the physiological properties, and the utilization of carbon sources are shown in Tables 1, 2, and 3. The color of vegetative mycelia showed yellow to brown and the aerial mass color showed red series. Melanoid pigment was produced.

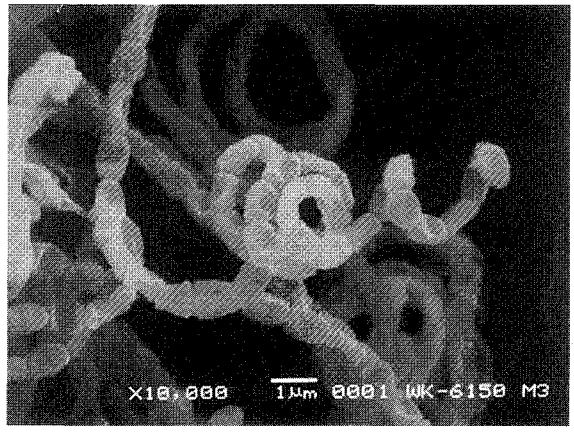
Based on the taxonomic properties described above, strain WK-6150 is considered to belong to the genus *Streptomyces*[2].

Fig. 1. Structures of oligomycins G (**1**) and SC-II (**2**).



	R
Oligomycin G (**1**)	H
Oligomycin SC-II (**2**)	OH

Fig. 2. Scanning electron micrograph of spore chains of strain WK-6150 grown on oatmeal agar at 27°C for 14 days.



Bar represents 1 μm.

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Table 1. Cultural characteristics of strain WK-6150.

Medium		Cultural characteristics
Yeast extract-malt extract agar [a]	G:	Good, bisque (3ec)
	R:	Adobe brown (3lg)
	AM:	Abundant, ashes of rose (7ge)
	SP:	None
Oatmeal agar [a]	G:	Good, camel (3ie)
	R:	Adobe brown (3lg)
	AM:	Abundant, rosewood (5ge)
	SP:	None
Inorganic salts-starch agar [a]	G:	Moderate, biscuit (2ec)
	R:	Camel (3ie)
	AM:	Poor, pink tint (7ba)
	SP:	None
Glycerol-asparagine agar [a]	G:	Moderate, biscuit (2ec)
	R:	Light ivory (2ca)
	AM:	Moderate, orchid tint (10ba)
	SP:	None
Glucose-asparagine agar	G:	Moderate, ivory (2db)
	R:	Ivory (2db)
	AM:	None
	SP:	None
Peptone-yeast extract-iron agar [a]	G:	Moderate, covert tan (2ge)
	R:	Covert tan (2ge)
	AM:	None
	SP:	None
Tyrosine agar [a]	G:	Moderate, covert tan (2ge) to beaver (3li)
	R:	Covert tan (2ge) to covert brown (2nl)
	AM:	None
	SP:	Dark brown (2pn)
Sucrose-nitrate agar [b]	G:	Moderate, pearl (3ba)
	R:	Pearl(3ba)
	AM:	Trace, sand (3cb)
	SP:	None
Glucose-nitrate agar [b]	G:	Poor, pearl pink (3ca)
	R:	Pearl pink (3ca)
	AM:	None
	SP:	None
Glycerol-calcium malate agar [b]	G:	Good, ivory (2db)
	R:	Bisque (3ec)
	AM:	None
	SP:	None
Glucose-peptone agar [b]	G:	Moderate, pearl pink (3ca)
	R:	Pearl pink (3ca)
	AM:	None
	SP:	None
Nutrient agar [b]	G:	Moderate, bisque (3ec)
	R:	Bamboo (2gc)
	AM:	None
	SP:	None

a: Medium recommended by ISP.
b: Medium recommended by S. A. WAKSMAN.
Abbreviations: G, growth of vegetative mycelium; R, reverse side color; AM,aerial mycelium; SP, soluble pigment.

The strain was deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, under the name *Streptomyces* sp. WK-6150, and the accession No. is FERM BP-7036.

A slant culture of the strain WK-6150 was inoculated into two 500-ml Erlenmeyer flasks, each containing 100 ml of a seed medium consisting of glucose 0.1%, starch 1.0%, peptone (DIFCO) 0.3%, meat extract (Kyokuto Pharmaceutical Industrial Co.) 0.3%, yeast extract (Oriental Yeast Co.) 0.1%, and $CaCO_3$ 0.3% (pH 7.0 before sterilization). After incubation on a rotary shaker at 27°C for 3 days, 2 ml of this seed culture was transferred into each of 100-Erlenmeyer flasks of 500-ml capacity containing 100 ml of a producing medium consisting of glucose 5.0%, Polypepton (Nihon Pharmaceutical Co.)

Table 2. Physiological properties of strain WK-6150.

Melanin formation	
Tyrosine agar	+
Peptone-yeast extract-iron agar	—
Tryptone-yeast extract broth	+
Reduction of nitrate	—
Liquefaction of gelatin (21~23°C)	—
Hydrolysis of starch	+
Coagulation of milk (27°C)	—
Peptonization of milk (27°C)	+
Decomposition of cellulose	—
Temperature range for growth	4~29°C

+: Positive, —: Negative

Table 3. Utilization of carbon sources by strain WK-6150.

Utilized:	L-Arabinose, D-Glucose
Weakly utilized:	D-Fructose
Not utilized:	*i*-Inositol, D-Mannitol, Melibiose, Raffinose, L-Rhamnose, Sucrose, D-Xylose

0.4%, yeast extract 0.1%, meat extract 0.4%, NaCl 0.25%, and $CaCO_3$ 0.5% (pH 7.0 before sterilization). The fermentation was carried out at 27°C for 6 days on a rotary shaker.

The fermentation broth (10 liters) was centrifuged at 3,000 rpm for 10 minutes. The supernatant was extracted with ethyl acetate. The mycelium cake was extracted with MeOH and the solvent was evaporated. Then the residual aqueous solution was extracted with ethyl acetate. The both ethyl acetate extracts were concentrated *in vacuo* to give a crude material (881 mg). This material was charged on a column of silica gel and eluted with $CHCl_3$-MeOH (50:1~10:1). The fractions showed activity against *Artemia salina* and *Caenorhabditis elegans* were combined (583 mg) and further purified by an ODS column eluted with 90% MeOH to yield a yellow powder (142 mg). 60 mg of the powder was purified on a reversed-phase HPLC (Pegasil-ODS, Senshu Scientific Co., Ltd.) eluted with 85% acetonitrile to give 14.7 mg of **1** and 19.1 mg of **2**.

Structures of compounds **1** and **2** were deduced to be similar to oligomycins by comparing the NMR and UV spectra.

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

Appearance	White powder
MP (°C)	125~128
α_D^{25}	–62.7 (*c* 0.3, MeOH)
Molecular formula	$C_{44}H_{74}O_{10}$
FAB-MS (*m/z*)	785 $(M+Na)^+$
HR-FAB-MS:	calcd 785.5179 $(C_{44}H_{74}O_{10}Na)^+$
	found 785.5157 $(M+Na)^+$
UV λ_{max}^{MeOH} nm (ε)	225 (63,600), 231 (58,300), 235 (48,000), 241 (32,700)
IR ν_{max} (KBr) cm^{-1}	2966, 1701, 1460, 1385, 1284, 987

Compound **2** was identified as oligomycin SC-II[3] (BE-56384)[4], reported as an antitumor compound, by its physico-chemical properties and NMR data.

The physico-chemical properties of **1** are summarized in Table 4. The FAB-MS of **1** gave a molecular ion peak at *m/z* 785 $(M+Na)^+$, and the high resolution FAB-MS revealed its molecular formula as $C_{44}H_{74}O_{10}$.

The structure of **1** was deduced on the basis of 1D and 2D NMR experiments. The analysis of ^{13}C NMR and DEPT spectra of **1** revealed the presence of 10 methyls, 8 methylenes, 23 methines, one ketal carbon (δ_C 99.6), and two carbonyl carbons (δ_C 165.3, 216.2). The ^{13}C and ^1H NMR spectral data for **1** are summarized in Table 5. The correlations observed in ^1H-^1H COSY and HMBC experiments are shown in Fig. 3. In addition to these correlations, comparison of its chemical shifts with those of oligomycin SC-II and oligomycin A[5] revealed that the structure of **1** was the same as that of compound **2** except for the 12-hydroxyl group. Thus the structure of **1** was elucidated as 12-dehydroxy-oligomycin SC-II. Recently, new oligomycins Mer-5504A1~A4 were reported[6]. Mer-5504A4 has an ethyl side chain at C-26 instead of the methyl side chain of **1**.

Insecticidal and nematocidal activities of **1** were studied by a microplate assay using brine shrimp *Artemia salina* and free-living nematode *Caenorhabditis elegans*. The nauplii larvae hatched from eggs were used for the brine shrimp assay. *C. elegans* was cultivated on an agar plate covered with *Escherichia coli* for 3~4 days at 20°C and the grown nematodes were used for the assay. These organisms and **1** were incubated in 96-well microplates at 20°C. After 48 hours, the motilities were assessed visually under a microscope (×40) in comparison to controls.

Table 5. The ^{13}C and ^{1}H NMR data for **1**.

Position	^{13}C	^{1}H
1	165.3 s	
2	122.5 d	5.83 dd (1H, *J*=1, 16 Hz)
3	151.1 d	6.77 dd (1H, *J*=10, 16 Hz)
4	42.1 d	2.52 m
5	81.0 d	3.63 m
6	36.2 d	1.92 m
7	80.4 d	3.75 m
8	41.5 d	1.51 m
9	67.3 d	4.09 m
10	49.1 t	2.62 dd (1H, *J*=10, 18.5 Hz), 2.78 dd (1H, *J*=2, 18.5 Hz)
11	216.2 s	
12	50.5 d	2.74 dd (1H, *J*=7, 10 Hz)
13	71.5 d	3.90 m
14	34.4 d	1.66 m
15	37.7 t	2.03 m, 2.16 m
16	131.4 d	5.43 ddd (1H, *J*=4, 11, 15 Hz)
17	133.2 d	6.12 ddd (1H, *J*=1.5, 10.5, 15 Hz)
18	132.0 d	6.01 dd (1H, *J*=10.5, 15 Hz)
19	137.3 d	5.18 dd (1H, *J*=10, 15 Hz)
20	47.2 d	1.86 m
21	32.3 t	1.48 m, 1.75 m
22	32.2 t	0.98 m, 1.68 m
23	69.2 d	3.92 m
24	36.9 d	2.09 m
25	76.9 d	4.86 dd (1H, *J*=5, 11 Hz)
26	38.7 d	1.78 m
27	99.6 s	
28	26.8 t	1.23 m, 1.95 m
29	27.4 t	1.42 m, 2.17 m
30	31.4 d	1.56 m
31	68.0 d	4.15 dt (1H, *J*=3, 10 Hz)
32	43.7 t	1.30 m, 1.54 m
33	64.1 d	3.97 m
34	25.4 q	1.18 d (1H, *J*=6.5 Hz)
35	18.6 q	1.14 d (1H, *J*=6.5 Hz)
36	4.6 q	0.84 m
37	9.9 q	1.01d (1H, *J*=6.5 Hz)
38	13.6 q	0.86 m
39	13.1 q	0.88 m
40	29.7 t	1.24 m, 1.39 m
41	12.3 q	0.85 m
42	6.6 q	0.91 d (1H, *J*=7 Hz)
43	12.3 q	0.99 d (1H, *J*=6.5 Hz)
44	11.5 q	0.94 d (1H, *J*=7 Hz)

The acetone-d_6 signals (2.06 ppm for ^{1}H and 29.8 ppm for ^{13}C) were used as references.
The coupling constants (Hz) are in parentheses.

Compound **1** affected the growth of *A. salina* at 0.2 μg/ml and *C. elegans* at 1 μg/ml.

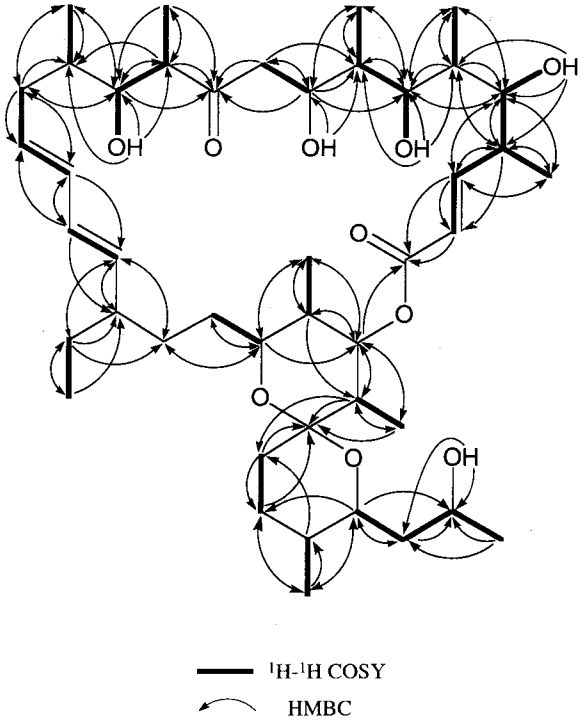
In vitro nematocidal activity of **1** was also studied against parasitic nematodes *Trichinella spiralis* and *Nippostrongylus brasiliensis*[7] comparing with oligomycin A. *T. spiralis* larvae were isolated from infected Bor CFW1 mice three weeks after oral infection. Twenty larvae per estimation were incubated with the compounds at 37°C for 72 hours. Thereafter the motility of the larvae was examined visually. The level of activity of the test compound was assessed on a scale 0~3 where 3=full activity (all larvae dead); 2=partial activity (<100% but>50% of the larvae dead or paralyzed larvae); 1=weak activity (>50% of larvae still alive); 0=no activity (number of living larvae equals that in the control).

Adult *N. brasiliensis* worms were isolated from the small intestine of female Wistar rats. Five worms (mixed sexes) were incubated with the compounds at 37°C for six days,

which was used for estimation of acetylcholinesterase (AChE) activity. Anthelmintic activity was ranked after incubation using the following scale: 3=full activity (95~100% AChE inhibition); 2=partial activity (75~95% inhibition); 1=weak activity (50~75% inhibition) and 0=no activity (<50% inhibition).

Both oligomycin A and **1** were partially active against *T. spiralis* in the range of 1~100 μg/ml (Table 6). As for *N. brasiliensis*, **1** showed weak activity at 10 and 100 μg/ml, but oligomycin A did not show activity even at 100 μg/ml.

Fig. 3. Structure of **1** elucidated by ^{1}H-^{1}H COSY and HMBC.



Compounds **1** and **2** showed antifungal activity against *Pyricularia oryzae*, *Aspergillus niger*, and *Mucor racemosus*, but showed no activity against bacteria and yeasts (10 μg/disk, paper disk method).

The IC_{50} values of **1** and oligomycin A against P388 cells were 2.5 ng/ml and 0.65 ng/ml, respectively.

Experimental

NMR spectra were obtained with Varian 400 MHz spectrometer using acetone-d_6 as a solvent. Mass spectrometry was conducted on a JEOL JMS-AX505 HA spectrometer. UV and IR spectra were measured with a Beckman DU640 spectrophotometer and a Horiba FT-210 Fourier transform infrared spectrometer, respectively. Optical rotation was recorded on a JASCO model DIP-181 polarimeter. Melting point was measured with a Yanaco micro melting point apparatus MP-S3.

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Table 6. *In vitro* activities of **1** and oligomycin A against *Trichinella spiralis* and *Nippostrongylus brasiliensis* at different concentrations.

	Concentration	*Trichinella spiralis*	*Nippostrongylus brasiliensis*
Oligomycin G (**1**)	100 μg/ml	2	1
	10	2	1
	1	2	0
	0.1	0	
Oligomycin A	100 μg/ml	2	0
	10	2	
	1	2	
	0.1	0	

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