OLIGOMYCIN E, A NEW ANTITUMOR ANTIBIOTIC PRODUCED BY *STREPTOMYCES* SP. MCI-2225

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A new oligomycin, oligomycin E (**1**), and known oligomycins A (**2**)[1] and B (**3**)[2], were obtained from the culture broth of *Streptomyces* sp. MCI-2225 as cytotoxic compounds against HeLa cells (a uterine carcinoma cell).

This paper reports the fermentation, isolation and structure of oligomycin E (**1**). Since no report has been made on cytotoxicity of oligomycins [A (**2**), B (**3**), C (**4**)[1], D (rutamycin) (**5**)[3,4] and rutamycin B (**6**)[5]] against HeLa cells, this paper also deals with the cytotoxicity of **1** and **2**~**4** in addition to their antibacterial and antifungal activities.

Microorganisms

Streptomyces sp. MCI-2225 was isolated from a soil of Tasmania, Australia. Morphological, cultural and physiological characteristics of this strain will be described in detail elsewhere.

Fermentation

Streptomyces sp. MCI-2225 on an agar slant was inoculated on a seed medium (40 ml) in a 200-ml Erlenmeyer flask. The seed medium (pH 7.0) was composed of maltose syrup 4%, soybean oil 0.3%, soybean meal 2%, Fermamedia 1%, Sungrowth L (Sungrowth Co., Osaka) 0.5%, $CaCO_3$ 0.3%, $FeSO_4 \cdot 7H_2O$ 0.001%, $CoCl_2 \cdot 6H_2O$ 0.0001% and $NiCl_2 \cdot 6H_2O$ 0.0001%. The flasks were incubated on a rotary shaker (210 rpm) for 4 days at 26°C. The seed culture was transferred into the medium described above in a 500-ml Erlenmeyer flask, and the flasks were incubated on a rotary shaker (210 rpm) for 6 days at 28°C.

Biological Assay

Using oligomycin E (**1**) and oligomycins A, B and C (**2**~**4**) obtained commercially (Sigma, St. Louis), the following biological assays were carried out.

For cytotoxicity against HeLa S_3 cells, the method was essentially according to that of MIRABELLI *et al.*[6]. MIC values for antibacterial activity against the bacteria shown in Table 4 were determined by the standard agar dilution method. Antifungal activity against *Pyricularia oryzae* F67-54 (rice blast disease fungus) was evaluated by the method of AKATSUKA *et al.*[7].

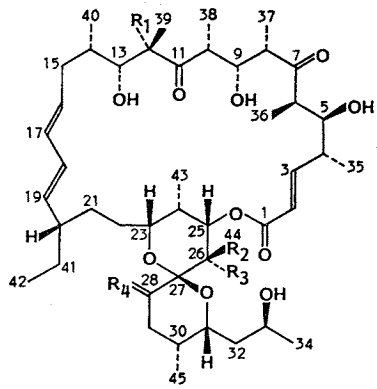
Isolation

The isolation procedure was monitored by cytotoxicity against HeLa cells. The filtered broth (10.8 liters) adjusted to pH 9.0 was extracted with EtOAc (5 liters × 2). A brown paste (7.9 g) from the concentrated extract was chromatographed over Silica gel (Merck, Kieselgel 60, 400 g) eluting with a $CHCl_3$ - MeOH mixture (95 : 5). The active fractions were combined, and concentrated to give a yellowish oil (5.1 g). Two hundred and fifty mg of the oil was applied on a Pre-sep C18 cartridge (Gasukuro Kogyo Co., Tokyo), and eluted successively with 15 ml each of 20%, 75% and 100% MeOH. This procedure was repeated twenty times to separate all of the yellowish oil. The concentrate of the active 75% MeOH fractions gave a colorless oily residue (424 mg), which was finally purified by HPLC (column: 10.7 × 250 mm packed with Unisil Q C18 (5 μm) (Gasukuro Kogyo Co.); eluent: 80% acetonitrile; flow rate: 5 ml/minute. Components with retention times of 7.9, 9.6 and 12.5 minutes were active, and gave oligomycin E (**1**) (76 mg), compounds I (**3**) (73 mg) and II (**2**) (60 mg), respectively by concentration. Oligomycin E (**1**) was colorless plates (52 mg) (recrystallized from EtOH). Compounds I (**3**) (58 mg) and II (**2**) (48 mg) were colorless prisms when recrystallized from MeOH - water.

Structure

The physico-chemical properties of oligomycin

Fig. 1. Structures of oligomycins.



Oligomycin E	(1)	R_1=OH,	R_2=CH_3,	R_3=OH,	R_4=O
Oligomycin A	(2)	R_1=OH,	R_2=CH_3,	R_3=H,	R_4=H_2
Oligomycin B	(3)	R_1=OH,	R_2=CH_3,	R_3=H,	R_4=O
Oligomycin C	(4)	R_1=H,	R_2=CH_3,	R_3=H,	R_4=H_2
Oligomycin D	(5)	R_1=OH,	R_2=H,	R_3=H,	R_4=H_2
Rutamycin B	(6)	R_1=H,	R_2=H,	R_3=H,	R_4=H_2

Table 1. Physico-chemical properties of oligomycin E (1).

Nature	Neutral, colorless plates
MP (°C)	120.5~121.5
$[\alpha]_D^{25}$	−49.1° (*c* 1.05, dioxane)
Formula	$C_{45}H_{72}O_{13}$
Anal Calcd for $C_{45}H_{72}O_{13}\cdot H_2O$:	C 64.42, H 8.89.
Found:	C 64.46, H 9.12.
FAB-MS (*m/z*)	821 $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	219 (sh), 224 (29,100), 232 (27,100), 240 (sh)
IR ν_{max}^{KBr} cm^{-1}	3456, 1703, 1644

E (**1**) is summarized in Table 1. The molecular formula of **1** was determined to be $C_{45}H_{72}O_{13}$ by fast atom bombardment mass specturm (FAB-MS) ($(M+H)^+$ *m/z* 821) and elemental analysis.

The IR spectrum of **1** indicated the existence of hydroxyl (3456 cm^{-1}) and carbonyl (1703 cm^{-1}) groups. The conjugated diene system was indicated by the IR (1644 cm^{-1}) and UV(λ_{max}^{MeOH} 224 nm) spectra[1]. These spectral properties suggested **1** to be an oligomycin.

In the ^1H and ^{13}C NMR spectra, similar signal patterns were observed between **1** and oligomycin B (**3**). The comparison of the NMR data between **1** and **3** (see Tables 2 and 3), especially the following signals, led us to determine the structure of **1** as 26-hydroxyoligomycin B (Fig. 1): ^1H NMR, 25-H: d in **1** vs. dd in **3**; 26-H: No signal in **1** vs. dq in **3**; 44-H_3: s in **1** vs. d in **3**; ^{13}C NMR, C-26: δ 74.05 in **1** vs. δ 31.27 in **3**; C-44: δ 21.52 in **1** vs. δ 11.69 in **3**.

The relative stereochemistry of the bicyclic spiroketal moiety of **1** (Fig. 1) was confirmed by the two-dimensional homonuclear nuclear Overhauser effect correlation spectroscopy (NOECOSY) experiment. Cross peaks were observed between: 23-H and 25-H; 23-H and 31-H; 24-H and 25-H; and 25-H and 44-H_3, but no cross peak between 43-H_3 and 44-H_3. Oligomycin E (**1**) is the first oligomycin having an oxidized C-26.

Compounds I and II were respectively identified as oligomycins B (**3**) and A (**2**) by comparison of their physico-chemical properties (mp, $[\alpha]_D$, IR, UV and NMR) with those of the authentic specimens.

Biological Activities

Biological activities of the oligomycins are listed in Table 4. The order of cytotoxic potency (IC_{50}) against HeLa cells is **2**>**1**=**3**>**4**. **1** is effective

Table 2. ^{1}H NMR data of oligomycins E (**1**) and B (**3**) (400 MHz, $CDCl_3$).

Position	Chemical shift[a] (*J*, Hz)	
	1	**3**
2	5.89 (d, 15.4)	5.84 (d, 15.4)
3	7.00 (dd, 15.4, 9.8)	6.69 (dd, 15.4, 10.2)
4	2.42 (ddd, 9.8, 9.8, 6.8)	2.40 (ddd, 10.3, 10.2, 6.8)
5	3.83 (d, 9.8)	3.81 (d, 10.3)
6	2.86 (q, 7.3)	2.76 (q, 6.8)
8	2.72 (qd, 7.3, 3.5)	2.76 (qd, 7.3, 3.0)
9	4.14 (dd, 8.9, 3.5)	3.99 (dd, 8.4, 3.0)
10	3.63 (dq, 8.9, 6.8)	3.65 (dq, 8.4, 6.8)
13	3.90 (s)	3.93 (s)
14	1.91[b]	1.87[b]
15	2.01 (ddd, 14.0, 10.9, 10.9), 2.20	2.06 (ddd, 14.0, 11.0, 11.0), 2.15
16	5.48 (ddd, 14.4, 10.9, 3.8)	5.45 (ddd, 13.9, 11.0, 3.7)
17	6.06 (dd, 14.4, 11.0)	6.05 (dd, 13.9, 11.0)
18	5.95 (dd, 14.6, 11.0)	5.94 (dd, 14.6, 11.0)
19	5.23 (dd, 14.6, 9.5)	5.18 (dd, 14.6, 9.5)
20	1.87[b]	1.84[b]
21	1.41[b], 1.63[b]	1.34[b], 1.61[b]
22	1.16[b], 1.73[b]	1.08[b], 1.63[b]
23	4.18 (br d, 10.0)	4.05[b]
24	2.16[b]	2.15[b]
25	5.00 (d, 5.9)	4.95 (dd, 11.7, 5.1)
26	—	2.53 (dq, 11.7, 6.8)
29	2.18, 3.18 (dd, 14.2, 5.6)	2.18, 3.04 (dd, 14.7, 5.9)
30	2.30[b]	2.20[b]
31	4.53 (br d, 10.2)	4.53 (d, 10.8)
32	1.44[b], 1.65[b]	1.38[b], 1.70 (ddd, 13.7, 10.8, 2.2)
33	4.03[b]	4.08[b]
34	1.28 (d, 6.8)	1.29 (d, 6.8)
35	1.21 (d, 6.8)	1.19 (d, 6.8)
36	1.04 (d, 7.3)	1.04 (d, 6.8)
37	1.06 (d, 7.3)	1.07 (d, 7.3)
38	1.08 (d, 6.8)	1.09 (d, 6.8)
39	1.14 (s)	1.13 (s)
40	1.01 (d, 7.3)	1.01 (d, 6.8)
41	1.30[b], 1.44[b]	1.28[b], 1.40[b]
42	0.85 (t, 7.3)	0.84 (t, 7.3)
43	1.07 (d, 6.8)	0.86 (d, 6.8)
44	1.29 (s)	0.87 (d, 6.8)
45	0.98 (d, 7.3)	0.96 (d, 7.3)

[a] Chemical shift in ppm from TMS.

[b] These signals are with unreadable coupling constants because of overlapping with other signals.

—: No signal.

against Gram-positive bacteria, which is different from the other oligomycins. However, all the oligomycins are inactive against Gram-negative bacteria. Although **2**, **3** and **4** show significant antifungal activity (IC_{50}) against *Pyricularia oryzae*, **1** exhibits weak activity (34% inhibition at 100 μg/ml). The order of the antifungal potency is **4**$\geq$**2**>**3**$\gg$**1**. These activity orders in the cytotoxicity and antifungal activity suggest that there are some relationships between the activities and the oxidation level at C-12, C-26 and C-28.

No report has been seen on antibacterial activity of the known oligomycins, but C-26 oxidized oligomycin E (**1**) is active against Gram-positive bacteria.

From these activity-oxidation level relation-

Table 3. ^{13}C NMR data of oligomycins E (**1**) and B (**3**) (100 MHz, $CDCl_3$).

Position	1	3	Position	1	3
1	165.44	165.19	24	35.59	35.80
2	122.03	122.47	25	74.20	75.84
3	150.03	148.91	26	74.05	31.27
4	40.43	40.29	27	97.64	100.15
5	73.14	73.01	28	207.89	203.21
6	45.17	46.41	29	45.03	44.11
7	219.77	220.05	30	37.98	37.04
8	46.30	46.03	31	68.01	67.18
9	72.51	72.74	32	41.49	41.70
10	42.11	41.92	33	64.31	64.58
11	220.80	220.36	34	25.11	25.03
12	83.04	83.11	35	17.96	17.94
13	72.51	72.04	36	9.06	9.37
14	33.53	33.66	37	8.64	8.42
15	38.47	38.38	38	14.00	14.02
16	129.77	129.92	39	21.18	21.10
17	132.27	132.23	40	14.44	14.58
18	130.56	130.68	41	28.53	28.71
19	137.38	137.16	42	12.12	12.14
20	45.92	46.15	43	8.61	5.97
21	31.23	31.19	44	21.52	11.69
22	30.37	30.78	45	12.67	12.81
23	70.94	71.15			

Chemical shift in ppm from TMS.

Table 4. Biological activities of oligomycins E (**1**), A (**2**), B (**3**) and C (**4**).

Organism	1	2	3	4
		IC_{50} (μg/ml)[a]		
Uterine carcinoma cell HeLa S_3	0.014	0.008	0.015	0.106
		MIC (μg/ml)		
Bacteria				
Staphylococcus aureus FDA 209PJC 1	6.25	>100	>100	>100
S. aureus Terajima	12.5	>100	>100	>100
S. aureus MS353	12.5	>100	>100	>100
Bacillus subtilis ATCC 6633	100	>100	>100	>100
Micrococcus luteus ATCC 9341	25	>100	>100	>100
Escherichia coli NIHJ JC 2	>100	>100	>100	>100
Klebsiella pneumoniae PCI 602	>100	>100	>100	>100
Salmonella typhimurium IID 971	>100	>100	>100	>100
Serratia marcescens IAM 1184	>100	>100	>100	>100
Pseudomonas aeruginosa IFO 3445	>100	>100	>100	>100
Morganella morganii IFO 3848	>100	>100	>100	>100
Proteus vulgaris HX 19	>100	>100	>100	>100
Providencia rettgeri IFO 3850	>100	>100	>100	>100
Enterobacter aerogenes ATCC 13048	>100	>100	>100	>100
		IC_{50} (μg/ml)[b]		
Rice blast fungus *Pyricularia oryzae* F67-54	131	3.5	16.9	2.2

[a] Concentration causing 50% inhibition of cell growth.
[b] Concentration causing 50% inhibition of spore germination.

ships, the oxidation level at C-12, C-26 and C-28 is expected to be an important structural factor for various biological activities and to be related to the activity-hydrophilicity relationships.

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