ISOLATION OF OLIGOMYCIN A AS A RESULT OF SCREENING FOR ANTAGONISTS OF LIPIDS

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In the course of our screening for antagonists of lipids from microbial origin, we noticed the presence of an antimicrobial substance inhibited by oleic acid in a culture of *Streptomyces* sp. No. 178. This organism, isolated from soil, was similar to *Streptomyces chibaensis*. The substance named No. 178 was identified as oligomycin A and inhibited the growth of *Rhodotorula glutinis* 110 and several kinds of moulds. However, the antimicrobial activity was inhibited by the presence of oleic acid.

In this paper we wish to describe the isolation process, chemical characterization and identification as oligomycin A as well as the antagonism to various lipids of this substance.

The producing strain was cultivated in modified BENNETT's medium composed of 1% glucose, 0.1% Polypeptone, 0.1% meat extract, 0.1% yeast extract (adjusted to pH 7.0) for 3 days at 30°C. The culture filtrate was adjusted to pH 3. The active substance was extracted with ethyl acetate, adsorbed on a column of silica gel and eluted with chloroform - ethyl acetate (80: 20). After concentration *in vacuo*, it was obtained as colorless crystals, mp 148~150°C.

The color reaction of the active substance was positive to bromothymol blue, phosphomolybdic acid and potassium permanganate. Its UV spectrum showed absorption peaks at 226 nm ($E^{1\%}_{1cm}$ 490), 232 nm ($E^{1\%}_{1cm}$ 460) and 241 nm ($E^{1\%}_{1cm}$ 280) in methyl alcohol. At higher concentration a broad absorption from 280 nm to 300 nm appeared. The IR spectrum showed absorption

Fig. 1. Comparison of IR spectra of No. 178 substance and authentic oligomycin A.

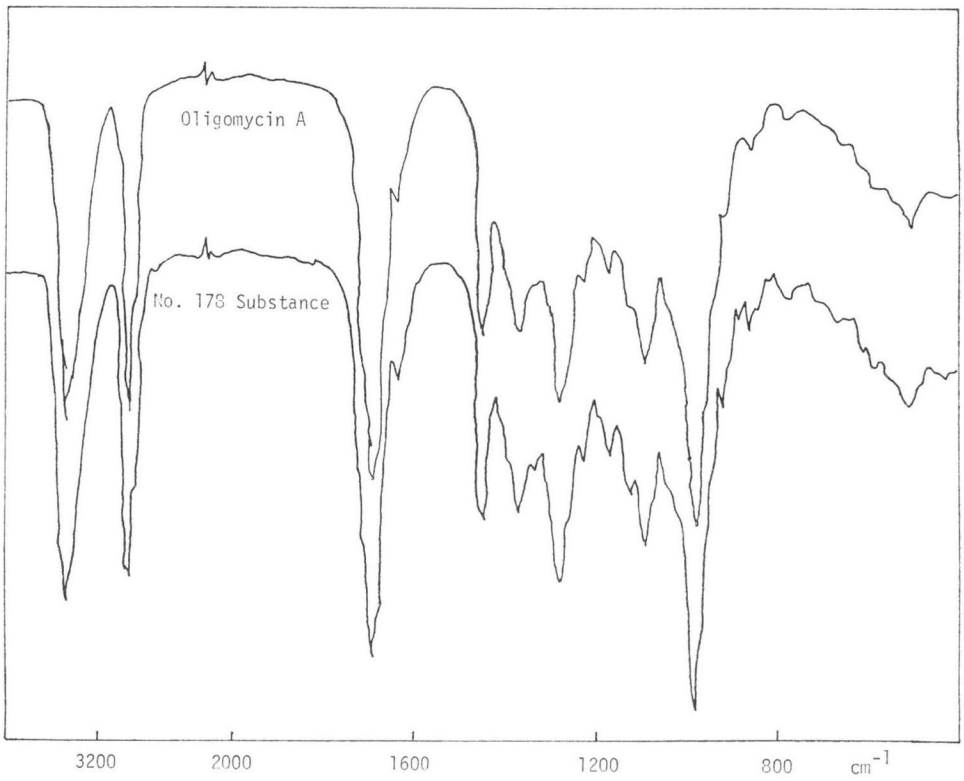

Table 1. Comparison of physico-chemical characteristics of No. 178 substance and authentic oligomycin A.

	No. 178 substance	Oligomycin A
Elementary analysis	C: 65.34% H: 9.40 N: 0	C: 67.0 % H: 9.50 N: 0
mp	148~150°C	150~151°C
UV	225, 232, 241 280~300 nm	225, 232, 240 285 nm
IR	3475, 1695, 1635 cm^{-1}	3470, 1700, 1638 cm^{-1}
TLC* (Rf)	1) 0.45 2) 0.40 3) 0.28	1) 0.46 2) 0.40 3) 0.28

* Development solvent systems
1) Benzene - acetone (5: 1)
2) Chloroform - methanol - acetic acid (97: 3: 0.1)
3) Chloroform - ethyl acetate (3: 1).

bands at 3475 cm^{-1} (hydroxyl), 1695 cm^{-1} (carbonyl) and 1635 cm^{-1} (unsaturation). The optical rotation of No. 178 substance was $[\alpha]_D^{13} -72.6°$ (*c* 1, chloroform).

Anal. Found: C, 65.34; H, 9.40; N, 0%.

From the data mentioned above, No. 178 substance was assumed to be very similar to oligomycin A,[1,2] which is well known as an inhibitor of energy metabolism. The assumption was supported by comparison of the IR spectra of No. 178 substance and authentic oligomycin A as shown in Fig. 1. Moreover, the physico-chemical data of No. 178 substance were consistent with authentic oligomycin A and the two were indistinguishable on silica gel TLC with various solvent systems (Table 1).

The biological activity of No. 178 substance was examined by conventional paper disc methods on synthetic medium containing 15 g glucose, 1 g NH_4NO_3, 1 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.5 g $CaCl_2 \cdot 2H_2O$, 10 mg $FeCl_3 \cdot 6H_2O$, 10 mg $ZnSO_4 \cdot 7H_2O$ and 10 g agar in 1 liter water (pH 5.5) using *R. glutinis* 110 as the test organism.

As shown in Table 2, the antimicrobial activity of No. 178 substance was diminished in proportion to an increase in the concentration of oleic acid.

Table 2. Antagonistic activity of oleic acid to No. 178 substance.

Oleic acid	No. 178 substance	
	100 mcg/ml	200 mcg/ml
0 mcg/ml	18 mm*	19 mm
50	17	18
100	14	15
200	10	12
400	none	none

* Diameter of inhibitory zone.

Table 3. Reversal of inhibition of No. 178 substance by some specific lipids.

Substance (400 mcg/ml)	Diameter of inhibitory zone (mm) Conc. of No. 178 substance	
	125 mcg/ml	250 mcg/ml
None	17	18
Myristic acid	14	15
Palmitic acid	15	16
Stearic acid	13	15
Oleic acid	none	none
Tween 40	13	13
Tween 80	12	13
Lecithin (soybean)	none	none
Mevalonic acid	16	19
Phytol	none	none
Squalene	12	16
Ergosterol	15	18
Cholesterol	16	18

The inhibitory effects of fatty acids, steroids and isoprenoids on the activity of No. 178 substance were then examined. The experimental conditions were the same as those described above for oleic acid, but the concentrations of the lipids in the medium were always 400 mcg/ml. As shown in Table 3, the activity of No. 178 substance was slightly reversed by Tween 40 and Tween 80, but markedly inhibited by oleic acid, soybean lecithin and phytol. The activity of No. 178 substance was not reversed by myristic acid, palmitic acid, stearic acid and steroids.

The effects of vitamins on the activity of No. 178 substance were also tested. As shown in Table 4, vitamin D_2 and vitamin E had a remarkable inhibitory effect, while water-soluble

vitamins did not exhibit such inhibitory activities.

Similar experiments were carried out using *Aspergillus niger* IFO 4416 as the test organism. The results obtained in this case were consistent with those described above.

Finally, antimycin A[3] and dicyclohexylcarbodiimide,[4] inhibitors of energy metabolism, were tested for antagonistic actions of lipids against their activities. Similar results were obtained as in the case of No. 178 substance.

It is concluded that some specific lipid substances antagonize the actions of certain inhibitors of energy metabolism similar to oligomycin A in fungal physiology.

Table 4. Reversal of inhibition of No. 178 substance by vitamins.

Substance (400 mcg/ml)	Diameter of inhibitory zone (mm) Conc. of No. 178 substance	
	125 mcg/ml	250 mcg/ml
None	18	20
Vitamin A	14	15
Vitamin D_2	none	none
Vitamin E	none	none
Vitamin K_1	13	15
Folic acid	17	19
Riboflavin	16	19
Vitamin B_1	15	18
Nicotinamide	16	18
Pyridoxine	17	19

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