ANTIBIOTIC DE-3936, A POLYETHER ANTIBIOTIC IDENTICAL WITH LONOMYCIN

TAXONOMY, FERMENTATION, ISOLATION AND CHARACTERIZATION

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Antibiotic DE-3936 was isolated from the fermentation broth of a streptomycete No. 9735-1, which is identified as a strain of *Streptomyces hygroscopicus*. The antibiotic is a hydrophobic compound having the molecular formula of $C_{44}H_{75}O_{14}Na$ and is active against Gram-positive bacteria, mycobacteria, mycoplasma and protozoa, especially coccidia. Its chemical and biological properties indicate that antibiotic DE-3936 belongs to the group of polyether antibiotics and is identical with lonomycin.

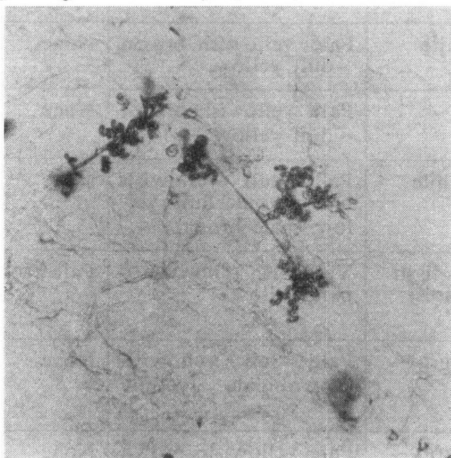
In the course of our screening for new antibiotics, we found that a streptomycete, strain No. 9735-1, isolated from a soil sample collected at Musashino-City, Tokyo, produced an antibiotic complex which was mainly active against Gram-positive bacteria. The main component was designated as antibiotic DE-3936. From its physico-chemical properties and biological activities, antibiotic DE-3936 was considered to be one of the polyether antibiotics such as nigericin[1], X-206[2], A-204 A[3], A-28695 A and B[4], septamycin[5,6] and lonomycin[7]. Recently its structure has been elucidated by X-ray crystallographic analysis of its silver salt by YAMAZAKI *et al.*[8] as shown in Fig. 1. As a result, it was proved that antibiotic DE-3936 is identical with lonomycin, the structure of which has been determined independently by ŌTAKE *et al.*[9]

Taxonomic studies on strain No. 9735-1 were carried out on its morphological, cultural and physiological characteristics. As a result of detailed comparisons with known species of *Streptomyces*, the strain was identified as a strain of *Streptomyces hygroscopicus* (JENSEN) WAKSMAN and HENRICI (1948); it was therefore named *Streptomyces hygroscopicus* No. 9735-1. The strain No. 9735-1 has been deposited in the Fermentation Research Institute, Chiba, Japan, and assigned accession number FERM-P No. 3159. This paper describes the taxonomic characteristics of the strain, as well as fermentation, isolation, and physico-chemical and biological properties of antibiotic DE-3936.

Description of the Producing Strain

Strain No. 9735-1 was isolated from a soil sample collected at Musashino-City, Tokyo. Taxonomic studies were generally carried out in accordance with the methods adopted by the International Streptomyces Project (ISP)[10] and SHINOBU[11]. The media used in this study were prepared according to the recommendation of SHIRLING and GOTTLIEB[10], WAKSMAN[12] and SHINOBU[11].

Plate 1. Photomicrograph of aerial mycelia of strain No. 9735-1 cultured on glucose asparagine agar for 10 days (×800)



(1) Morphological Characteristics

Morphological characteristics of the strain were observed with cultures incubated at 28°C for 10～14 days on various media, such as ammonium CZAPEK's agar, glucose asparagine agar, inorganic salts-starch agar, tyrosine agar and glycerol starch glutamate agar. Microscopical observations of morphological characteristics were made with both optical and electron microscopes.

The following data were mainly observed with cultures on glucose asparagine agar: Substrate mycelium is flexuous or wavy, and does not fragment into bacillary or coccoid forms. Conidiophores are formed as short branches located along straight or flexuous main axial hyphae of aerial mycelia, and terminated in mostly closed spirals of two or more turns, sometimes in hook- and loop-like spirals, seldom in open spirals (Plate 1). Conidia are oval to short cylindrical (0.8～1.0 μ× 1.0～1.5 μ), sometimes non-segmented, with warty surface (Plate 2). Whirls, zoospores, sporangia or sclerotia were not observed.

(2) Cultural Characteristics

All cultures were incubated at 28°C for 21 days and observations were carried out every 7 days after inoculation. The color terms recorded for mature cultures were described according to 'Guide to Color Standard'[13] and 'Dictionary of Color Names'[13]. The cultural characteristics are shown in Table 1.

(3) Physiological Characteristics

The physiological characteristics, including the utilization of carbon sources, investigated with the methods of PRIDHAM and GOTTLIEB[14], are shown in Tables 2 and 3.

From the above results, the characteristics of strain No. 9735-1 are summarized as follows: (1) The strain No. 9735-1 belongs to the genus *Streptomyces*. (2) The aerial mycelium exhibits hygroscopic properties. (3) The conidiophores terminate in mostly closed spirals. The conidia surface is warty. (4) The color of the aerial mycelium is brownish gray on most media, and no soluble pigment is produced on most media. (5) Tyrosinase reaction, milk

Plate 2. Electronmicrograph of conidia of strain No. 9735-1 cultured on glucose asparagine agar for 12 days. (×8,000)

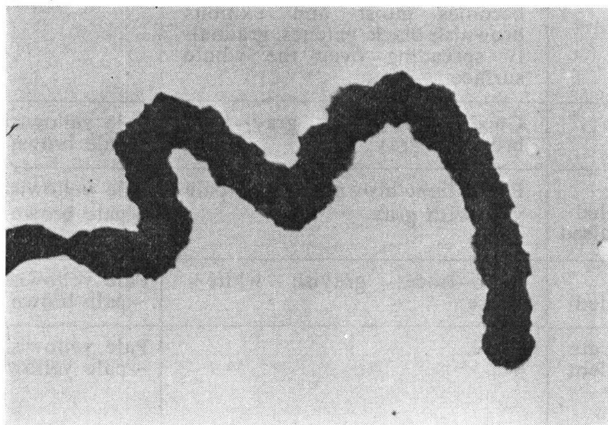


Table 1. Cultural characteristics of strain No. 9735-1

Medium	Growth	Aerial mycelium	Substrate mycelium	Soluble pigment
Sucrose CZAPEK's agar	Good~moderate	Poor; brownish white~white	Pale yellowish brown ~dull yellow	None
Glycerol CZAPEK's agar	Moderate	Poor; light brownish gray~white	Pale yellowish brown ~dull yellow	None
Ammonium CZAPEK's agar	Moderate	None, sometimes trace; white	Pale dull yellowish brown~pale dull yellow~pale brown	None
Glucose-asparagine agar	Moderate	Moderate; brownish gray~light brownish gray; becomes moist	Yellowish brown~pale yellow	Pale brown
Glycerol-asparagine agar	Moderate	Moderate; light brownish gray~brownish white	Pale dull yellowish brown~pale yellow	None
Calcium-malate agar	Moderate	Poor; light brownish gray~brownish white; sometimes partially yellowish white	Pale yellowish brown ~dull yellow	None
Inorganic salts-starch agar	Moderate	Moderate; light brownish gray~brownish white, sometimes partially purplish gray or yellowish gray; becomes moist and exhibits dark brown patches	Pale yellowish brown ~pale yellow	None
Tyrosine agar	Good	Good~moderate; light brownish gray~brownish white, sometimes partially yellowish white; becomes moist	Brown~pale yellowish brown	At first reddish brown, later pale brown
Glycerol starch glutamate agar	Moderate ~poor	Poor~trace; brownish white~white	Light brownish gray ~dull yellow	None
Nutrient agar	Moderate; wrinkled	None	Pale yellow	None
Glucose nutrient agar	Moderate	None	Pale yellowish brown ~pale yellow	None
Yeast extract-malt extract agar	Good	Good; brownish gray~light brownish gray, sometimes partially pale yellowish gray; becomes moist and exhibits brownish black patches, gradually spreading over the whole surface	Yellowish brown~dull yellow	None
Oatmeal agar	Good	Good; brownish gray~light brownish gray	Pale yellowish brown ~pale brown	None
Potato plug	Good; wrinkled and raised	Poor; light brownish gray~pale yellowish gray	Pale yellowish brown ~pale brown	None
Whole egg medium	Poor; wrinkled	Poor~trace; grayish white~white	Pale yellowish brown ~pale brown	None
Glucose nutrient broth	Moderate; flocculent in the bottom	None	Pale yellowish brown ~pale yellow	None

Table 2. Physiological characteristics of strain No. 9735-1

Optimum temperature for growth	26~30°C
Optimum pH for growth	6~ 7
Tyrosinase reaction	Negative
Hydrolysis of starch	Positive
Reduction of nitrate	Positive
Liquefaction of gelatin	Positive (20°C)
Coagulation of milk	Negative
Peptonization of milk	Positive
Cellulose decomposition	Negative
Chromogenicity	Negative

coagulation and cellulose decomposition are negative. Nitrate reduction, starch hydrolysis, gelatin liquefaction and milk peptonization are positive. (6) The chromogenicity is negative (non-chromogenic type). (7) Carbon sources are generally utilized well.

(4) Comparison of Strain No. 9735-1 with Other Known Species

Among the known species of *Streptomyces* described in 'BERGEY's Manual of Determinative Bacteriology' 7th Ed.[15] and 8th Ed.[16], WAKSMAN's 'The Actinomycetes; Vol. II[12], the ISP reports[17] and other literature, *Streptomyces hygroscopicus* (JENSEN) WAKSMAN and HENRICI is closely related to strain No. 9735-1. However, there were some differences in the following points: Formation of aerial mycelium on nutrient agar, soluble pigment on sucrose CZAPEK's agar and glucose asparagine agar, nitrate reduction and carbon source utilization. TRESNER and BACKUS[18] pointed out the following three fundamental characteristics of *Streptomyces hygroscopicus*: (1) The brownish-gray (Mouse Gray to Benzo Brown of RIDGWAY, 1912) color of the spore in mass. (2) The tightly wound coils of the spore-bearing hyphae. (3) The characteristic black hygroscopic areas on certain media. Strain No. 9735-1 has such fundamental characteristics of *Streptomyces hygroscopicus*.

Table 3. Carbon source utilization of strain No. 9735-1

Carbon source	Growth	Carbon source	Growth
L-Arabinose	+	Starch	+++
D-Xylose	±	Inulin	++
L-Rhamnose	+++	Dulcitol	−
D-Glucose	+++	Inositol	−
D-Galactose	+++	D-Sorbitol	−
D-Fructose	++	Glycerol	+++
D-Mannose	+	Salicin	−
Sucrose	+++	D-Mannitol	+++
Lactose	+++	Na-Citrate	−
D-Maltose	±	Na-Succinate	+
Trehalose	+	Na-Acetate	−
Raffinose	+++	Negative control	−

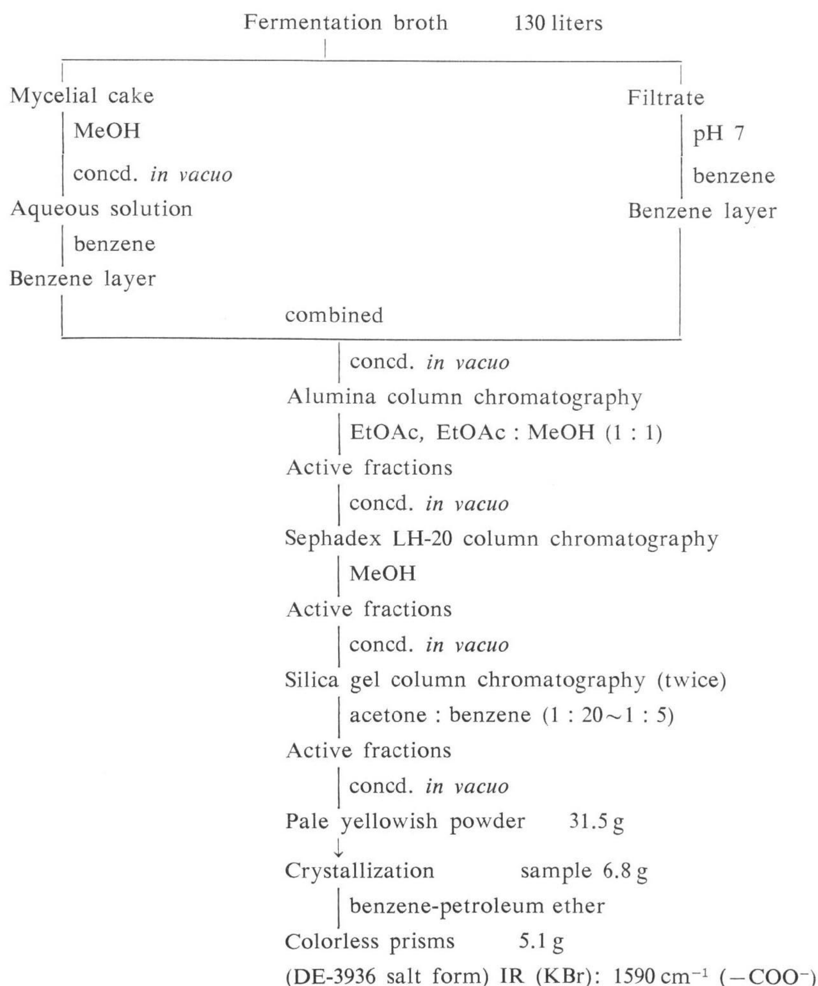
+++: good growth ++: moderate growth
+~±: poor growth −: no growth

Therefore, it is reasonable to conclude that strain No. 9735-1 is one of the strains of *Streptomyces hygroscopicus* (JENSEN) WAKSMAN and HENRICI (1948), and it was named *Streptomyces hygroscopicus* No. 9735-1.

Fermentation and Isolation

Streptomyces hygroscopicus No. 9735-1 was inoculated into 500-ml SAKAGUCHI-flasks each containing 100 ml of the following medium: 2 % glucose, 0.5 % peptone, 0.3 % meat extract, 0.3 % dry yeast, 0.5 % NaCl and 0.3 % $CaCO_3$ (pH 7). The flasks were incubated at 28°C for 3 days on a reciprocal shaker, and the resulting culture (1 liter) was transferred to 130 liters of fermentation medium in a 200-liter fermentor. Fermentation medium was composed of 2.5 %

Chart 1. Isolation and purification procedures for antibiotic DE-3936



glucose, 0.5 % cotton seed meal, 0.5 % corn steep liquor, 0.5 % NaCl and 0.3 % $CaCO_3$ (pH 7). The fermentation was carried out for 3~6 days under the following conditions: temperature 30°C, aeration 70 liters per minute and agitation 200 rpm.

Antibiotic DE-3936 could be extracted with common organic solvents from both mycelial cake and broth filtrate, and the extract was purified by alumina column chromatography using a solvent system of ethylacetate-methanol. Further purification was achieved by column chromatography on Sephadex LH-20 (solvent: methanol) and on silica gel (solvent system: acetone-benzene), and by crystallization from such a solvent system as benzene-petroleum ether, benzene-*n*-hexane and methanol-water. The procedures for isolation, purification and crystallization are summarized in Chart 1.

Physico-Chemical Properties

Antibiotic DE-3936 obtained as described above is usually a mixture of sodium and potassium salts, and occasionally of ammonium salt, of carboxylic acid (ν_{max}^{KBr} 1590 cm^{-1}). The

sodium and potassium salts, methylester and free acid of DE-3936 were prepared.

Antibiotic DE-3936 sodium salt crystallizes as colorless prisms melting at 173~176°C with decomposition, is soluble in most organic solvents but practically insoluble in water. The specific rotation is $[\alpha]_D^{25}$ +67.0° (*c* 1, chloroform) and $[\alpha]_D^{25}$ +49.8° (*c* 1, methanol). The antibiotic exhibits no characteristic UV absorption in methanol solution. It gives positive sulfuric acid, *p*-anisaldehyde, antimony trichloride, vanillin-sulfuric acid and iodine vapor reactions on silica gel plates, and negative MOLISCH, anthrone and ninhydrin reactions.

The free acid of antibiotic DE-3936 crystallizes as colorless prisms melting at 109~114°C, and the specific rotation is $[\alpha]_D^{20}$ +66.6° (*c* 1, chloroform). The pKa′ value is 5.9 in 66 % acetone. Its solubility and color reactions are similar to those of the sodium salt.

As shown in Fig. 2, the IR spectrum of the sodium salt (in chloroform) indicates the presence of hydroxyl group (3150 cm^{-1}), —C—O—C— (1080 cm^{-1}) and carboxylate (1580 cm^{-1}). The peak at 1580 cm^{-1} shifts to 1730 cm^{-1} for the free acid. The molecular weight and the molecular formula were determined by mass spectrometry[10], high resolution mass spectrometry and elemental analysis. The mass spectrum of the sodium salt showed the molecular ion at *m/e* 850, and fragment ions due to loss of $-CH_3$, $-OCH_3$ and CO_2 appeared at *m/e* 835, 819 and 806, respectively. The mass spectrum of the potassium salt exhibited identical fragmentation patterns in the region of above *m/e* 800, with the appropriate 16-mass units shift. That is, the molecular and fragment ions were *m/e* 866 (M^+), 851 (M^+-CH_3), 835 (M^+-OCH_3) and 822 (M^+-CO_2). On the other hand, the molecular ion of the free acid was not observed, and the first peak was observed at *m/e* 766 corresponding to the formula $C_{43}H_{74}O_{11}$. This peak was due to the loss of both CO_2 and H_2O from the molecule. In the mass spectrum of the methylester, the dehydration peak from the molecule was found at *m/e* 824. As a result of mass spectrometry, the most likely molecular formula was $C_{44}H_{76}O_{14}$ for the free acid, $C_{44}H_{75}O_{14}Na$ for the sodium salt and $C_{45}H_{78}O_{14}$ for the methylester.

Fig. 1. The structure of antibiotic DE-3936 Na salt

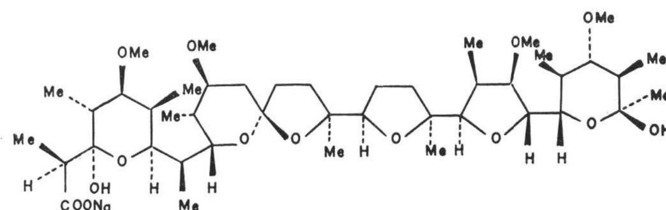
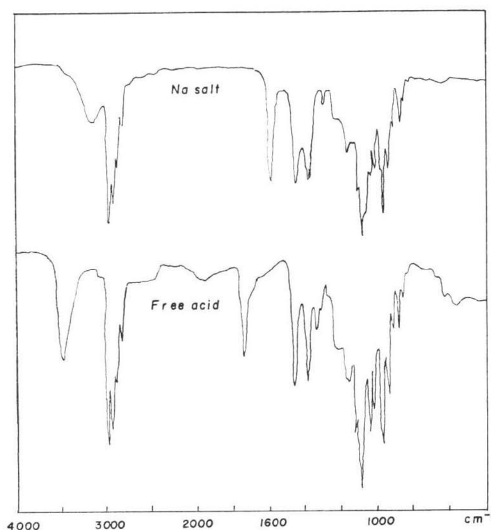


Fig. 2. IR absorption spectra of antibiotic DE-3936 ($CHCl_3$)



Elemental analysis supported the results of mass spectrometry:

Fig. 3. NMR spectrum of antibiotic DE-3936 sodium salt ($CDCl_3$, 100 MHz)

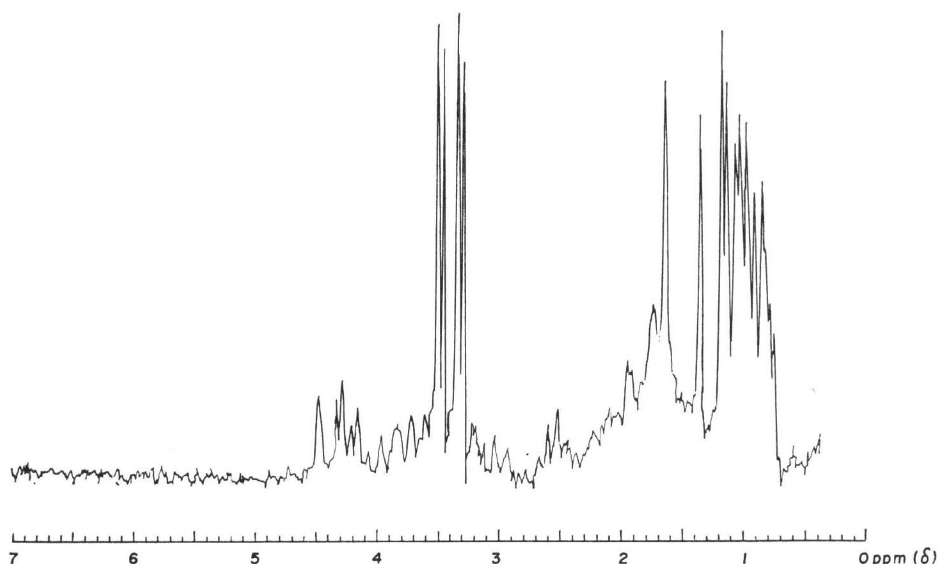
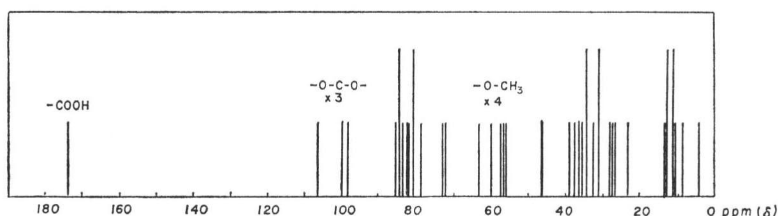


Fig. 4. ^{13}C NMR spectrum of antibiotic DE-3936 free acid ($CDCl_3$)



Anal. Free acid; Calcd. for $C_{44}H_{76}O_{14} \cdot C_6H_6 \cdot H_2O$: C 64.91; H 9.15
Found: C 64.65; H 9.05

Sodium salt; Calcd. for $C_{44}H_{75}O_{14}Na$: C 62.10; H 8.88; Na 2.70
Found: C 62.52; H 8.90; Na 2.51

The NMR spectrum of the sodium salt in $CDCl_3$ (Fig. 3) showed the presence of four methoxyl groups at $\delta 3.3 \sim 3.5$. The ^{13}C NMR of the free acid in $CDCl_3$ (Fig. 4) indicated the presence of four methoxyl groups at δ 56.0, 56.7, 57.3 and 60.0, one carboxyl group at δ 173.8, three —O—C—O— at δ 98.5, 100.0 and 106.6 and many $-C-CH_3$, and confirmed that antibiotic DE-3936 possesses forty four carbon atoms. These chemical properties suggest that antibiotic DE-3936 belongs to the group of polyether antibiotics. The unusual solubility properties (very hydrophobic nature) are common to polyether antibiotics.

Biological Properties

The antimicrobial spectrum of antibiotic DE-3936 is shown in Table 4. The antibiotic is active against Gram-positive bacteria, mycobacteria and mycoplasma, but does not inhibit the growth of Gram-negative bacteria and fungi. It exhibits activity also against protozoa, especially coccidia, and is effective in the treatment of coccidiosis in chickens at dosages of $50 \sim 125$

Table 4. Antimicrobial spectrum of antibiotic DE-3936 sodium salt

Test organism	MIC*(mcg/ml)	Medium**
Staphylococcus aureus FDA 209 P	12.5	I
Staphylococcus aureus Smith	6.25	I
Staphylococcus aureus 39 (PC-G, SM, TC, Macrolides-R)	3.12	I
Streptococcus faecalis ATCC 8043	1.56	I
Streptococcus pneumoniae DP-1	1.56	I
Bacillus subtilis ATCC 6633	3.12	I
Sarcina lutea ATCC 9341	3.12	I
Mycobacterium ATCC 607	12.5	I
Escherichia coli NIHJ	>100	I
Pseudomonas aeruginosa No. 15	>100	I
Mycoplasma hominis DH	100	I
Mycoplasma gallisepticum GP-31	25	I
Mycoplasma pulmonis M-53	50	I
Piricularia oryzae	>100	II
Trichophyton mentagrophytes	>100	II
Candida albicans	>100	II

* MIC was determined by agar dilution method.
** Medium I; Heart infusion agar, Medium II; SABOURAUD's agar

mg/kg of feed. The acute toxicity (LD_{50}) of the sodium salt in mice is 45.8 mg/kg orally, 13.0 mg/kg intraperitoneally and 37.5 mg/kg subcutaneously.

Comparison with Other Known Polyether Antibiotics

Antibiotic DE-3936 was compared with polyether antibiotics such as A-28695 A, septamycin, A-218[20] and lonomycin, all having four methoxyl groups and no characteristic UV absorption. Comparison of these antibiotics is shown in Table 5. The silica gel thin-layer chromatogram of antibiotic DE-3936 and related polyether antibiotics is shown in Fig. 5. The physico-chemical characteristics and chromatographic behavior of antibiotic DE-3936 were identical with those of lonomycin. The IR spectra of antibiotic DE-3936 (sodium salt) and lonomycin are shown in Fig. 6.

Experimental

General

Melting points were determined with a capillary melting point apparatus. UV spectra were obtained with a Hitachi 124 spectrophotometer. IR spectra were obtained with a Hitachi 285 grating infrared spectrophotometer. NMR spectra were obtained at 100 MHz with a JNM 4H-100. ^{13}C NMR spectra were obtained at 15 MHz with a JNM FX-60. Mass spectra were taken with JMS O1SG-2 mass spectrometer. Specific rotations were measured with a Perkin-Elmer 141 polarimeter.

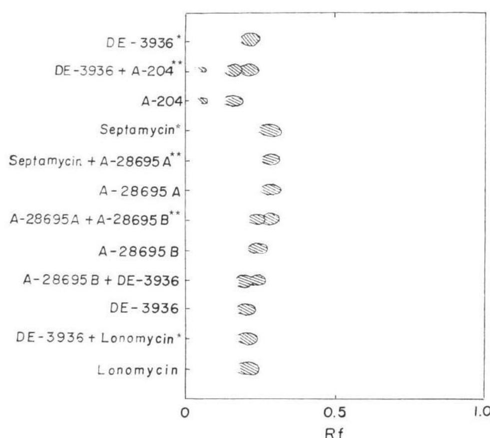
Thin-layer chromatography was carried out on silica gel (type 60, Merck) using ethylacetate as a solvent. Antibiotic DE-3936 was detected by spraying with a solution of vanillin (2 %) in ethanol - conc. sulfuric acid (100 : 1, v/v), and then heating at about 100°C for several minutes.

Table 5. Characterizations of the polyether antibiotics with four methoxyl groups

		DE-3936 (Na salt)	A-28695 A (Na salt)	Septamycin (Na salt)	A-218 (Na salt)	Lonomycin (Na salt)
Producing strain		*Streptomyces hygroscopicus* No. 9735-1	*Streptomyces albus* NRRL 3885	*Streptomyces hygroscopicus* NRRL 5678	*Streptomyces hygroscopicus* A-218	*Streptomyces ribosidificus* TM-481
mp (°C)		173～176	159～160	164～166	187～188	188～189
Specific rotation		$[\alpha]_D^{25}$ +67.0° (*c* 1, $CHCl_3$) $[\alpha]_D^{25}$ +49.8° (*c* 1, MeOH)	$[\alpha]_D^{25}$ +14.07° (*c* 1, MeOH)*	$[\alpha]_D^{20}$ +14.4° (*c* 1, MeOH)	$[\alpha]_D^{23}$ +52.3±0.8° (*c* 0.664, EtOH)	$[\alpha]_D^{20}$ +47° (*c* 1, MeOH)
Molecular formula		$C_{44}H_{75}O_{14}Na$		$C_{48}H_{81}O_{16}Na$	$C_{46}H_{79}O_{15}Na$	$C_{44}H_{75}O_{14}Na$
MW		850 (M^+)	874 (titration)	937 (osmometry)	921 (osmometry)	846 (osmometry)
Elemental analysis (Found)		C 62.52 H 8.90 Na 2.51	C 63.31** H 8.83 O 28.03	C 61.7 H 9.0 Na 2.4	C 61.85 H 8.95 Na 2.66	C 61.93 H 8.64 Na 2.68
UV absorption		none	none	none	none	none
Solubility	soluble in	most organic solvents		benzene, chloroform, acetone, MeOH	most organic solvents	EtOAc, benzene, chloroform, MeOH, EtOH, acetone, *n*-hexane, petroleum ether, ether
	insoluble in	water		water	water	water
Acute toxicity LD_{50}(mg/kg, mouse)		13.0 (IP), 45.8 (PO) 37.5 (SC)	41.1 (PO)		10～20 (IP)	8.28 (IP), 4.86 (IV)

* mixed Na and K salt, ** free acid

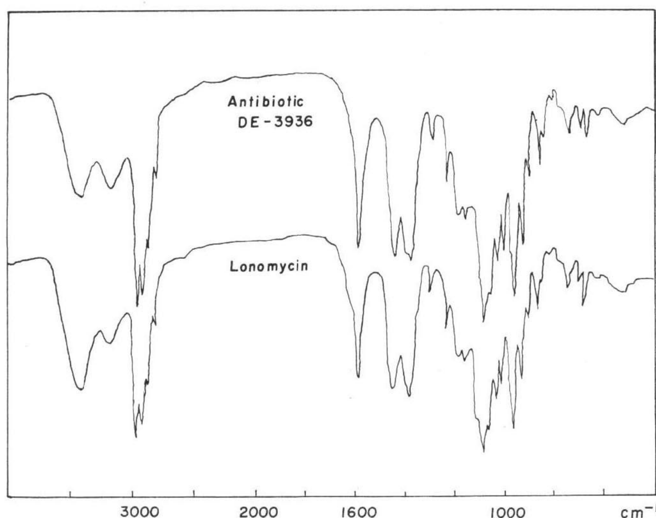
Fig. 5. Silica gel thin-layer chromatogram of antibiotic DE-3936 and related polyether antibiotics
Solvent: EtOAc-benzene (1:1) Plate: Silica gel 60 F254 (Pre-coated TLC plate, Merck) Detection: Vanillin-H_2SO_4
* Na salt ** mixed Na and K salts



Isolation

Mycelial cake (wet weight, 2.6 kg) and 120 liters of filtrate were obtained from about 130 liters of whole broth. The mycelial cake was suspended in 15 liters of methanol, and the mixture was allowed to stand overnight after stirring for about ten minutes, and then filtered. The residual mycelial cake was re-extracted with 5 liters of methanol. The methanol extracts thus obtained were combined and concentrated to remove the methanol. Two liters of aqueous residue was extracted four times with 3 liters of benzene. Culture filtrate (pH 7) was extracted twice with benzene (firstly; 35 liters, secondly; 20 liters). The benzene layers obtained above were combined, and concentrated to obtain a reddish brown oily residue. This oily substance was dissolved in benzene, and charged on a column of alumina oxide packed in benzene. The column was washed with benzene, and then eluted with ethylacetate and succesively with ethylacetate-methanol (1 : 1). The antibiotic fractions were combined and concentrated to an oily substance. The residue was dissolved in methanol and purified by Sephadex LH-20 column chromatography using methanol as an eluant. The effluent containing antibiotic DE-3936 was concentrated to dryness *in vacuo*. The oily residue was dissolved in benzene, and adsorbed on a silica gel column packed in benzene. After the column was washed with benzene and acetone-benzene (1:50), the antibiotic was eluted with acetone-benzene (1:20 to 1 : 5). Antibiotic fractions were concentrated *in vacuo*. Silica gel column chromatography was repeated twice, and 31.5 g of pale yellowish powder was obtained. During the purification, the antibiotic fractions were monitored carefully by silica gel thin-layer chromatography.

Fig. 6. IR absorption spectra of antibiotic DE-3936 and lonomycin (KBr)



Crystallization

The pale yellowish powder (6.8 g) obtained above was crystallized from benzene-petroleum

ether at 4°C, and 4.6 g of colorless prisms was obtained. The mother liquor was concentrated to yield more crystals. The residue was also crystallized from benzene - petroleum ether, and the resulting colorless prisms were filtered to give an additional 500 mg of crystals. The crystals thus obtained were in the salt form (mostly sodium and potassium salts) of antibiotic DE-3936.

Preparation of free acid

Antibiotic DE-3936 (1 g) obtained above was dissolved in 100 ml of acetone, and 50 ml of water was added to this solution. After the solution was adjusted to pH 2.9 with 0.5 N hydrochloric acid, the mixture was extracted with 50 ml of benzene three times. The benzene layer was washed with water, and evaporated to dryness *in vacuo* after drying with sodium sulfate. The resulting white powder was crystallized from benzene by adding petroleum ether at room temperature; 800 mg of colorless prisms was obtained. This crystals are in the free acid from the IR spectrum ($\nu_{max}^{CHCl_3}$ 1730 cm^{-1}) and elemental analysis.

Preparation of sodium salt

The free acid (3 g) was dissolved in 150 ml of acetone. To the solution was added 75 ml of water with stirring, and the pH was adjusted to pH 9.6 with 0.5 N sodium hydroxide. The mixture was concentrated *in vacuo* to remove the acetone. The resulting aqueous solution was extracted with benzene after addition of 1 g of sodium chloride. The benzene layer was washed with water, dried with sodium sulfate, and evaporated to dryness *in vacuo*. The white residue was crystallized from benzene - petroleum ether, and gave 2 g of colorless prisms.

Preparation of potassium salt

The potassium salt was prepared by the same procedure as for the sodium salt. The free acid (1 g) afforded 780 mg of colorless prisms melting at 169~173°C.

Anal. Calcd. for $C_{44}H_{75}O_{14}K \cdot C_6H_6$: C 63.53; H 8.64; K 4.14
Found: C 64.06; H 8.63; K 3.97

Preparation of methylester

An amorphous methylester was obtained by treating the free acid in ether with an excess of ethereal diazomethane. The free acid (500 mg) was dissolved in 20 ml of diethylether and ethereal diazomethane was excessively added at room temperature. The reaction mixture was washed with sodium bicarbonate aqueous solution and water after treating with acetic acid, and dehydrated with anhydrous sodium sulfate. The methylester was obtained as white amorphous powder (460 mg) to evaporate the ether layer.

Anal. Calcd. for $C_{45}H_{78}O_{14}$: C 64.11; H 9.33
Found: C 64.64; H 9.42
NMR ($CDCl_3$): $-COOCH_3$ δ3.75 (s)

Added in Proof

After we presented the work orally at the 199th Scientific Meeting of the Japan Antibiotics Research Association (Sept. 19, 1975), emericid[21] (31,559 RP)[22] came to our attention. Direct comparison of DE-3936 with emericid by silica gel thin-layer chromatography and IR spectra revealed that both antibiotics are identical.

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