ENDUSAMYCIN, A NOVEL POLYCYCLIC ETHER ANTIBIOTIC PRODUCED BY A STRAIN OF *STREPTOMYCES ENDUS* SUBSP. *AUREUS*

JOHN R. OSCARSON†, JON BORDNER†, WALTER D. CELMER†, WALTER P. CULLEN†,*, LIANG H. HUANG†, HIROSHI MAEDA, PETER M. MOSHIER†, SATOSHI NISHIYAMA, LAURA PRESSEAU†, RIICHIRO SHIBAKAWA and JUNSUKE TONE

Central Research, Pfizer Inc.,
Nagoya, Japan and Groton, Connecticut 06340, U.S.A.†

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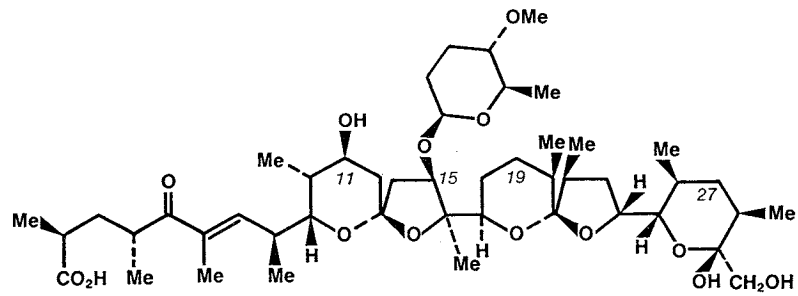
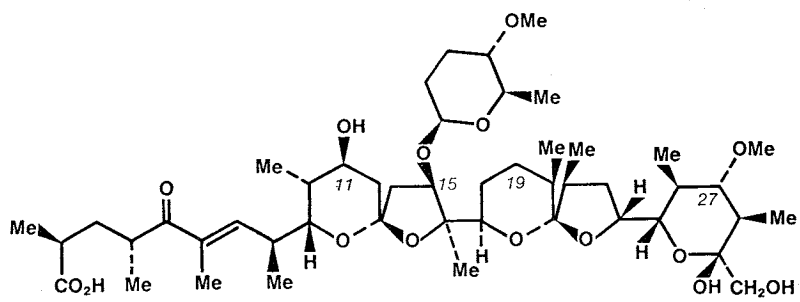
Endusamycin formerly called CP-63,517 ($C_{47}H_{77}O_{14}Na$), is a novel polycyclic ether antibiotic produced by a new strain of *Streptomyces endus* subsp. *aureus* (ATCC 39574). Recovery, fractionation and purification were achieved using standard procedures. Forms include the endusamycin free acid, mp 95~105°C, λ_{max} 232 nm (log E 4.16), $[\alpha]_D^{25}$ +47.4° (*c* 0.5, methanol) and a crystalline sodium salt, mp 215~220°C, λ_{max} 232 nm, (log E 4.15), $[\alpha]_D^{25}$ +25° (*c* 0.5, methanol). The structure is shown below, Fig. 1. Endusamycin exhibited; antibacterial activity, *in vitro* against Gram-positive and anaerobic bacteria, effectiveness against coccidia in poultry, and stimulation of propionic acid production in an *in vitro* system.

A screen programmed to detect specific metabolites such as ionophores in fermentation broths will detect many duplicate compounds in the course of screening. When the ionophore antibiotic

Fig. 1. The structure of endusamycin and X-14934A.



Endusamycin



X-14934A

Table 1. The cultural characteristics of *Streptomyces endus* subsp. *aureus* ATCC 39574.

Agar medium	Amount of growth; texture of color	Color of colony surface; color of aerial mycelium	Color of colony reverse; soluble pigment
Yeast extract - malt extract (ISP-2)	Good; raised, wrinkled	White, pale yellow to pale pink gray (1 ea, 1½ ca, near gray series 3 dc, 5 de, 5 fe); same	Brown (2 pg, 3 ne); yellowish brown (2 lc, 3 nc)
Oatmeal (ISP-3)	Moderate to good; slightly raised smooth, velvet	Off white, gray to pink gray (near gray series 3 fe, 5 fe, 7 fe, 7 ih); same	Pale yellow (2 ca) to gray (near gray series 3 dc, 5 fe); pale yellowish to yellowish (1½ ea, 1½ ga)
Inorganic salts - starch (ISP-4)	Moderate to good; raised, wrinkled	Whitish yellow to pink gray (1½ ca, near gray series 1 ba, 5 fe, 7 fe, 7 ih); same	Yellowish to pink gray (1½ ga, near gray series 5 fe); yellowish (1½ ga, 1½ ea)
Glycerol - asparagine (ISP-5)	Poor to moderate; thin, smooth or appearing as isolated colonies	Off white (near gray series 2 ba); same	Colorless to pale yellowish (1½ ca); no soluble pigment
CZAPEK - sucrose	Moderate; thin, smooth, with circular or curved lines	Pale off white (near 1½ ca, near gray series 2 cb); no aerial mycelium	Cream (1½ ca); cream (1½ ca)
Glucose - asparagine	Good; moderately raised, wrinkled or granular	Gray, pink gray (near gray series 3 fe, 5 fe) to yellowish (1 ea, 1 ga); same	Yellowish gray to gray (2 gc, 2 ge, near gray series 3 fe, 3 ih); yellowish (1½ ia, 1½ la)
Gordon - Smith tyrosine	Moderate; moderately raised, wrinkled or appearing as isolated colonies	Off white (near gray series 2 ba); off white	Pale yellow (1½ ca, 1½ ea); pale yellow (2 ea)
Calcium - malate	Moderate; thin, smooth or appearing as isolated colonies	White to off white (near gray series 1 ba); sparse, white to off white	Cream (1½ ca); cream (1½ ca)
Casein	Good; moderately raised, finely wrinkled	White to pale grayish cream (near 2 ec, 3 ec); no aerial mycelium	Pale lavender (3 ec); pale lavender (4 ec)
Bennett	Good; raised, wrinkled	White, pale yellow to pink gray (1 ea, near gray series 5 fe, 7 fe, 7 ih); same	Lavender gray (4 ig, 4 li, 5 ig, 5 li); yellowish (1½ na)
Emerson	Good; raised, wrinkled or appearing as isolated colonies	White to off white; white to off white	Yellowish brown (2 ea, 2 lc); brown (3 lc)
Nutrient	Moderate; raised, wrinkled or appearing as isolated colonies	White; white	Cream (1½ ca); no soluble pigment
Gelatin	Good; moderately raised, wrinkled	White to cream (1½ ca); white	Cream (2 ca); no soluble pigment

The color scheme used was from the Color Harmony Manual, 4th Ed., 1958, Container Corporation of America, Chicago, IL, U.S.A., 1985.
Cultural characteristics studied on various media according to WAKSMAN[14], SHIRLING and GOTTLIEB[15].

endusamycin (CP-63,517)[1] was first isolated and its novelty assessed, it was found to be a member of a family of polyethers that are analogs of dianemycin[2]; CP-53,607[3] (X-14931A[4]), lenoremycin[5], A-130B and C[6], X-14934A[7], CP-60,993[8], leuseramycin[9], TM-531B and C[10] and moyukamycin[11]. These and many other family members as yet undisclosed, or to be discovered, show the diversity in nature of the secondary metabolites produced by members of the genus *Streptomyces*.

Table 2. Biochemical properties of *Streptomyces endus* subsp. *aureus*[a] ATCC 39574.

Production of melanin	−
Production of hydrogen sulfide	+
Liquefaction of gelatin	+
Hydrolysis of starch	+
Reduction of nitrate	+
Digestion of casein	+
Digestion of calcium malate	+
Coagulation and clearing of milk	+
Decomposition of cellulose	−
Digestion of tyrosine	−
Carbohydrate utilization:	
Glucose	+
Arabinose	+
Fructose	+
Inositol	+
Mannitol	+
Raffinose	+
Rhamnose	+
Sucrose	+
Xylose	+

[a] For methodology of the biochemical tests, see HUANG[16].

Taxonomy

The culture, N497-34, was isolated from a soil sample collected in Okayama Prefecture, Japan. The culture is characterized (Tables 1 and 2) by the gray color of the spores in mass, the negative melanin reaction, the spiral spore chains, and spores with a warty surface. Cell wall analysis of the culture indicated that the whole-cell hydrolysates contain LL-diaminopimelic acid but no characteristic sugars. These features place the culture in the genus *Streptomyces*. When compared with the descriptions of known species of *Streptomyces*, the culture N497-34 closely resembled *Streptomyces endus* Anderson and Gottlieb subsp. *aureus* Tomita, Nakano, Sato, Shirahata, Yoshida and Morimoto NRRL 12174[12,13]. The former but not the latter grows at 45°C and coagulates milk. Minor culture variations found during side-by-side comparisons of the two cultures indicate that culture N497-34 is a new strain of this culture. It has been deposited with the American Type Culture Collection with the accession number ATCC 39574. Fermentation of NRRL 12174 under the same conditions as ATCC 39574 failed to produce any detectable endusamycin or anticoccidial activity when the lyophilized whole broth was tested *in vivo* against an *Eimeria* infection in chickens or extracted into solvent and examined by bioassay and TLC.

Production and Isolation

The culture was maintained on ATCC 172 medium consisting of glucose 1%, soluble starch 2%, yeast extract 0.5%, NZ-Amine A 0.5%, calcium carbonate 0.1% and agar 1.5%. The inoculum was grown in JD medium consisting of Cerelose 0.1%, casein 0.5%, starch 0.5%, corn steep liquor 0.5%, calcium carbonate 0.3% and cobalt chloride 0.0002%. A 5% inoculum was used to seed a production run in CL-13-NZ medium consisting of Cerelose 2%, soya flour 1%, NZ-Amine YTT 0.5%, sodium sulfate 0.05%, cobalt chloride 0.0002% and calcium carbonate 0.2%. The fermentations were run at 28°C for 120 to 144 hours. Antibiotic titers were followed using a disc assay on a sensitive strain of *Staphylococcus aureus* ATCC 6538 or *Bacillus subtilis* ATCC 6633. Productivity could also be followed by extracting aliquots of the broth into chloroform, separating, then concentrating the solvent, spotting the concentrate on a silica gel TLC plate and developing it in neat ethyl acetate or chloroform - methanol (9 : 1). Endusamycin could be visualized by spraying with 3%

Fig. 2. Isolation and purification of endusamycin.

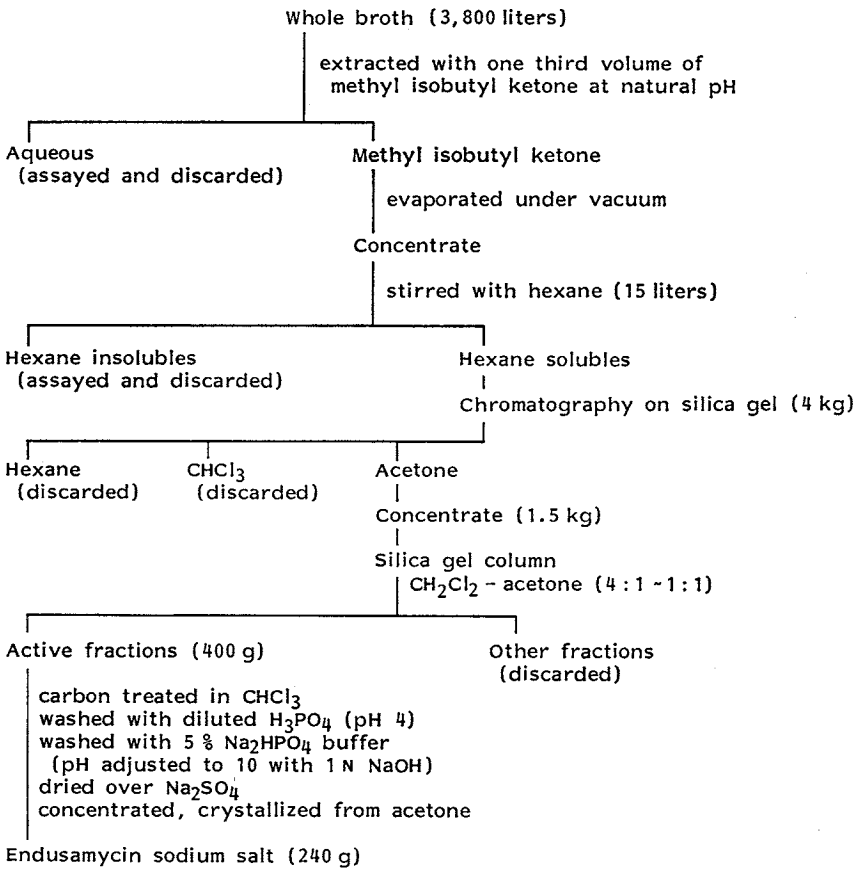


Table 3. Physico-chemical properties of the endusamycin sodium salt and free acid.

Property	Na^+ salt	H^+ free acid
MP (°C)	215~220	95~105
$[\alpha]_D^{25}$ (*c* 0.5, MeOH)	+25.0°	+47.4°
UV λ_{max}^{MeOH} nm (log E)	232 (4.15)	232 (4.16)
MW	889.12	867.13
Empirical formula	$C_{47}H_{77}O_{14}Na$	$C_{47}H_{78}O_{14}$
Elemental *Anal* Calcd:	C 63.49, H 8.89, O 25.04, Na 2.58.	C 65.10, H 9.07, O 25.83.
Found:	C 62.45, H 8.61, O 26.36, Na 2.58.	C 64.05, H 8.93, O 27.03.
Color reaction[a]	Green	Green
Solubility Soluble:	Hexane, $CHCl_3$, MeOH, acetone	Hexane, $CHCl_3$, acetone, MeOH
Insoluble:	H_2O	H_2O

[a] 3% Vanillin in 3 : 1 EtOH 85% H_3PO_4, heat to 80°C.

vanillin in 3 : 1 ethanol 85% phosphoric acid and heating to 80°C; the spot turns green. The antibiotic can also be visualized with UV light at 254 nm, or by overlaying the TLC with *B. subtilis* in agar and incubating overnight at 37°C.

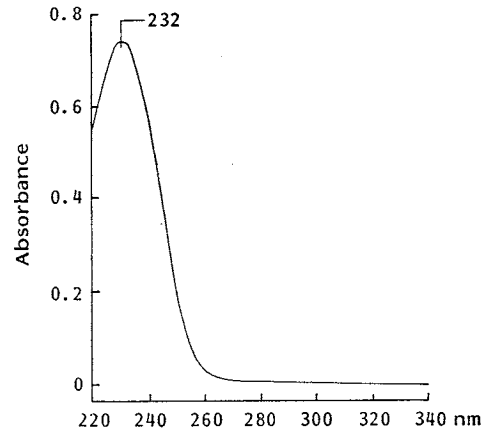
The antibiotic was isolated (Fig. 2) from a 3,800-liter of whole broth by extraction at natural pH into methyl isobutyl ketone. The extract was clarified, concentrated to a syrup, then triturated with hexane. The hexane concentrate was chromatographed on silica gel using a hexane to acetone

step-wise gradient. Activity was followed by bioassay and TLC, the active cuts being concentrated and rechromatographed on silica gel with a methylene chloride - acetone step-wise gradient. Active cuts were combined, concentrated, treated with Darco G60 carbon in chloroform, acidified with phosphoric acid, then washed with pH 9 sodium phosphate buffer, dried over anhydrous sodium sulfate, concentrated and the sodium salt of endusamycin crystallized from acetone. The yield of the first crop was 240 g.

Physico-chemical Characterization

Endusamycin is characterized as a monocarboxylic acid. Elemental analysis suggested a molecular formula of $C_{47}H_{78}O_{14}$ for the free acid and $C_{47}H_{77}O_{14}Na$ for the sodium salt. Some physico-chemical properties are listed in Table 3. The UV and IR spectra are shown in Figs. 3 and 4, respectively.

Fig. 3. The UV spectrum of endusamycin sodium salt in MeOH.



Endusamycin is a new polyether antibiotic. The structure was determined by its characteristic ^1H NMR (Fig. 5). X-Ray crystallographic analysis of the corresponding rubidium salt revealed the complete structure and the absolute configuration of endusamysin as indicated in Fig. 6. It contains the α,β-unsaturated ketone, two spiroketals and sugar moiety seen in many polyethers such as dianemycin and lenoremycin. A comparison of the ^{13}C NMR chemical shifts obtained for endusamycin and dianemycin is shown in Table 4. Among the known polyethers

Fig. 4. IR spectrum (KBr) of endusamycin sodium salt.

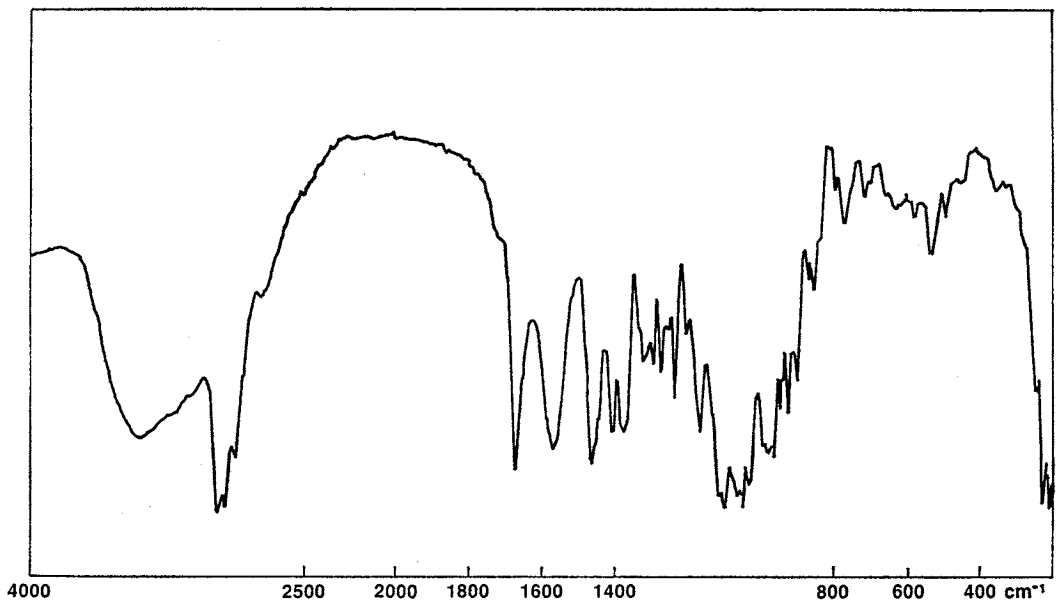


Fig. 5. The ^1H NMR spectrum of endusamycin in $CDCl_3$.

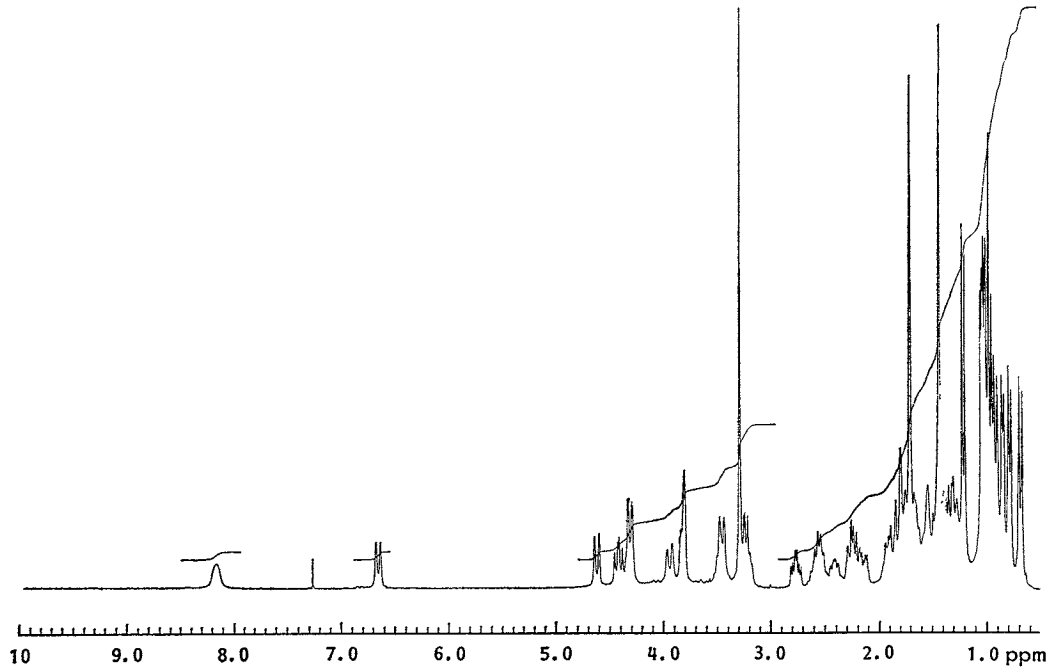


Fig. 6. Stereoscopic drawing of the absolute configuration of the endusamycin Rb salt.

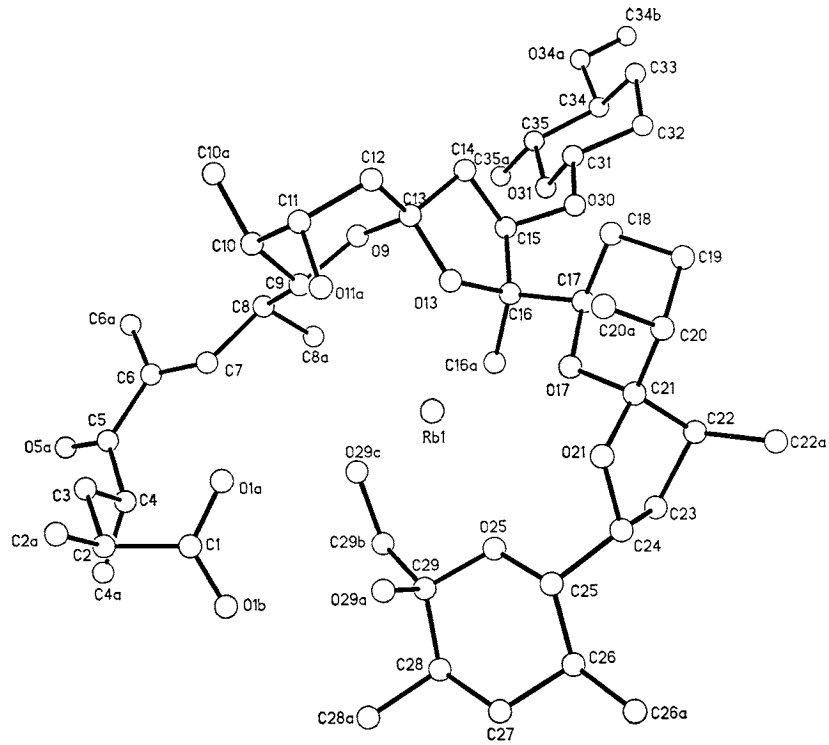


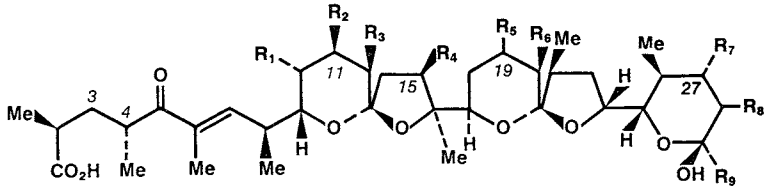
Table 4. ^{13}C Chemical shifts of endusamycin and dianemycin in $CDCl_3$.

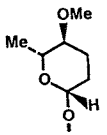
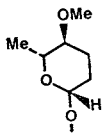
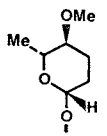
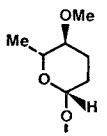
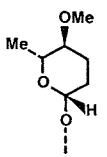
Carbon	Functionality	Endusamycin Na^+ salt[a]	Dianemycin Na^+ salt
1	COO	183.8	183.8
2	CH	39.9 (2.47)	40.2
3	CH_2	41.5 (1.01, 1.72)	41.5
4	CH	37.5 (3.49)	37.5
5	C=O	206.3	206.2
6	C=	133.7	133.6
7	CH=	144.5 (6.70)	144.9
8	CH	35.9 (2.64)	37.8
9	CHO	69.7 (4.68)	69.6
10	CH	36.0 (1.83)	35.9
11	CHO	70.2 (3.87)	70.4
12	CH_2	33.7 (1.76, 1.85)	34.0
13	OCO	104.2	106.9
14	CH_2	44.9 (1.88, 2.30)	39.5
15	CHO or CH_2	84.3 (4.48)	32.2
16	CO	69.7	86.6
17	CHO	78.9 (3.51)	75.7
18	CH_2	19.3 (1.50, 1.71)	25.4
19	CH_2 or CHO	27.0 (1.61)	79.2
20	CH	30.0 (1.97)	34.6
21	OCO	110.6	109.8
22	CH	35.3 (2.59)	35.9
23	CH_2	29.8 (1.39, 2.29)	29.9
24	CHO	78.5 (4.37)	77.9
25	CHO	72.9 (3.88)	73.2
26	CH	32.9 (1.31)	32.9
27	CH_2	36.5 (1.36, 1.49)	36.5
28	CH	35.9 (1.50)	35.9
29	OCOH	98.6	98.5
30	CH_2OH	65.2 (3.27, 4.00)	65.3
2-CH_3		19.4 (1.02)	19.5
4-CH_3		14.4 (1.09)	16.9
6-CH_3		11.1 (1.77)	11.2
8-CH_3		16.9 (1.07)	14.4
10-CH_3		9.9 (0.74)	10.0
16-CH_3		24.8 (1.49)	26.6
20-CH_3		12.9 (1.04)	13.1
22-CH_3		14.7 (0.97)	16.1
26-CH_3		17.4 (0.84)	17.7
28-CH_3		16.6 (0.90)	16.7

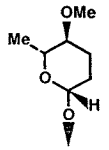
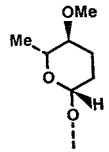
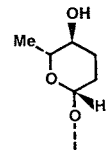
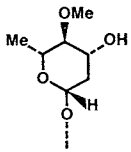
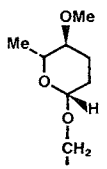
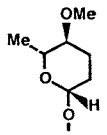
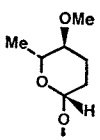
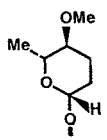
		Deoxy sugar at:	
		C-15	C-19
1′	OCHO	101.5 (4.36)	102.4
2′	CH_2	30.4 (1.46, 1.83)	30.6
3′	CH_2	26.8 (1.31, 2.19)	27.0
4′	CHO	79.9 (2.83)	79.9
5′	CHO	74.6 (3.30)	74.5
6′	CH_3	18.2 (1.27)	18.5
4′-OCH_3		56.8 (3.35)	56.7

[a] Values in parentheses are ^1H shifts in ppm from TMS in $CDCl_3$ solution.

Table 5. The structures of polyether antibiotics containing an α,β-unsaturated ketone and two spiroketals.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	ref
CP-53,607 (X-14931A)[a]	CH_3	OH	H	H	H	CH_3	H	◄CH_3	CH_2OH	3, 4
Lenoremycin (A-130A)	H		CH_3	H	H	CH_3	H	◄CH_3	CH_2OH	5, 6
A-130C	H		CH_3	H	H	CH_3	H	---CH_3	CH_2OH	6
Endusamycin (CP-63,517)	CH_3	OH	H		H	CH_3	H	◄CH_3	CH_2OH	1
X-14934A (CP-47,224)	CH_3	OH	H		H	CH_3	OCH_3	◄CH_3	CH_2OH	7, 17
Dianemycin	CH_3	OH	H	H		CH_3	H	◄CH_3	CH_2OH	2

CP-60,993	CH_3	OH	H	H		CH_3	H	◄CH_3	CH_2OH	8
Leuseramycin	CH_3	OH	H	H		CH_3	H	◄CH_3	CH_3	9
TM-531B[b]	CH_3	OH	H	H		CH_3	H	◄CH_3	CH_2OH	10
TM-531C[b]	CH_3	OH	H	H		CH_3	H	◄CH_3	CH_2OH	10
Moyukamycin[c]	CH_3	OH	H	H	H		H	◄CH_3	CH_3	11
CP-80,219	CH_3	OH	H	H	H	CH_3		◄CH_3	CH_2OH	18
A-130B[d]	H		CH_3	H	H	CH_3		◄CH_3	CH_2OH	6

[a] No glycoside moiety.
[b] The first polyethers to contain sugars other than 4-*O*-methyl amicetose.
[c] Has a double bond at C(3)=C(4).
[d] Diglycoside.

endusamycin is structurally most similar to X-14934A. The two compounds differ only at position 27, where the methoxy group present in X-14934A is absent in endusamycin (Fig. 1). The absolute stereo configuration of endusamycin at position 15 as determined by the X-ray analysis of the rubidium salt is the same as that of X-14934A.

A comparison of the structures of the polyethers reported in the literature to contain an α,β-unsaturated ketone and two spiroketals (Table 5) is arranged according to the appearance of the sugar moiety on the ring system with the only diglycoside, A-130B, being last. The relationship between the aglycone CP-53,607 and the other members of the glycosylated series can be readily seen.

Endusamycin and X-14934A are unique in that the common glycoside moiety 4-*O*-methyl amicetose is attached at position 15 rather than at position 19 as seen in dianemycin and its analogs, or at position 11 as seen in lenoremycin and its analogs.

Biological Characterization

Endusamycin, as indicated in Table 6 has an excellent spectrum of activity against Gram-positive microorganisms and good activity against many anaerobes and organisms such as *Treponema hyodysenteriae* of interest in the animal health field.

The polyether was active against *Eimeria tenella* and *Eimeria acervulina* coccidia when administered in feed at 10 to 40 μg/g (Table 7). Chickens were protected from lesions but showed

Table 6. Antimicrobial spectrum of endusamycin.

Test organisms	MIC (μg/ml)
Staphylococcus aureus 01A005	0.39
S. aureus 01A110	0.39
S. aureus 01A539	0.78
S. aureus 01A543	3.12
S. epidermidis 01B087	0.78
S. epidermidis 01B111	0.39
Streptococcus pyogenes 02C054	0.10
Erysipelothrix rhusco 04A005	0.39
Lactobacillus casei 09B001	0.39
L. catenaforme 09C001	0.39
Corynebacterium pyogenes 11D001	12.5
Peptococcus sp. 17B001	$\leq$0.10
Haemophilus parahaemolyticus 54B002	25
Pasteurella multocida 59A013	50
P. multocida 59A048	0.39
P. haemolytica 59B018	0.39
P. haemolytica 59B046	0.39
Bordetella bronchiseptica 73A006	0.39
B. bronchiseptica 73A016	6.25
Bacteroides vulgatus 78E032	25
Fusobacterium plauti 84G001	0.39
F. necrophorum 84C004	25
Moraxella bovis 93A001	50
Treponema hyodysenteriae 94A001	6.25
T. hyodysenteriae 94A002	6.25

Table 7. Efficacy data or endusamycin sodium salt against coccidial infections in chickens.

Drug	Species	Dose (μg/g of feed)	Lesion Control[a] (%)	Weight gain (%)
Endusamycin	*Eimeria tenella*	40	100	1
		30	100	13
		20	100	20
		10	67	50
	E. acervulina	40	25	0
		30	50	0
		20	75	60
		10	50	0
Monensin	*E. tenella*	100	62	70
		50	17	95
	E. acervulina	100	90	87
		50	30	40

[a] The criteria for evaluation, CHAPPEL *et al.*[19].

poor weight gains and feed intake. Endusamycin had an LD_{50} of 7.5 mg/kg orally in male rats.

The antibiotic also induced a change in the proportion of volatile fatty acids (acetate, propionate and butyrate) produced in the rumen by increasing the molar proportion of propionate in the rumen fluids (Table 8).

Table 8. The *in vitro* stimulation of rumen propionic acid production by the sodium salt of endusamycin.

Compound	Dosage (μg/g)	Rumen propionic acid (untreated control=100%)
Endusamycin	20	196
	10	195
	5	193
	2.5	183
Monensin	10	130
Salinomycin	10	165

Criteria for evaluation, KELLOGG[20].

Experimental

Preparation of Free Acid of Endusamycin

Endusamycin sodium salt (102 mg) was dissolved in 58 ml of $CHCl_3$. The dissolved sample was combined with 50 ml of pH 2 HCl solution. The mixture was shaken vigorously for approximately 2 minutes. The organic phase was separated and extracted once with deionized, distilled H_2O, and then evaporated to a white foam. IR indicated that the free acid had been formed.

Preparation of Rb Salt of Endusamycin

The free acid of endusamycin was dissolved in 50 ml of $CHCl_3$. $RbCO_3$ (150 mg in 49 ml distilled, deionized H_2O) was added to the $CHCl_3$, and the mixture was shaken vigorously for 2 minutes. The organic phase was separated and extracted once with deionized, distilled H_2O, and then evaporated to a white solid. The Rb salt was recrystallized using MeOH, EtOAc, diethyl ether, heating the solution slightly, adding a few drops of hexane and allowing the solution to evaporate slowly.

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References

1) OSCARSON, J. R.; W. D. CELMER, L. R. CHAPPEL, W. P. CULLEN, L. H. HUANG, H. MAEDA, S. NISHIYAMA, R. SHIBAKAWA, J. TONE & K. TSUKUDA: CP-63,517, a novel polycyclic ether antibiotic produced by a strain of *Streptomyces endus* subsp. *aureus*. Program and Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 1008, p. 272, New York, Oct. 4~7, 1987
2) HAMILL, R. L.; M. M. HOEHN, G. E. PITTENGER, J. CHAMBERLIN & M. GORMAN: Dianemycin, an antibiotic of the group effecting ion transport. J. Antibiotics 22: 161~164, 1969
3) CELMER, W. D.; W. P. CULLEN, R. SHIBAKAWA & J. TONE (Pfizer): A new polycyclic ether antibiotic (CP-53,607). U.S. 4,361,649, Nov. 30, 1982
4) WESTLEY, J. W.; C. LIU, L. H. SELLO, N. TROUPE, J. F. BLOUNT, A.-M. CHIU, L. J. TODARO, P. A. MILLER & M. LIU: Isolation and characterization of antibiotic X-14931A, the naturally occurring 19-deoxyaglycone of dianemycin. J. Antibiotics 37: 813~815, 1984
5) ANTEUNIS, M. J. O.; N. A. RODIOS & G. VERHEGGE: Solution conformation of dianemycin, its sodium salt and of lenoremycin-Na^+ (Ro 21-6150 or A-130A). Bull. Soc. Chim. Belg. 86: 609~632, 1977
6) TSUJI, N.; Y. TERUI, K. NAGASHIMA, K. TORI & L. F. JOHNSON: New polyether antibiotics, A-130B and A-130C. J. Antibiotics 33: 94~97, 1980
7) LIU, C.-M.; J. W. WESTLEY, N. J. PALLERONI, L. H. SELLO, T. E. HERMANN, R. EVANS, Jr., M. LIU & E. SCHILDKNECHT: X-14934A, a novel polyether antibiotic produced by *Streptomyces*. Program and Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 1007, p. 272, New York, Oct. 4~7, 1987
8) CELMER, W. D.; W. P. CULLEN, J. C. RUDDOCK, H. MAEDA & J. TONE (Pfizer): 19-*Epi*-dianemycin and process therefore. U.S. 4,707,493, Nov. 17, 1987
9) MIZUTANI, T.; M. YAMAGISHI, H. HARA, A. KAWASHIMA, S. ŌMURA, M. ŌZEKI, K. MIZOUE, H. SETO & N. ŌTAKE: Studies on the ionophorous antibiotics. XXIV. Leuseramycin, a new polyether antibiotic

produced by *Streptomyces hygroscopicus*. J. Antibiotics 33: 137~143, 1980

10) MIZUTANI, T.; M. YAMAGISHI, K. MIZOUE, A. KAWASHIMA, S. ŌMURA, M. ŌZEKI, H. SETO & N. ŌTAKE: Studies on the ionophorous antibiotics. XXVII. The structures of TM-531B (4'-*O*-demethyldianemycin) and TM-531C (3'-hydroxydianemycin), new polyether antibiotics containing sugars other than 4-*O*-methyl amicetose. J. Antibiotics 34: 1369~1373, 1981
11) NAKAYAMA, H.; H. SETO, N. ŌTAKE, M. YAMAGISHI, A. KAWASHIMA, T. MIZUTANI & S. ŌMURA: Studies on the ionophorous antibiotics. XXVIII. Moyukamycin, a new glycosylated polyether antibiotic. J. Antibiotics 38: 1433~1436, 1985
12) TOMITA, F.; H. NAKANO, T. SATO, K. SHIRAHATA, M. YOSHIDA & M. MORIMOTO (Kyowa Hakko): Jpn. Kokai 4975 ('82), Nov. 1, 1982
13) NAKANO, H.; M. YOSHIDA, K. SHIRAHATA, S. ISHII, Y. ARAI, M. MORIMOTO & F. TOMITA: Senacarcin A, a new antitumor antibiotic produced by *Streptomyces endus* subsp. *aureus*. J. Antibiotics 35: 760~762, 1982
14) WAKSMAN, S. A. (*Ed.*): The Actinomyces. Vol. 2. Classification, Identification and Descriptions of Genera and Species. Williams & Wilkins Co., Baltimore, 1961
15) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
16) HUANG, L. H.: *Actinomadura macro* sp. nov., the producer of antibiotics CP-47,433 and CP-47,434. Int. J. Syst. Bacteriol. 30: 565~568, 1980
17) CELMER, W. D.; C. E. MOPPETT, W. P. CULLEN, J. R. OSCARSON, L. H. HUANG, R. SHIBAKAWA & J. TONE (Pfizer): Polycyclic ether antibiotic (CP-47,224) produced by a strain of *Streptomyces hygroscopicus*. U.S. 4,150,152, Apr. 17, 1979
18) BORDNER, J.; L. A. PRESSEAU & J. P. DIRLAM: The structure of CP-80,219. in preparation
19) CHAPPEL, L. R.; H. L. HOWES & J. E. LYNCH: The site of action of a broad-spectrum aryltriazine anticoccidial, CP-25,415. J. Parasitol. 60: 415~420, 1974
20) KELLOGG, D. W.: Analysis of rumen fluid volatile fatty acids by chromatography with Porapak Q.S. J. Dairy Sci. 52: 1690~1692, 1969