CP-82,009, A POTENT POLYETHER ANTICOCCIDIAL RELATED TO SEPTAMYCIN AND PRODUCED BY *Actinomadura* sp.

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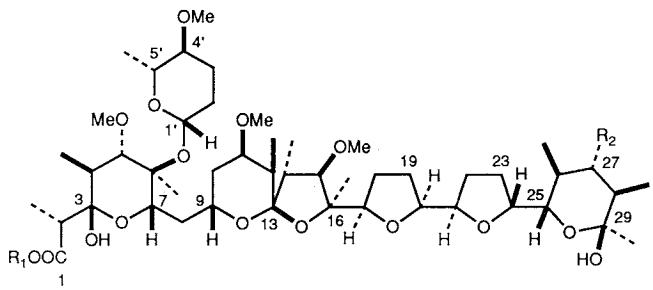
A new polyether antibiotic CP-82,009 ($C_{49}H_{84}O_{17}$) was isolated by solvent extraction from the fermentation broth of *Actinomadura* sp. (ATCC 53676). Following purification by column chromatography and crystallization, the structure of CP-82,009 was elucidated by spectroscopic (NMR and MS) methods. The absolute stereochemistry was determined by a single crystal X-ray analysis of the corresponding rubidium salt. CP-82,009 is among the most potent anticoccidial agents known, effectively controlling the *Eimeria* species that are the major causative agents of chicken coccidiosis at doses of 5 mg/kg or less in feed. It is also active *in vitro* against certain Gram-positive bacteria, as well as the spirochete, *Serpulina* (*Treponema*) *hyodysenteriae*.

Polyether antibiotics have been an important class of drugs in veterinary medicine for over 20 years. For example, monensin[1], lasalocid[1] and salinomycin[2] are marketed as anticoccidal agents for poultry, and are used as growth permittants in cattle and swine. Narasin[1] and maduramicin[3] are also used as anticoccidial agents.

In the course of screening actinomycetes for novel antimicrobial substances, a new strain of *Actinomadura* sp. was found to produce a new polyether antibiotic, CP-82,009 (**1**). This compound, which was very potent against *Eimeria* coccidia in chickens, was shown to be structurally similar to septamycin[4]. The present paper describes the taxonomy and fermentation studies on the producing organism of CP-82,009, as well as the isolation, characterization and biological testing of this antibiotic[5].



CP-82,009 free acid (**1**) $R_1=H$ $R_2=OMe$
CP-82,009 Na-salt (**2**) $R_1=Na$ $R_2=OMe$
Septamycin $R_1=H$ $R_2=H$

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Taxonomy of the Producing Strain

The CP-82,009 producing strain, *Actinomadura* sp. N742-34, was isolated from a soil sample collected in Hongo Town, Toyama City, Toyama Prefecture, Japan. It was found to have narrow hyphae of the actinomycetes, an aerial mycelium upon which spore chains are produced, and an unfragmented substrate mycelium. The culture is further characterized by the white to pale gray aerial mycelium; the green-yellow, yellow-brown to brown substrate mycelium; and the short straight to flexuous spore chains with a warty surface (Figs. 1 and 2). However, the culture did not produce spores on any media except for inorganic salts-starch agar. After seven weeks of incubation on this medium, a few small patches of spore chains were produced. They were short with 3 to 9 spores per spore chain and were straight, flexuous, curved to hooked. The spores were globose, oval to elliptical and measured $0.9 \sim 1.3\,\mu m$ diameter or $1.0 \sim 1.6 \times 0.8 \sim 1.1\,\mu m$, respectively. The physiological properties and carbohydrate utilization are shown in Tables 1 and 2. The whole-cell hydrolysates indicate the presence of *meso*-diaminopimelic acid, madurose, glucose, galactose and ribose. Thus, the culture belongs in the genus *Actinomadura*, as defined by

Fig. 1. Scanning electron micrograph of a cluster of short spore chains of *Actinomadura* sp. ATCC 53676 grown on inorganic salts-starch agar for seven weeks at 28°C, ×9,000.

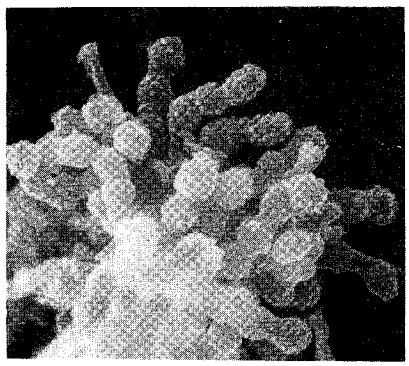
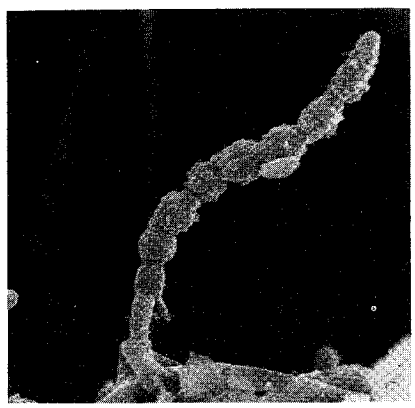


Fig. 2. Scanning electron micrograph of a spore chain of *Actinomadura* sp. ATCC 53676 grown on inorganic salts-starch agar for seven weeks at 28°C, ×10,000.



Note a short sporophore at the base of the spore chain and the warty nature of the spore surface.

Table 1. Physiological properties of *Actinomadura* sp. N742-34.

Production of:		Resistance to:	
Gelatinase	+	Lysozyme	−
Melaninase	−	Reduction of:	
Tyrosinase	+	Organic nitrate	−
Urease	−	Dextrose nitrate	+
H_2S	−	Utilization of:	
Milk:		Acetate	+
Coagulation	+	Propionate	+
Clearing	+	Pyruvate	+
Decomposition of:		Growth at:	
Adenine	−	21°C	+
Cellulose	−	28°C	+
Esculin	−	37°C	+
Hippurate	−	45°C	−
Hydrolysis of:			
Casein	+		
Hypoxanthine	−		
Starch	+		
Xanthine	−		

Table 2. Carbohydrate utilization of *Actinomadura* sp. N742-34.

Adonitol	−	α-Methyl-D-glucoside	−
Arabinose	−	Melezitose	−
Cellobiose	−	Melibiose	−
Dulcitol	−	Raffinose	−
Erythritol	−	Rhamnose	+
Fructose	−	Ribose	−
Galactose	−	Salicin	−
Glucose	+	Sorbitol	−
Glycerol	−	Sorbose	−
Inositol	−	Starch	+
Lactose	−	Sucrose	+
Maltose	−	Trehalose	+
Mannitol	−	Xylose	−
Mannose	−		

LECHEVALIER[6].

Growth was good on yeast extract-malt extract agar, BENNETT's agar, EMERSON's agar, and GAUZE's organic medium 2; moderate to good on oatmeal agar, CZAPEK-sucrose agar, glucose-asparagine agar, GORDON and SMITH's tyrosine agar, casein agar, gelatin agar, starch agar, and GAUZE's mineral medium 1; poor on inorganic salts-starch agar, nutrient agar, glycerol-asparagine agar, potato carrot agar, and tap water agar; and none on calcium malate agar.

Aerial mycelium was generally none or sparse and colorless or white to pale gray. The surface color of the colonies was yellowish green (1 ic)[7] on most media used, cream on CZAPEK-sucrose agar (1 1/2 ca), and brown (3 ne) on starch agar. The reverse color of the colonies was yellowish to yellowish brown (2 ga, 3 lc) on most of the media used, colorless to cream (1 1/2 ca) on CZAPEK-sucrose agar, and brown (3 le) on gelatin agar. The soluble pigment was generally lacking; yellowish (2 lc) on yeast extract-malt extract agar, GORDON and SMITH's tyrosine agar, and BENNETT's agar; yellowish brown (3 lc) on casein agar and EMERSON's agar; and pale cream (1 1/2 ca) on GAUZE's mineral medium 1.

Among the more than forty species of *Actinomadura*, five resemble strain N742-34 in morphological and/or biochemical properties: *A. citrea*, *A. cremea*, *A. flava*, *A. livida*, and *A. macra*. Both *A. citrea* and *A. flava* produce a lemon-yellow substrate mycelium, as does strain N742-34. *A. flava* produces long spore chains and spores with a smooth surface, whereas strain N742-34 forms short spore chains and spores with a warty surface. Unlike strain N742-34, *A. citrea* utilizes arabinose, xylose, mannitol, and fructose.

A. livida forms spore chains in the form of hooks or spirals with a single turn, whereas strain N742-34 forms straight or flexuous spore chains. On oatmeal and GAUZE's mineral medium 1, *A. livida* forms a pale violet soluble pigment, but strain N742-34 forms a cream soluble pigment.

A. cremea differs from strain N742-34 in positive utilization of arabinose, fructose, mannitol, xylose, adonitol, glycerol, lactate, and succinate; negative utilization of sucrose and starch; and decomposition of esculin.

A. macra is closely similar to strain N742-34 in most of the biochemical properties. A few differences were noted. Unlike the former, the latter utilizes rhamnose and starch, coagulates milk, and decomposes casein. In addition, strain N742-34 produces mostly a yellow-green, yellow-brown or brown substrate mycelium and spores with a warty surface; whereas *A. macra* produces mostly a cream or gray substrate mycelium and spores with a smooth surface.

On the basis of the above, strain N742-34 is considered as a member of the genus *Actinomadura* and designated *Actinomadura* sp. It has been deposited at the American Type Culture Collection under the accession No. ATCC 53676.

Fermentation

Actinomadura sp. ATCC 53676 was maintained on an ATCC 172 medium (g/liter: glucose (10), soluble starch (20), yeast extract (5), NZ-Amine A (1), calcium carbonate (1) and agar (20); pH 7.0 (with KOH) for 7~9 days at 28°C), and the inoculum was grown in JDYTT medium (g/liter: Cerelose (10), corn starch (5), corn steep liquor (5), NZ-Amine YTT (5), cobalt chloride (0.002) and calcium carbonate (3); pH 7.2 for 5~7 days at 28~36°C; 150~200 rpm). A 3%-inoculum was used to seed a production run in C′ medium (g/liter: Cerelose (10), corn starch (10), soybean flour (10), corn fermentable solids (5), sodium chloride (5), cobalt chloride (0.002) and calcium carbonate (1); pH 7.2 for 5~7 days at 30°C; 1,700 rpm (jar), 600 rpm (tanks)). The antibiotic titers were followed by using a disc assay on a sensitive strain of

Bacillus subtilis ATCC 6633. The presence of CP-82,009 was followed by TLC on silica gel plates using ethyl acetate as the eluent. The ionophore was visualized as a rose-red coloration using vanillin - EtOH - H_3PO_4 spray reagent (3 g vanillin in 75 ml of EtOH and 25 ml of 85% H_3PO_4), followed by heating to 80°C.

Isolation

Work-up of a tank fermentation of whole broth was carried out by extracting the approximately 100 liters of broth with 50 liters of methyl isobutyl ketone. The organic extract was separated, and concentrated *in vacuo*. The resulting oil was chromatographed on 500 g of silica gel using ethyl acetate. The active cuts were combined and then concentrated *in vacuo*, dissolved in ethyl acetate, treated with Darco G60 and filtered. The filtered solution was shaken first with phosphoric acid, then with pH 9.0 sodium phosphate buffer to form the sodium salt. After drying over anhydrous Na_2SO_4, the solvent was concentrated *in vacuo*, and the resulting product was crystallized from ethyl acetate - heptane to afford 0.95 g of CP-82,009 Na-salt (**2**). Further purification was accomplished by flash chromatography using a column of 100 g of silica gel, 32~63 μm, and employing a gradient of 95 : 5 to 50 : 50 chloroform - ethyl acetate, and 0.85 g of product as **2** was obtained following the removal of solvent *in vacuo*.

Structural Determination

CP-82,009 free acid (**1**) was obtained by treatment of a chloroform solution of the sodium salt **2** with an aqueous solution of HCl. CP-82,009 Rb-salt was prepared from the free acid **1**, and the resulting crystals were suitable for X-ray crystallographic studies (see below). The physico-chemical properties of the free acid **1** and its sodium salt **2** are summarized in Table 3.

Spectroscopic data and elemental analyses were consistent with $C_{49}H_{84}O_{17}$ for the free acid **1**, and $C_{49}H_{83}O_{17}Na$ for the sodium salt **2**. For example, in the positive FAB-MS, diagnostic cationized molecules *m/z* 967 ($(M+Na)^+$) and 989 ($(M+2Na-H)^+$) were detected for **2**. Furthermore, **2** gave a base peak at *m/z* 905, *i.e.*, 62 daltons less than the corresponding metal-adduct molecular ion, which is common for polyethers having a β-hemiketal carboxylic acid group ($(M+Na-CO_2-H_2O)^+$)[8]. The ^{13}C and ^1H NMR spectral data for **2**, including a polarization transfer (DEPT)[9] experiment revealed the following groups. CH_3 (10), CH_2 (8), CH (6), CH_3O (5), O–CH (13), C–O (2), O–CH–O (1), O–C–O (3), and –COONa (1) (see Table 4). These groups accounted for all the hydrogens in **2** except for two exchangeable ones,

Table 3. Physico-chemical properties of CP-82,009 free acid (**1**) and Na-salt (**2**).

Property	1	2
MP (°C)	95~102	175~177
$[\alpha]_D^{25}$ (*c* 1.0, MeOH)	+20.2°	+12.8°
Empirical formula	$C_{49}H_{84}O_{17}$	$C_{49}H_{83}O_{17}Na$
MW	945.2	967.2
Elemental analysis	$C_{49}H_{84}O_{17}\cdot H_2O$	$C_{49}H_{83}O_{17}Na\cdot H_2O$
Calcd:	C 61.10, H 9.00	C 59.74, H 8.70
Found:	C 61.53, H 9.31	C 59.47, H 8.54
IR ($CHCl_3$)	3414, 2928, 2876, 2828, 1675 (–CO_2H), 1457, 1378, 1287, 1163, 1089, 1054, 953	2926, 2829, 1599 (–CO_2Na), 1458, 1378, 1163, 1086, 976
Solubility		
Soluble:	Organic solvents	Organic solvents
Insoluble:	H_2O	H_2O

Table 4. ^{13}C and ^{1}H NMR chemical shift data for the Na-salts of CP-82,009 and septamycin in $CDCl_3$.

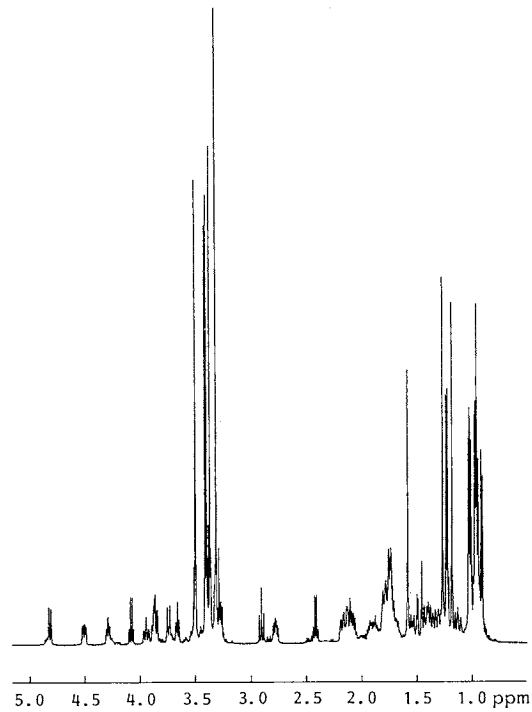
Carbon	CP-82,009		Septamycin[a]	
	^{13}C shift[b]	^{1}H shift[c]	^{13}C shift[b]	^{1}H shift[c]
1 COONa	180.77 (0)	—	180.5 (0)	—
2 CH	45.22 (1)	2.41	45.4 (1)	2.47
3 O–C–O	99.62 (0)	—	99.5 (0)	—
4 CH	40.71 (1)	1.73	40.7 (1)	1.76
5 O–CH	88.79 (1)	3.29	88.7 (1)	3.34
6 C–O	80.25 (0)	—	80.2 (0)	—
7 O–CH	67.54 (1)	3.75	67.4 (1)	3.79
8 CH_2	32.53 (2)	1.43, 1.72	32.5 (2)	1.48, 1.78
9 O–CH	61.57 (1)	3.95	61.5 (1)	3.99
10 CH_2	31.13 (2)	1.13, 2.12	31.1 (2)	1.16, 2.15
11 O–CH	79.75 (1)	3.32	79.6 (1)	3.35
12 CH	36.92 (1)	1.74	36.9 (1)	1.76
13 O–C–O	106.70 (0)	—	106.6 (0)	—
14 CH	46.13 (1)	2.07	46.0 (1)	2.12
15 O–CH	94.74 (1)	3.51	94.7 (1)	3.54
16 C–O	83.26 (0)	—	83.2 (0)	—
17 O–CH	79.06 (1)	3.87	79.1 (1)[d]	3.92
18 CH_2	25.63 (2)	1.75, 1.88	25.6 (2)	1.77, 1.93
19 CH_2	23.01 (2)	1.70	23.0 (2)	1.72
20 O–CH	79.30 (1)	4.31	79.2 (1)[e]	4.56
21 O–CH	83.49 (1)	3.66	83.3 (1)[f]	3.70
22 CH_2	29.18 (2)	1.37, 1.92	29.2 (2)	1.38, 1.93
23 CH_2	24.17 (2)	1.79, 2.11	24.2 (2)	1.80, 2.16
24 O–CH	80.25 (1)	4.30	80.4 (1)	4.30
25 O–CH	73.95 (1)	3.85	75.4 (1)	3.82
26 CH	39.41 (1)	1.25	32.7 (1)	1.32
27 O–CH/CH_2	84.70 (1)	2.90	36.8 (2)	1.48
28 CH	46.32 (1)	1.38	39.6 (2)	—[g]
29 O–C–O	98.40 (0)	—	96.8 (0)	—
2-Me	11.54 (3)	1.01	11.5 (3)	1.06
4-Me	12.00 (3)	0.98	12.0 (3)	1.01
5-OMe	61.72 (1)	3.51	61.2 (3)	3.54[h]
6-Me	9.95 (3)	1.18	9.9 (3)	1.22
11-OMe	58.94 (3)	3.41	58.9 (3)	3.47[h]
12-Me	12.54 (3)	1.02	12.6 (3)	0.98
14-Me	11.51 (3)	0.96	11.5 (3)	1.00
15-OMe	60.18 (3)	3.37	60.1 (3)	3.41[h]
16-Me	28.43 (3)	1.58	28.4 (3)	1.62
26-Me	13.14 (3)	0.91	17.3 (3)	0.82
27-OMe	59.93 (3)	3.40	—	—
28-Me	12.69 (3)	0.94	16.9 (3)	0.90
29-Me	26.58 (3)	1.26	26.4 (3)	1.28
Deoxysugar (Deo)[i]				
1′ O–CH–O	96.63 (1)	4.81	96.5 (1)	4.86
2′ CH_2	31.90 (2)	1.54, 1.78	31.8 (2)	1.55, 1.84
3′ CH_2	27.69 (2)	1.32, 2.18	27.7 (2)	1.32, 2.22
4′ O–CH	80.25 (1)	2.78	80.2 (1)	2.81
5′ O–CH	74.33 (1)	3.28	74.3 (1)	3.33
4′-OMe	56.82 (3)	3.31	56.8 (3)	3.35[h]
5′-Me	18.54 (3)	1.23	18.5 (3)	1.26

[a] Assignments are based on ref 13.
[b] In ppm from TMS in $CDCl_3$ solution; number of attached protons are in parentheses.
[c] In ppm from TMS in $CDCl_3$ solution.
[d] Originally assigned C-20 in ref 13.
[e] Originally assigned C-21 in ref 13.
[f] Originally assigned C-17 in ref 13.
[g] Not ascertained in ref 13.
[h] Tentatively assigned in ref 13 and may be interchanged.
[i] 4-Methylamicetose.

which were assumed to be free hydroxy functions on δ_C 99.62 and 98.40 based on deuterium induced upfield shifts observed in the ^{13}C NMR spectrum of **2** (see Experimental). Both the δ_C 99.62 and 98.40 were assigned as hemiketal carbons, and δ_C 106.70 to a ketal carbon by process of elimination.

In our efforts to elucidate the structures of unknown ionophores, we have found that it is helpful to estimate the number of rings (R) and the number of oxygen links (E). This is done by the NMR method developed by WHIPPLE *et al.* [10], and we have previously illustrated its use for another ionophore[11], *i.e.*, CP-84,657. In the present case, where there are three exchangeable protons (including Na^+ for the sodium salt **2**), the estimated number of rings (R) is 7, and the number of oxygen links (E) is 13 for **2**.

Fig. 3. ^1H NMR spectrum of CP-82,009 Na-salt (**2**) in $CDCl_3$ (500 MHz).

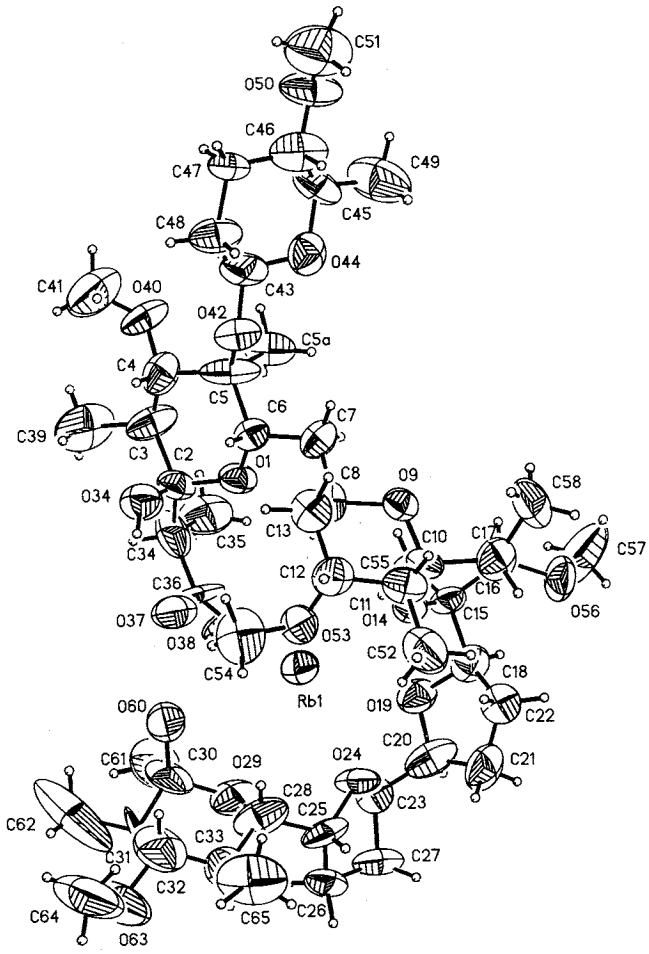


With a knowledge of R, E, and the empirical formula of CP-82,009, coupled with other information such as the presence of one ketal and two hemiketal functions, and a sugar moiety, a comparison with known polyether antibiotics can be readily made. Indeed, among the known ionophores, CP-82,009 is similar to a group of antibiotics designated "group 3b" by SETO and ŌTAKE[12], namely, etheromycin (CP-38,295), A204A, carriomycin, septamycin, 6016, K-41A, and K-41B. In particular, CP-82,009 is closely related to septamycin. A comparison of the ^{13}C and ^1H NMR chemical shifts for CP-82,009 Na-salt (**2**) (obtained by using ^{13}C DEPT, COSY, HETCOR experiments in a previously described manner[11]) with those values reported for the sodium salt of septamycin (A28695A)[13] is shown in Table 4. Many of the ^{13}C and ^1H signals of **2** essentially correspond to those of septamycin Na-salt. However, some marked changes in the ^{13}C chemical shifts between the two polyethers were observed that are centered at C-27, which is consistent with the addition of a methoxy group at C-27 in **2**. The structure elucidated for **2** based on NMR studies, *i.e.*, 27-methoxyseptamycin, was confirmed by the X-ray analysis of the corresponding Rb-salt as discussed below.

X-Ray Analysis of CP-82,009 Rb-salt

The three-dimentional structure of the rubidium salt to **1** was determined by X-ray crystallography with a crystal that measured $0.14 \times 0.14 \times 0.19$ mm. A 1 Å data set (maximum $\sin\theta/\lambda = 0.5$) was collected on a Nicolet R3m/μ diffractometer, and trial structure was obtained by direct methods revealing the following lattice parameters: $a = 15.646(4)$ Å, $b = 15.646(3)$ Å and $c = 50.32(1)$ Å with $\alpha = 90.0°$, $\beta = 90.0°$ and $\gamma = 90.0°$. The space group was determined to be $P4_32_12$ with 8 molecules per unit cell. The molecular formula was $C_{49}H_{83}O_{17}Rb$ with a calculated density of 1.11 g/cm^3. There were 3,701 reflections collected, and of those reflections 2,519 (68%) with $I > 3.0\sigma$ were adjudged observed. This trial structure refined routinely. Hydrogens were calculated wherever possible. The methyl hydrogens and the hydrogens on

Fig. 4. Crystal structure of CP-82,009 Rb-salt.



oxygen were located by difference Fourier techniques. The hydrogen on oxygen 60 could not be located satisfactorily. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least-squares refinement were all less than 0.1 of their corresponding standard deviations. The final R-index was 0.095. A final difference Fourier revealed no missing or misplaced electron density. The rubidium ion was used to establish the absolute configuration utilizing the method of IBERS and HAMILTON[14], and HAMILTON[15].

The computer generated perspective drawing of the final X-ray model of CP-82,009 Rb-salt is shown in Fig. 4. The anion of the ionophore is wrapped around the central metal ion in a fashion typical of complexed ionophores, generating a number of short Rb-O bond distances. With the exception of the additional methoxy group at C_{27}, the absolute configuration determined for CP-82,009 Rb-salt is the same as that reported for the parent strucrure septamycin, as established by X-ray crystal structure analysis of the *p*-bromophenacyl derivative[4].

Biological Activity

CP-82,009 is among the most potent ionophores known in terms of anticoccidial activity. We conducted anticoccidial efficacy titrations against five major pathogenic species of poultry coccidia, with salinomycin

Table 5. Anticoccidial activity of CP-82,009, salinomycin and maduramicin in chickens[a].

Treatment	Dose (mg/kg)	*Eimeria tenella*		*Eimeria necatrix*		*Eimeria acervulina*		*Eimeria maxima*		*Eimeria brunetti*	
		% WG	% LC	% WG	% LC	% WG	% LC	% WG	% LC	% WG	% LC
Uninfected, untreated	0	100	100	100	100	100	100	100	100	100	100
Infected, untreated	0	64	(3.8)[b]	49	(3.1)	75	(3.2)	54	(2.8)	44	(2.9)
CP-82,009	10	80	100	86	100	69	100	83	97	80	93
	5	98	100	94	100	89	94	77	84	82	52
	2.5	107	79	91	79	90	67	86	69	63	17
Salinomycin	60	95	92	76	100	93	91	71	93	95	87
Maduramicin	5	83	92	96	100	103	94	87	100	95	100

Abbreviations: WG, weight gain; LC, lesion control.
[a] Data are averages for 2~4 tests.
[b] Lesion score, maximum of 4.0 for all species.

and maduramicin as positive controls (Table 5). CP-82,009 exhibited broad spectrum efficacy and toleration, comparable to commercial controls, at doses of 5 or 10 mg/kg in feed. Substantial efficacy was seen against most species at a 2.5 mg/kg dose. CP-82,009 appeared to have potency similar to maduramicin and CP-84,657 (12-methylportmicin), and superior to four other commercial anticoccidial ionophores (*i.e.*, salinomycin, narasin, lasalocid and monensin), when compared in the same test system[11]. We do not have comparable data for the parent structure septamycin, however, published information[16] would indicate that CP-82,009 is *ca.* 6 times more potent than septamycin.

Table 6. *In vitro* antimicrobial activity of CP-82,009.

Test organism	MIC (μg/ml)
Streptococcus suis 02T001	<0.20
Serpulina hyodysenteriae 94A007	<0.20
Actinomyces pyogenes 14D002	<0.20
Clostridium perfringens 10A006	<0.20
Erysipelothrix rhusiopathiae 04A005	<0.20
Staphylococcus aureus 01A106	1.56
Campylobacter fetus 49A001	50
Bacteroides fragilis 78C024	50
Fusobacterium necrophorum 84C004	50
Pasteurella haemolytica 59B046	>100
Actinobacillus pleuropneumoniae 54B004	>100
Escherichia coli 51A538	>100
Pasteurella multocida 59A006	>100
Salmonella choleraesuis 58B015	>100

The results of the *in vitro* antibacterial testing of CP-82,009 are summarized in Table 6. In general, polyether antibiotics are highly effective against Gram-positive bacteria and a number of anaerobic bacteria, but exhibit no activity against Gram-negative aerobes. Activity versus *Serpulina* (*Treponema*) *hyodysenteriae*, a causative agent in swine dysentery, is often observed. CP-82,009 does in fact exhibit the expected excellent activity against a number of Gram-positive bacteria, as well as the spirochete, *S. hyodysenteriae*. No activity was observed versus *Escherichia coli*, *Salmonella choleraesuis* and *Actinobacillus* (*Haemophilus*) *pleuropneumoniae*.

Experimental

General Methods

The media for characterization of the culture and some biochemical tests are those used by HUANG[17]. The utilization of organic acid; the acid production from carbohydrates; the hydrolysis of hippurate and esculin; the resistance to lysozyme; and the decomposition of adenine, hypoxanthine, xanthine, and urea are those described by GORDON *et al.*[18]. The methods of whole-cell amino acid and sugar analyses were described by BECKER *et al.*[19] and by LECHEVALIER[6].

MP's were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Spectral data were recorded on the following instruments: NMR, Bruker WM-250 spectrometer (modified to incorporate a pulse programmer and Aspect-3000 data system) and a Bruker AM-500 spectrometer, using 50 mg samples dissolved in 0.5 ml of $CDCl_3$ (spectra were recorded at 24°C); IR, Perkin-Elmer 1420 spectrophotometer, FAB-MS, VG Analytical 70/250-S mass spectrometer in the positive ion mode using a dithiothreitol-dithioerythritol (3:1) matrix; and optical rotations, Perkin-Elmer 141 polarimeter.

Isotope shift measurements for **2** in $CDCl_3$ solution consisted of identically measuring the ^{13}C spectrum following successive washes with H_2O-D_2O (1:1), H_2O and finally D_2O. In the latter two instances, the washes were repeated several times prior to recording the ^{13}C spectrum.

CP-82,009 Na-salt

The sodium salt of CP-82,009 was obtained as described above from fermentation. The physico-chemical properties are given in Table 3. ^{13}C and ^1H NMR chemical shift data (in $CDCl_3$) and assignments are summarized in Table 4.

CP-82,009 Free Acid

The free acid of CP-82,009 was prepared by vigorously shaking a $CHCl_3$ solution of the corresponding sodium salt with an equal volume of HCl at pH 2 in a separatory funnel. The phases were separated, and the $CHCl_3$ layer was washed with water and then evaporated under vacuum to give the free acid (Table 3).

CP-82,009 K-salt

To prepare the potassium salt of CP-82,009, the free acid (229 mg) was dissolved in $CHCl_3$ (100 ml). Potassium carbonate (75 mg) in 100 ml of water was added and the resulting mixture was allowed to stir for 15 minutes and was then placed in a separatory funnel and vigorously shaken for several minutes. The organic phase was separated and evaporated under vacuum to afford CP-82,009 K-salt as a white solid; mp 175~179°C, $[\alpha]_D^{25}$ +13.8° (*c* 1.0, MeOH). ^1H and ^{13}C NMR spectra were consistent with the desired structure.

Anal Calcd for $C_{49}H_{83}O_{17}K \cdot H_2O$: C 58.78, H 8.56.
Found: C 59.17, H 8.89.

CP-82,009 Rb-salt

To prepare the rubidium salt of CP-82,009, the free acid (100 mg) was dissolved in $CHCl_3$ (100 ml). Rubidium carbonate (150 mg in 100 ml of water) was added to the $CHCl_3$ solution and the mixture was shaken vigorously in a separatory funnel for several minutes. The organic phase was separated and extracted one time with deionized, distilled water, and then evaporated to afford a white solid. The rubidium salt was recrystallized by slow evaporation from ether and used directly for single crystal X-ray analysis.

Single Crystal X-Ray Analysis of CP-82,009 Rb-salt

The refined structure was plotted using the SHELXTL[20] plotting package (Fig. 4). Coordinates, anisotropic temperature factors, distances and angles are available as supplementary material.

Anticoccidial and Antimicrobial Assays

Anticoccidial testing was conducted according to CHAPPEL *et al.*[21], using the lesion scoring system of JOHNSON and REID[22] for all species.

MICs were determined as described by DIRLAM *et al.*[23] except that all anaerobes were tested on Tryptose Agar (Difco) supplemented with 5% bovine blood (TBA) and incubated 48 hours at 39°C in a Coy (Ann Arbor, Mich.) anaerobe chamber containing an N_2-CO_2-H_2 (80:10:10) atmosphere. MICs for aerobes were determined in an identical manner except that Brain-Heart Infusion Agar (Difco) was used, and plates were incubated aerobically at 37°C for 18~20 hours.

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