CP-84,657, A POTENT POLYETHER ANTICOCCIDIAL RELATED TO PORTMICIN AND PRODUCED BY *ACTINOMADURA* SP.

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A new polyether antibiotic CP-84,657 ($C_{45}H_{78}O_{14}$) was isolated by solvent extraction from the fermentation broth of *Actinomadura* sp. (ATCC 53708). Following purification by column chromatography and crystallization, the structure of CP-84,657 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-84,657 is among the most potent anticoccidal 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, *Treponema hyodysenteriae*.

Interest in polyether antibiotics has remained at a high level for over 20 years, owing largely to the commercial importance of this class of drugs in veterinary medicine. For example, monensin[1], lasalocid[1] and salinomycin[2] are marketed as anticoccidial agents for poultry, and are used as growth permittants in cattle or swine. Narasin[1] and maduramicin[3] are also used as anticoccidial agents.

In the process of screening actinomycetes for novel antimicrobial substances, a new strain of *Actinomadura* sp. was found to produce a new polyether antibiotic, CP-84,657 (**1**). This compound, which was very potent versus *Eimeria* coccidia in chickens, was shown to be structurally similar to portmicin[4], Upon completion of this work[5], studies were presented by Hamill *et al.*[6] on the discovery of a new polyether antibiotic, A82810, produced by *Actinomadura fibrosa*. Although an X-ray analysis of A82810 has not been reported, based on ^{13}C and ^1H NMR data it appears that CP-84,657 and A82810 are identical. The present paper describes the taxonomy and fermentation studies on the producing organism of CP-84,657, as well as the isolation, characterization and biological testing of this antibiotic.
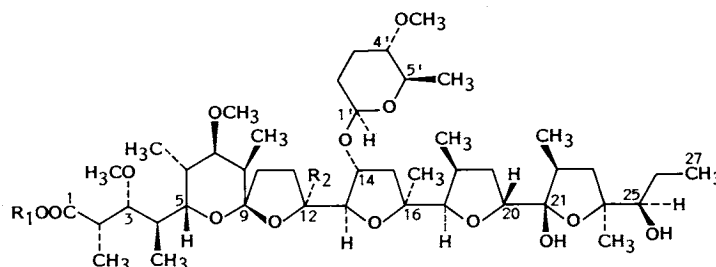
CP-84,657 free acid (**1**) $R_1 = H$ $R_2 = CH_3$
CP-84,657 Na-salt (**2**) $R_1 = Na$ $R_2 = CH_3$
Portmicin $R_1 = H$ $R_2 = H$

Fig. 1. Scanning electron micrograph of spore chains of culture N777-1 on inorganic salts - starch agar at 21 days, ×15,000.
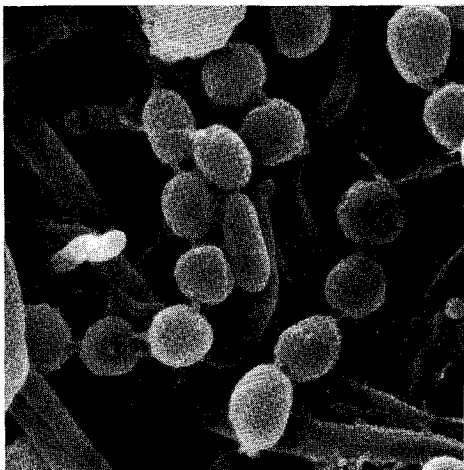

Fig. 2. Scanning electron micrograph of a spore chain of culture N777-1 on inorganic salts - starch agar at 21 days, ×20,000.

Table 1. Physiological properties of *Actinomadura* sp. N777-1.

Production of:		Utilization of:	
Amylase	−	Acetate	+
Cellulase	−	Benzoate	−
Esculinase	+	Citrate	−
Gelatinase	+	Dextrin	−
Nitrate reductase	−	Lactate	−
Melanin	−	Malate	−
H₂S	−	Mucate	−
Milk coagulation	−	Oxalate	−
Milk peptonization	−	Phenol	−
Hydrolysis of:		Propionate	−
Adenine	−	Pyruvate	+
Xanthine	+	Succinate	−
Hypoxanthine	+	Growth at:	
Casein	+	21°C	+
Tyrosine	−	28°C	+
Calcium malate	−	37°C	+
Hippurate	−	45°C	+

Table 2. Carbohydrate utilization of *Actinomadura* sp. N777-1.

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

Taxonomy of the Producing Culture

The CP-84,657 producing strain, *Actinomadura* sp. N777-1, was isolated from a soil sample collected in Tuzla, Istanbul, Turkey. The culture is characterized by the cream substrate mycelium; the short, colorless aerial mycelium; the short spore chains which are straight, curved or hooked; and the spores with a smooth surface (Figs. 1 and 2). The spores were globose, oval to elliptical and measured $0.8 \sim 1.4 \, \mu m$ i.d. or $1.1 \sim 1.8 \times 0.8 \sim 1.2 \, \mu m$, 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, mannose and ribose. Thus, the culture belongs in the genus *Actinomadura*, as defined by LECHEVALIER[7].

    Growth was good on yeast extract - malt extract agar, casein agar, BENNETT's agar, gelatin agar, starch agar, and GAUZE's organic medium 2; moderate to good on CZAPEK - sucrose agar and tyrosine agar; moderate on oatmeal agar, glucose - asparagine agar and EMERSON's agar; poor to moderate on glycerol - asparagine agar, nutrient agar, potato - carrot agar and GAUZE's mineral medium 1; poor on

inorganic salts - starch agar and tap water agar; and scant on calcium malate agar.

Aerial mycelium was generally none or sparse and colorless. The surface color of the colonies was cream (2ca)[8] on most of the media used, cream to pale yellowish (2ca, 2ea) on EMERSON's agar, and off-white (ngs 2ba) on potato - carrot agar. The reverse color of the colonies was cream (2ca) on most of the media used; cream to pale yellowish (2ca, 2ea) on yeast extract - malt extract agar, casein agar, BENNETT's agar, gelatin agar, starch agar and GAUZE's organic medium 2; and yellowish (2ic) on EMERSON's agar. The soluble pigment was generally lacking or cream (2ca) on oatmeal agar; pale yellowish (2ea) on tyrosine agar, BENNETT's agar and gelatin agar; and yellowish (2ga) on casein agar.

The known species of *Actinomadura* which show similar cream substrate mycelium and/or similar biochemical properties include *Actinomadura cremea* subsp. *rifamycini*, *Actinomadura madurae* subsp. *simaoensis*, and *Actinomadura routienii*. The culture differs from *A. cremea* subsp. *rifamycini* in the smooth spores, the failure to reduce nitrate, the failure to utilize raffinose, and the utilization of arabinose and rhamnose.

The culture differs from *A. madurae* subsp. *simaoensis* in the cream rather than colorless to orange brown substrate mycelium, the colorless rather than colorless to pink-white aerial mycelium, the failure to reduce nitrate, the failure to decompose tyrosine, and the ability to decompose xanthine. Compared with *A. routienii*, it differs in its absence of pseudosporangia, failure to hydrolyze starch, failure to coagulate milk, and ability to utilize mannitol, fructose, and glycerol.

The culture is similar to *A. albolutea* in most of the biochemical tests, but differs from the latter in its failure to hydrolyze starch, failure to coagulate milk, cream rather than brown to dark brown substrate mycelium, and short rather than long spore chains.

On the basis of the data presented above, the culture 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 53708.

Fermentation

Actinomadura sp. ATCC 53708 was maintained on an ATCC 172 medium (g/liter: Glucose (10), soluble starch (20), yeast extract (5), NZ-Amine A (5), calcium carbonate (1) and agar (20); pH 7.0 (with KOH) for 7∼10 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-84,657 was followed by TLC on silica gel plates using ethyl acetate as the eluent. The ionophore was visualized as a purple 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 large tank fermentation of whole broth was carried out by extracting the approximately 4,000 liters of whole broth with 1,800 liters of methyl isobutyl ketone, separating the solvent on a Podbielnack extractor and concentrating the solvent to a thin syrup *in vacuo*. The concentrate was triturated two times

with an equal volume of methanol, separated from the methanol insoluble oil, and the methanol triturate was concentrated *in vacuo* to a syrup. The syrup was extracted two times with hexane, and the combined hexane extracts were washed with acetonitrile (the acetonitrile layer was retained for future recovery). The hexane was concentrated *in vacuo*, and the residue was chromatographed in several batches on silica gel. The silica gel was desorbed on a filter funnel first with hexane, then with methylene chloride, EtOAc, and finally with acetone. The active cuts, which were in the methylene chloride and EtOAc eluates, were concentrated and dissolved in the hexane. The hexane solution was washed with acid water, and extracted with a 1% solution of N-methyl-D-glucamine in water. The aqueous phase was salted with sodium chloride and extracted two times with an equal volume of EtOAc. The organic layers were combined, treated with Darco G60, filtered, and then washed with pH 9.0 sodium phosphate buffer and dried over anhydrous Na_2SO_4. Following concentration of the extracts *in vacuo*, the resulting product was crystallized from ether to afford 50.8 g of analytically pure CP-84,657 Na-salt (**2**).

Structural Determination

CP-84,657 free acid (**1**) was obtained by treatment of a chloroform solution of the sodium salt **2** with an aqueous solution of HCl. CP-84,657 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 $C_{45}H_{78}O_{14}$ for the free acid **1**, and $C_{45}H_{77}O_{14}Na$ for the sodium salt **2**. For example, in the positive FAB-MS, diagnostic cationized molecules m/z 865 ($(M+Na)^+$) and 887 ($(M+2Na-H)^+$) were detected for **2**. The ^{13}C and ^1H NMR spectral data for **2**, including a ^{13}C DEPT experiment[9], revealed the following groups: CH_3 (11), CH_2 (8), CH (6), CH_3O (3), O–CH (10), C–O (3), O–CH–O (1), O–C–O (2), and –COONa (1) (see Table 4). These groups accounted for all the hydrogens in **2** except for two exchangeable ones, which were assumed to be free hydroxy functions on δ_C 105.73 and 74.42 based on deuterium induced upfield shifts observed in the ^{13}C NMR spectrum of **2** (see Experimental). Therefore, δ_C 105.73 was assigned to a hemiketal carbon, and δ_C 109.98 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 method developed by WHIPPLE *et al.*[10] in which two equations are used to solve for the two parameters, R and E. The first Eq (Eq 1) for "unsaturated sites" (Unsat.) is a modification of the formula commonly used in mass spectral studies, where,

$$\text{Unsat.} = D + R = 1/2[2(C) + 2 - (H + h)] \qquad \text{(Eq 1)}$$

Table 3. Physico-chemical properties of CP-84,657 free acid (**1**) and Na-salt (**2**).

Property	1	2
MP (°C)	87~90	245~249
$[\alpha]_D^{25}$ (c 1.0, CH_3OH)	$-6.9°$	$-19.0°$
Empirical formula	$C_{45}H_{78}O_{14}$	$C_{45}H_{77}O_{14}Na$
MW	843.1	865.1
Elemental *Anal*	$C_{45}H_{78}O_{14} \cdot \frac{1}{2}H_2O$	$C_{45}H_{77}O_{14}Na$
Calcd:	C 63.43, H 9.34	C 62.48, H 8.97
Found:	C 63.35, H 9.53	C 62.89, H 9.22
IR ($CHCl_3$) cm^{-1}	2954, 2928, 2873, 1725 (–COOH), 1456, 1380, 1163, 1098, 1021, 990, 979, 945	2956, 2930, 2876, 1568 (–COONa), 1456, 1396, 1379, 1359, 1162, 1074, 1025, 990, 978, 938

D = number of unsaturated bonds, R = number of rings, C = number of carbons, H = number of non-exchangeable hydrogens, and h = number of exchangeable hydrogens (including M^+ for a salt form, *i.e.*, Na^+ for compound **2**)

Table 4. ^{13}C and ^1H NMR chemical shift data for CP-84,657 Na-salt (**2**) in $CDCl_3$.

Index		^{13}C Shift[a]	^1H Shift[b]	^1H-^1H Connections[c]	^{13}C-^1H Connections[d]	Assignment
1	COONa	183.25 (0)			21, 39	1
2	O–C–O	109.98 (0)			5, 42	9
3	O–C–O	105.73 (0)			26, 27, 41	21
4	O–CH–O	98.96 (1)	4.40	31		1'
5	O–CH	87.21 (1)	2.93	28, 32	18, 42	7
6	C–O	86.52 (0)			22, 26, 36	24
7	O–CH	86.08 (1)	3.56	14	30, 33	13
8	C–O	85.68 (0)			14, 33	12
9	O–CH	84.73 (1)	4.06	29	37	17
10	C–O	83.18 (0)			9, 23, 37	16
11	O–CH	82.30 (1)	3.34	21, 24	19, 39, 45	3
12	O–CH	80.07 (1)	2.78	15, 34	20, 38	4'
13	O–CH	78.50 (1)	4.21	27	27	20
14	O–CH	74.92 (1)	4.41	7, 23	7, 23	14
15	O–CH	74.68 (1)	3.27	12, 38	38	5'
16	O–CH	74.42 (1)	3.53	35	36, 44	25
17	O–CH	65.33 (1)	4.12	24	43, 45	5
18	OCH_3	60.24 (3)	3.48		5	7-OCH_3
19	OCH_3	57.69 (3)	3.34			3-OCH_3
20	OCH_3	56.82 (3)	3.33		12	4'-OCH_3
21	CH	44.11 (1)	2.58	11, 39	39	2
22	CH	38.91 (1)	1.88	26, 41	26, 41	22
23	CH_2	36.70 (2)	2.21	14	37	15
			1.42			
24	CH	36.31 (1)	2.25	11, 17, 45	45	4
25	CH_2	35.56 (2)	2.22			10
			1.84			
26	CH_2	35.33 (2)	1.97	22	22, 36	23
			1.47	22		
27	CH_2	35.29 (2)	2.10	13	40	19
			1.56	13		
28	CH	35.03 (1)	1.95	5, 42	5, 42	8
29	CH	34.93 (1)	2.30	9, 40	40	18
30	CH_2	31.24 (2)	2.18		33	11
			1.79			
31	CH_2	30.76 (2)	1.79	4, 34		2'
			1.47	4, 34		
32	CH	30.69 (1)	2.26	5, 43	43	6
33	CH_3	28.41 (3)	1.61		30	12-CH_3
34	CH_2	26.97 (2)	2.18	12		3'
			1.29	12, 31		
35	CH_2	26.02 (2)	1.35	16, 44	44	26
			1.06	16		
36	CH_3	24.90 (3)	1.11			24-CH_3
37	CH_3	20.95 (3)	1.19		23	16-CH_3
38	CH_3	18.17 (3)	1.23	15	12	5'-CH_3
39	CH_3	16.35 (3)	1.18	21		2-CH_3
40	CH_3	15.26 (3)	0.89	29		18-CH_3
41	CH_3	13.37 (3)	0.92	22	26	22-CH_3
42	CH_3	12.93 (3)	0.97	28	28	8-CH_3
43	CH_3	11.03 (3)	0.90	32		6-CH_3
44	CH_3	10.60 (3)	0.92	35		27
45	CH_3	10.08 (3)	1.03	24		4-CH_3

[a] In ppm from TMS in $CDCl_3$ solution; number of attached protons in parenthesis.
[b] In ppm from TMS in $CDCl_3$ solution.
[c] ^1H-^1H connections are listed by index number.
[d] Proton unit; ^{13}C-^1H connections are listed by index number.

In the present case, for $C_{45}H_{(75+h)}O_x$, no consideration is made for halogens or trivalent nitrogens, since these atoms can be ruled out based on the elemental and mass spectral analyses. Solving for R in Eq 1, where $D=1$ (i.e., one C=O group), gives $2R = (15-h)$. The second Eq (Eq 2) allows a determination of the number of single bonds between carbon and oxygen, N_{C-O}, where,

Table 5. Calculated number of rings (R), number of oxygen links (E), and total number of oxygens (N_O) as a function of exchangeable hydrogens (h) for $C_{45}H_{(75+h)}O_x$ using Eq 1 and 2.

h	R	E	N_O
1	7	11	13
3	6	10	14
5	5	9	15
7	4	8	16

$$N_{C-O} = n_c + n_v + 2n_k + n_a = h + 2E \qquad (Eq\ 2)$$

n_c = number of carboxy, n_v = number of vinyloxy, n_k = number of (hemi) ketal/acetal, and n_a = number of alkoxy groups

This Eq can be set equal to $(h+2E)$, excluding peroxides, where h and E are defined as above. Solving for N_{C-O} Eq 2, where $n_c=1$, $2n_k=6$, and $n_a=16$, gives $N_{C-O}=23=(h+2E)$. From Eq 1 and 2, values for R and E can be readily calculated, for $h=1$, 3, 5 and 7, as shown in Table 5. The total number of oxygens, N_O, that results from the addition of $(h+E)$ and the number of C=O groups, is also tabulated. Based on our experience, it is usually possible to select the correct solution for R and E by actually determining the value of h experimentally, i.e., from the ^{13}C NMR experiment as described in the Experimental using H_2O - D_2O. In the present case, $h=3$ when M^+ is included as an exchangeable proton. Thus, the number of rings (R) is 6, and the number of oxygen links (E) is 10 for compound 2. Quite often, N_O can be obtained from elemental and mass spectral analyses of an unknown structure. For 2, $N_O=14$ based on these data, and this is in agreement with the calculated value of $N_O=14$, where $h=3$ (Table 5).

With a knowledge of R and E, coupled with other information such as the number of methoxy groups, carbonyl groups, double bonds, MW, etc., characteristic of a given unknown polyether antibiotic, a comparison with representative structures from the over 120 known polyether ionophores can be readily made. From the data obtained for CP-84,657 Na-salt (2), it was apparent that the structure was very similar to portmicin[4]. Namely, it contained an additional methyl group relative to portmicin, which was readily observed as a singlet in the ^1H NMR spectrum at δ 1.61 ppm (Fig. 3). From a comparison of the ^{13}C and ^1H NMR data (including ^{13}C DEPT and HETCOR[11] experiments) obtained for CP-84,657 free acid (1) and those reported for portmicin free acid[12], both in benzene-d_6 solvent, the extra methyl appeared to perturb carbons 10 through 15, and some of the hydrogens attached to these carbons. From a consideration of the biogenesis of polyether antibiotics, methyl substitution at C-12 would be a likely possibility[13]. Indeed, ^{13}C and ^1H shift assignments from a detailed analysis of the NMR data obtained for 2, in $CDCl_3$ solvent, were consistent with methyl substitution at C-12 (Table 4). The NMR studies included two long-range HETCOR experiments. The first utilized the same standard pulse sequence employed for the one-bond correlations, with delays scaled to ^{13}C-^1H couplings of ca. 5 Hz. The second was an inverse experiment also scaled to long-range ^{13}C-^1H couplings and incorporating a one-band filter according to the method of BAX and SUMMERS, and BAX et al.[14,15]. Several assignments for 2 were made by analogy with the chemical shifts and assignments for portmicin, since a detailed NMR analysis had already been performed for this compound[12]. In particular, the carbon-carbon connectivities C-9 ~ C-10 and C-10 ~ C-11 were assumed. The ^{13}C shift at δ_C 31.24 was assigned as C-10 since it was the only carbon not assigned. The structure elucidated for 2 based on NMR studies, i.e., 12-methylportmicin, was confirmed

Fig. 3. ^{1}H NMR spectrum of CP-84,657 Na-salt (**2**) in CDCl$_3$ (500 MHz).
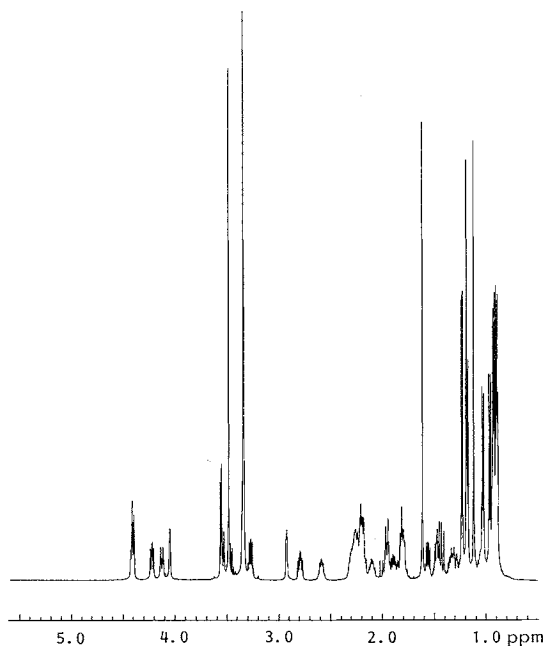
by the X-ray analysis of the corresponding Rb-salt as discussed below.

X-Ray Analysis of CP-84,657 Rb-Salt

The three-dimensional structure of the rubidium salt of **1** was determined by X-ray crystallography with a crystal that measured $0.21 \times 0.22 \times 0.25$ mm. A 1Å data set (maximum $\sin \theta/\lambda = 0.5$) was collected on a Nicolet R3m/μ diffractometer, and a trial structure was obtained by direct methods revealing the following lattice parameters: $a = 12.264$ (5) Å, $b = 16.13$ (1) Å and $c = 28.03$ (1) Å with $\alpha = 90.0°$, $\beta = 90.0°$ and $\gamma = 90.0°$. The space group was determined to be $P2_12_12_1$ with four molecules per unit cell. The molecular formula was $C_{45}H_{77}O_{14}Rb \cdot H_2O$ with a calculated density of 1.13 g/cm^3. There were 3,216 reflections collected, and of those reflections 3,036 (94%) with $I > 3.0\sigma$ were adjudged observed. This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogens on oxygen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The rubidium ion was used to establish the absolute configuration utilizing the method of IBERS and HAMILTON, and HAMILTON[16,17]. 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 0.069. A final difference Fourier revealed no missing or misplaced atoms.

The computer generated perspective drawing of the final X-ray model of CP-84,657 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. Furthermore, the crystal structure of CP-84,657 Rb-salt is very similar to that of portmicin Tl-salt as determined by SETO, CLARDY, and co-workers[12], and the space groups are the same. The additional methyl group at C-12 in CP-84,657 is directed away from the central metal ion and it therefore does not change the special arrangement observed for the rest of the molecule, relative to that observed for the parent structure.

Fig. 4.   Crystal structure of CP-84,657 Rb-salt.
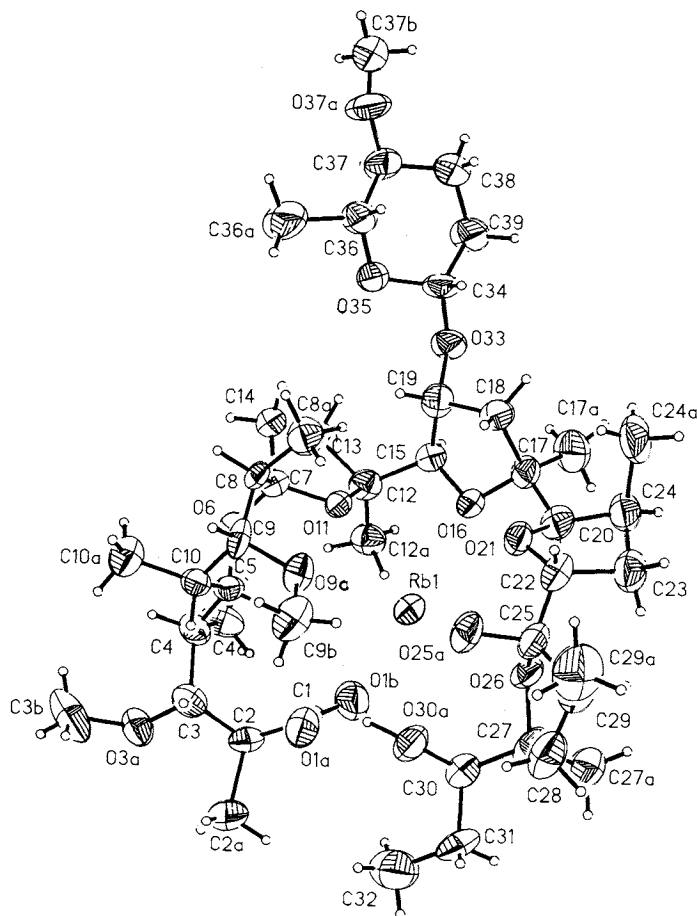
Biological Activity

CP-84,657 ranks among the most potent anticoccidial ionophores discovered. We conducted duplicate anticoccidial efficacy titrations against four major species of poultry coccidia, with salinomycin as a positive control (Table 6). CP-84,657 exhibited broad spectrum efficacy and toleration, comparable to salinomycin, at doses down to 2.5 mg/kg in feed. When we compared these results with contemporary trials using all five commercial anticoccidial ionophores in the same test system, we found the potency of CP-84,657 to be at least equivalent to the most potent agent, maduramicin. This is illustrated in Table 7 by data from titrations against *Eimeria tenella*. We do not have comparable data for the parent structure portmicin, however, published information[4] would indicate that portmicin is $2 \sim 10 \times$ less potent than CP-84,657.

The results of the *in vitro* antibacterial testing of CP-84,657 are summarized in Table 8. 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 Treponema hyodysenteriae*, a causative agent in swine dysentery, is often observed. CP-84,657 does indeed exhibit the expected excellent activity against a number of Gram-positive bacteria, as well as the spirochete, *T. hyodysenteriae*. No activity was observed *versus Escherichia coli, Salmonella choleraesuis* and *Actinobacillus (Haemophilus) pleuropneumoniae*.

Table 6.   Anticoccidial activity of CP-84,657 and salinomycin in chickens[a].

Treatment	Dose (mg/kg)	Eimeria tenella		E. acervulina		E. maxima		E. necatrix	
		WG (%)	LC (%)	WG (%)	LC (%)	WG (%)	LC (%)	WG (%)	LC (%)
Uninfected, untreated	0	100	100	100	100	100	100	100	100
Infected, untreated	0	64	(3.9)[b]	87	(3.0)	25	(3.0)	39	(3.8)
CP-84,657	10	99	100	80	100	87	91	78	100
	5	96	100	106	100	95	91	86	95
	2.5	97	83	95	96	90	79	95	90
	1.25	119	3	101	65	57	40	82	51
Salinomycin	60	98	94	93	100	85	76	94	95

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

Table 7.   Anticoccidial potency of CP-84,657 compared to 5 commercial ionophores against Eimeria tenella.

Treatment	Dose (mg/kg)	E. tenella mean lesion score[a] ($\pm$ SEM)	Number of tests	Treatment	Dose (mg/kg)	E. tenella mean lesion score[a] ($\pm$ SEM)	Number of tests
Unmedicated	0	3.30 $\pm$ 0.07	46	Salinomycin	60	0.36 $\pm$ 0.09	37
CP-84,657	10	0.00 $\pm$ 0.00	3		30	1.38 $\pm$ 0.21	36
	5	0.32 $\pm$ 0.32	4	Narasin	80	0.00 $\pm$ 0.00	4
	2.5	0.65	2		40	1.66 $\pm$ 0.38	4
	1.25	3.65	2	Lasalocid	100	0.09 $\pm$ 0.05	4
Maduramicin	5	0.38 $\pm$ 0.17	8		50	1.41 $\pm$ 0.51	4
	2.5	1.58 $\pm$ 0.29	8	Monensin	100	0.70 $\pm$ 0.25	4
					50	1.57 $\pm$ 0.53	4

[a]   Maximum lesion score 4.0.

Table 8.   In vitro antimicrobial activity of CP-84,657.

Test organism	MIC (μg/ml)	Test organism	MIC (μg/ml)
Streptococcus suis 02T001	<0.20	Bacteroides fragilis 78C024	50
Treponema hyodysenteriae 94A007	<0.20	Fusobacterium necrophorum 84C004	50
Actinomyces pyogenes 14D002	0.39	Pasteurella haemolytica 59B046	50
Clostridium perfringens 10A006	0.39	Actinobacillus pleuropneumoniae 54B004	>100
Erysipelothrix rhusiopathiae 04A005	0.78	Escherichia coli 51A538	>100
Staphylococcus aureus 01A106	3.12	Pasteurella multocida 59A006	>100
Campylobacter fetus 49A001	25	Salmonella choleraesuis 58B015	>100

Experimental

General Methods

The media for characterization of the culture and some biochemical tests are those used by HUANG[18]. 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.[19]. The methods of whole-cell amino acid and sugar analyses were described by BECKER et al.[20] and by LECHEVALIER[7].

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 $CDCl_3$ or C_6D_6 (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 1 : 1 H_2O - D_2O, 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-84,657 Na-Salt

The sodium salt of CP-84,657 was obtained from fermentation as described above. 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-84,657 Free Acid

The free acid of CP-84,657 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). The following ^{13}C NMR values were obtained for CP-84,657 free acid in C_6D_6 for comparison with those values reported for portmicin free acid[12] in C_6D_6 (number of attached hydrogens and ^1H chemical shifts are in parentheses): δ_C 176.31, 110.23, 106.66, 99.37 (1H, 4.40), 87.23 (1H, 3.76), 85.57, 85.57 (1H, 2.76), 85.38, 84.71 (1H, 4.26), 83.69, 81.85 (1H, 3.45), 80.42 (1H, 2.56), 79.45 (1H, 4.22), 77.21 (1H, 3.53), 75.89 (1H, 4.74), 75.14 (1H, 3.30), 67.73 (1H, 4.02), 58.44 (3H, 3.60), 57.77 (3H, 3.20), 56.37 (3H, 3.04), 40.74 (1H, 2.97), 38.72 (1H, 1.70), 37.78 (1H, 2.28), 37.46 (2H, 1.46, 2.70), 36.71 (2H, 1.92, 2.47), 36.59 (2H, 1.20, 2.30), 36.50 (1H, 2.09), 35.56 (1H, 1.92), 35.01 (2H, 1.26, 1.97), 32.66 (2H, 1.92, 2.40), 31.40 (2H, 1.43, 1.61), 30.51 (1H, 2.42), 28.10 (3H, 1.70), 27.31 (2H, 1.10, 1.84), 25.51 (2H, 1.24), 24.51 (3H, 0.92), 21.73 (3H, 1.20), 18.51 (3H, 1.37), 16.18 (3H, 1.47), 15.40 (3H, 0.75), 13.54 (3H, 1.16), 13.15 (3H, 1.10), 12.14 (3H, 1.36), 11.32 (3H, 1.16), and 10.97 (3H, 0.84).

CP-84,657 K-Salt

To prepare the potassium salt of CP-84,657, the free acid (130 mg) was dissolved in $CHCl_3$(100 ml). Potassium carbonate (100 mg) in 100 ml water was added and the resulting mixture was allowed to stir for several 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-84,657 K-salt as a white solid; mp 255~260°C, $[\alpha]_D^{25}$ −19.6° (c1.0, $CHCl_3$). ^1H and ^{13}C NMR spectra were consistent with the desired structure.

Anal Calcd for $C_{45}H_{77}O_{14}K$: C 61.34, H 8.81.
      Found: C 60.91, H 8.83.

CP-84,657 Rb-Salt

To prepare the rubidium salt of CP-84,657, the free acid (30 mg) was dissolved in $CHCl_3$ (50 ml). Rubidium carbonate (35 mg in 25 ml of water) was added to the $CHCl_3$ and the mixture was allowed to stir for 2 hours. The organic phase was separated and the aqueous layer was extracted with an equal volume of $CHCl_3$. The combined $CHCl_3$ extracts were 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-84,657 Rb-Salt

The refined structure was plotted using the SHELXTL[21] 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.*[22], using the lesion scoring system of JOHNSON and REID[23] for all species.

MICs were determined as described by DIRLAM *et al.*[24] 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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