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CP-82,996, a novel diglycoside polyether antibiotic related to monensin and produced by *Actinomadura* sp.

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SUMMARY

A new polyether antibiotic CP-82,996 ($C_{50}H_{86}O_{16}$) was isolated by solvent extraction from the fermentation broth of *Actinomadura* sp. (ATCC 53764). Following purification by silica gel column chromatography and crystallization, the structure of CP-82,996 was determined by a single crystal X-ray analysis. The structure is closely related to monensin, but is unique in that it contains two sugar groups, whereas monensin has none. The ^1H and ^{13}C NMR chemical shifts and assignments for CP-82, 996 were elucidated, and they were compared with those determined previously for monensin. CP-82,996 is active against certain Gram-positive bacteria, and is a very potent anticoccidial agent. It effectively controlled chicken coccidiosis caused by several *Eimeria* species at 5–10 ppm in feed, and is 10–20 times more potent than monensin.

INTRODUCTION

Interest in polyether antibiotics has remained at a high level for over 20 years, due largely to the commercial importance of this class of drugs in veterinary medicine. For example, monensin [14], lasalocid [14] and salinomycin [12] are marketed as anticoccidial agents for poultry, and are used as growth permittants in cattle or swine. Narasin [14] and maduramicin [13] are 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-82,996. This compound, which was very potent in vivo versus *Eimeria* coccidia in chickens, was shown to be structurally similar to monensin. This paper describes the taxonomy and fermentation studies on the producing organism, as well as the isolation, characterization and biological testing of CP-82,996 (structure shown in Fig. 1).

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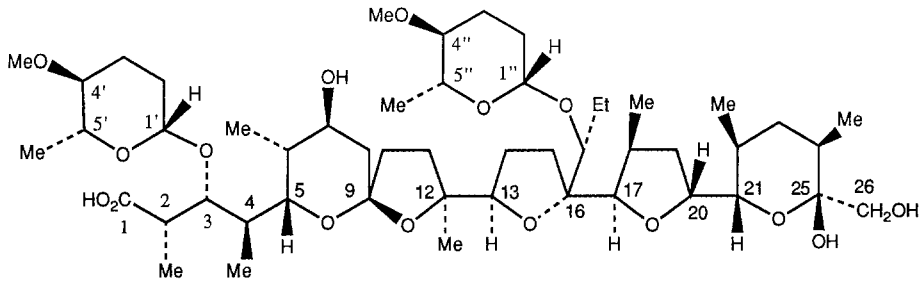
MATERIALS AND METHODS

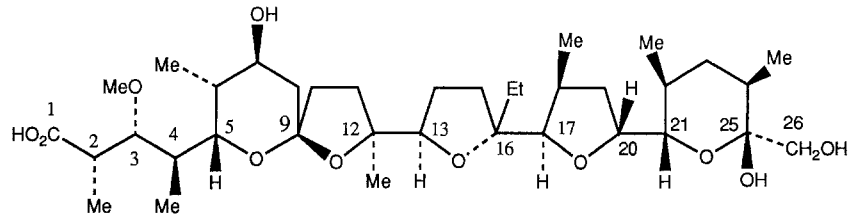
General. The media for characterization of the culture and some biochemical tests are those used by Huang [8]. The utilization of organic acid; the acid production from carbohydrates; the hydrolysis of hippurate and esculin; the resistance to lysozyme; and the decomposition of adenine, hypoxanthine, xanthine and urea are those described by Gordon et al. [7]. The methods of whole-cell amino acid and sugar analyses were described by Becker et al. [2] and by Lechevalier [9].

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Spectral data were recorded on the following instruments: NMR, Bruker WM-250 (250 MHz; equipped with an Aspect-3000 data system, using $CDCl_3$ solutions in a 5-mm dual $^{13}C/^1H$ probe); IR, Perkin-Elmer 1420; FAB-MS, VG-70/250-S; and optical rotations, Perkin-Elmer 141.

Taxonomy. The microorganism used to produce CP-82,996 was isolated from a soil sample collected in Nigeria. It was found to produce the narrow hyphae of the actinomycetes, an unfragmented substrate mycelium, and an aerial mycelium upon which short spore chains are



CP-82,996



Monensin

Fig. 1. The structure of CP-82,996 and monensin.

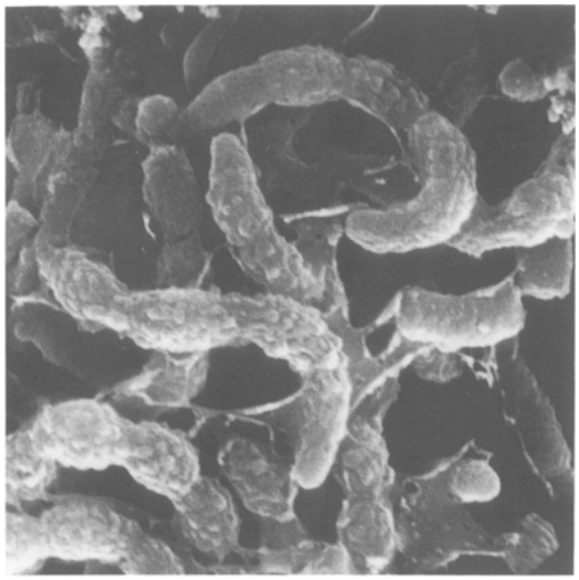


Fig. 2. Scanning electron micrograph of spore chains of *Actinomadura* sp. ATCC 53764 on inorganic salts-starch agar for 3 weeks at 28 °C. ×15000.

produced (Fig. 2). The culture is characterized (Table 1) by the white to cream (sometimes gray) colonies; the white (sometimes gray) aerial mycelium; the short spore chains which are flexuous, curved, hooked or looped; and the spores with a warty surface (Fig. 2). The colors of the substrate mycelium might range from colorless, cream, pale yellowish, yellowish, yellowish orange, yellowish gray to gray. Among all of the sugars tested, only glucose, sucrose, ribose, and starch were utilized. Hypoxanthine, but not adenine and xanthine, was decomposed. The culture did not produce melanin and hydrogen sulfide; did not hydrolyze esculin, hippurate, starch, and urea; and was sensitive to lysozyme. The whole-cell hydrolysates contained meso-diaminopimelic acid and the diagnostic sugar madurose. Thus, the culture belongs in the genus *Actinomadura*, as defined by Lechevalier [9].

The culture shows some similarity to the following known species of *Actinomadura* in cultural properties and/or biochemical properties: *A. atramentaria*, *A. livida*, and *A. macra*. It differs from *A. atramentaria* in the warty rather than smooth spores, the absence of dark brown

TABLE 1

Cultural characteristics of *Actinomadura* sp. ATCC 53764[a]

Medium	Growth and texture	Colony surface color[b]	Aerial mass color	Colony reverse color	Soluble pigment
Yeast extract-malt extract agar	Poor to moderate; raised, wrinkled	White, cream to gray (2 ca, ngs[c] 3 ih, 3 ml)	White to gray (ngs 3 ih, 3 ml)	Yellowish gray to gray (2 ie, 2 ig, ngs 3 ih)	Yellowish gray (2 ie)
Oatmeal agar	Moderate; slightly raised, smooth	White to cream (2 ca)	White	Cream (2 ca)	Cream (2 ca)
Inorganic salts-starch agar	Moderate; slightly raised, smooth	White	White	Colorless to cream (2 ca)	None
Glycerol-asparagine agar	Poor to moderate; slightly raised, smooth to granular	White	White	Colorless	None
Czapek-sucrose agar	Moderate to good; slightly raised, smooth	White to cream (2 ca)	White	Colorless to cream (2 ca)	None
Glucose-asparagine agar	Poor; thin to slightly raised, smooth to granular	White to cream (2 ca)	White	Colorless, cream to pale yellowish (2 ca, 2 ea)	None
Bennett's agar	Good; raised, wrinked	Gray (ngs 3 fe, 3 ih) with some white dots	White to gray (ngs 3 fe, 3 ih)	Grayish black (ngs 3 ih, 3 ml)	Yellowish gray (2 ig)
Emerson's agar	Poor to moderate; raised, wrinkled to granular	Cream, pale yellowish, yellowish gray to dark gray (2 ca, 2 ea, 2 ia, ngs 3 ih, 3 ml)	Gray (ngs 3 ih)	Yellowish to gray (ngs 2 ih)	Yellowish gray (2 le, 2 ie)
Nutrient agar	Moderate; slightly raised, smooth	Pale yellowish to yellowish (2 ea, 2 ga)	None	Same as surface	None
Gauze's Mineral medium 1	Moderate to good; slightly raised, smooth	White to cream (2 ca)	White	Cream (2 ca)	None
Gauze's organic medium 2	Moderate to good; moderately raised, smooth to wrinkled	Cream to pale yellowish (2 ca, 2 ea)	White, none to sparse	Same as surface	None

[a] Unless stated otherwise all readings were recorded after two weeks of incubation at 28 °C.
[b] The color scheme used was Color Harmony Manual, 4th Edn., 1958, Container Corporation of America, Chicago, IL, U.S.A.
[c] ngs, near gray series.

TABLE 2

Properties of CP-82,996 free acid and Na-salt salt

Property	Free acid	Na-salt
Melting point (°C)	115–117	265–267
$[\alpha]^{25}D$ (c = 1, $CHCl_3$)	+21.6°	31.2°
Empirical formula	$C_{50}H_{86}O_{16}$	$C_{50}H_{85}O_{16}Na$
Molecular weight	943.2	965.2
Elemental analysis		
found	C, 63.52; H, 9.36	C, 59.61; H, 8.56
calcd. for	$C_{50}H_{86}O_{16}$	$C_{50}H_{85}O_{16}Na \cdot 2H_2O$
	C, 63.67; H, 9.19	C, 59.98; H, 8.95
Infra-red ($CHCl_3$)	1730 cm^{-1} ($-CO_2H$)	1562 cm^{-1} ($-CO_2Na$)

TABLE 3

^{13}C and ^{1}H NMR chemical shift data for CP-82,996 Na-salt and monensin Na-salt

Carbon	CP-82,996 Na-salt		Monensin Na-salt[a]	
	^{13}C Shift[b]	^{1}H Shift[c]	^{13}C Shift[b]	^{1}H Shift[c]
1	181.41 (0)	–	181.20 (0)	–
9	106.80 (0)	–	107.03 (0)	–
25	98.32 (0)	–	98.31 (0)	–
16	89.11 (0)	–	85.90 (0)	–
12	85.46 (0)	–	85.26 (0)	–
17	84.32 (1)	3.80	84.94 (1)	3.94
3	83.84 (1)	3.82	83.11 (1)	3.19
13	81.98 (1)	3.49	82.52 (1)	3.54
16-$\underline{C}$H(O-Deo)CH_2CH_3	76.19 (1)	3.88	–	–
20	76.12 (1)	4.35	76.45 (1)	4.40
21	74.30 (1)	3.78	74.51 (1)	3.83
7	70.59 (1)	3.83	70.51 (1)	3.90
5	68.39 (1)	3.98	68.33 (1)	4.03
26	64.83 (2)	3.23, 3.93	64.92 (2)	3.30, 3.98
3-OMe	–	–	57.84 (3)	3.38
2	44.39 (1)	2.50	45.07 (1)	2.53
10	39.41 (2)	1.68, 1.96	39.28 (2)	1.70, 2.01
4	37.68 (1)	1.97	37.50 (1)	2.07
24	36.51 (1)	1.44	36.57 (1)	1.46
23	35.71 (2)	1.27, 1.45	35.75 (2)	1.24, 1.41
6	34.88 (1)	2.23	34.87 (1)	2.22
18	34.67 (1)	2.09	34.39 (1)	2.26
8	33.49 (2)	1.67, 1.84	33.56 (2)	1.69, 1.91
19	33.35 (2)	1.54, 2.12	33.33 (2)	1.55, 2.19
11	33.03 (2)	1.68, 1.93	33.25 (2)	1.72, 1.98
22	31.89 (2)	1.36	31.87 (1)	1.36
16-$\underline{C}H_2CH_3$	–	–	30.60 (2)	1.51, 1.60
12-Me	27.74 (3)	1.46	27.47 (3)	1.51
15	27.51 (2)	–[d], 2.18	29.91 (2)	1.47, 2.30
16-CH(O-Deo)$\underline{C}H_2CH_3$	25.49 (2)	2.05, 2.43	–	–
14	23.86 (2)	1.47, 1.74	27.26 (2)	1.54, 1.78
2-Me	16.91 (3)	1.23	16.72 (3)	1.24
22-Me	16.81 (3)	0.78	16.80 (3)	0.81
24-Me	16.10 (3)	0.83	16.03 (3)	0.85
18-Me	14.25 (3)	0.88	14.56 (3)	0.90
4-Me	10.71 (3)	1.18	10.97 (3)	1.18
6-Me	10.36 (3)	0.96	10.48 (3)	0.94
16-CH(O-Deo)$CH_2\underline{C}H_3$	10.03 (3)	0.84	–	–
16-$CH_2\underline{C}H_3$	–	–	8.14 (3)	0.94

substrate mycelium, the utilization of sucrose, the failure to produce melanin and to peptonize milk, and the ability to liquefy gelatin. Compared with *A. livida*, it differs in the white rather than cream aerial mycelium and the absence of the brown substrate mycelium.

The culture differs from *Actinomadura macra* in the presence of hooked or looped spore chains, the warty rather than smooth spores, the ability to digest casein, the failure to produce hydrogen sulfide, and utilization of ribose and starch.

TABLE 3 (continued)

Carbon	Deoxysugars (Deo)[e] of CP-82,996 Na-salt at C-3 and 16-CH(O-)CH_2CH_3	
	^{13}C Shift[b]	^{1}H Shift[c]
1′ or 1″	101.31 (1)	4.38
1″ or 1′	97.21 (1)	4.49
2′ or 2″	30.87 (2)	1.52, 1.74
2″ or 2′	30.79 (2)	1.45, 1.87
3′	27.16 (2)	1.44, 2.17
3″	27.16 (2)	1.44, 2.17
4′ or 4″	80.42 (1)	2.78
4″ or 4′	80.36 (1)	2.78
5′ or 5″	74.64 (1)	3.13
5″ or 5′	74.58 (1)	3.24
4′-OMe or 4″-OMe	56.87 (3)	3.32
4″-OMe or 4′-OMe	56.87 (3)	3.31
5′-Me or 5″-Me	18.32 (3)	1.22
5″-Me or 5′-Me	18.24 (3)	1.22

[a] Robinson et al [1].
[b] In ppm from TMS in $CDCl_3$ solution; number of attached protons in parentheses.
[c] In ppm from TMS in $CDCl_3$.
[d] Difficult to assign due to spectral crowding in the region of δ 1.5 ppm.
[e] 4-*O*-Methylamicetose.

On the basis of the above data, the culture is considered as a member of the genus *Actinomadura* and designated *Actinomadura* sp. It has been deposited with the American Type Culture Collection under the accession number ATCC 53764.

Fermentation and isolation of CP-82,996 Na-salt. Actinomadura sp. ATCC 53764 was maintained on an ATCC 172 medium (g/l: 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/l: 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 5–6 days at 28–36 °C, 150–200 rpm). A 5% inoculum was used to seed a production run in C′ medium (g/l: 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 7–10 days at 30 °C; 1700 rpm (jar), 600 rmp (tanks)). The antibiotic titers were followed by using a disc assay on a sensitive strain of *Bacillus subtilis* ATCC 6633. The presence of CP-82,996 was followed by TLC on silica gel plates using chloroform/2-propanol (95 : 5) as the eluent. The ionophore was visualized as a red-orange coloration using vanillin-EtOH-H_3PO_4 spray reagent (3 g vanillin in 85 ml of EtOH and 15 ml of 85% H_3PO_4), followed by heating to 80 °C.

Work-up of a tank fermentation was carried out by extracting the approximately 4000 liters of whole broth with 1400 liters of methyl isobutyl ketone. The organic extract was separated and concentrated under vacuum, initially in a vacuum pan and finally on a rotary evaporator to yield 12 liters of crude product as a syrup. The syrup was extracted twice with 25 liters of methanol. The extracts were combined and restripped to a second oil which was chromatographed on Sephadex LH20 using methanol as eluent, and monitored by TLC as described above. Product containing cuts were combined and chromatographed on silica gel, eluted sequentially with hexane, toluene, $CHCl_3$, ethyl acetate and acetone, again monitored by TLC. The desired product was contained in the $CHCl_3$ and ethyl acetate cuts. The latter were combined, concentrated to 85 g of residue and rechromatographed on silica gel using 1 : 1 ethyl acetate-hexane as eluent. Product fractions were combined, treated with activated carbon, filtered, shaken with pH 9.0 dibasic sodium phosphate buffer and dried over Na_2SO_4. The mixture was filtered and concentrated, and the resulting product was

crystallized from ethyl acetate-hexane to afford 2.03 g of analytically pure CP-82,996 Na-salt (properties are given in Table 2).

Preparation of CP-82,996 free acid. The free acid of the CP-82,996 was prepared by vigorously shaking a $CHCl_3$ solution of the corresponding sodium salt with an equal volume of hydrochloric acid at pH 2 in a separatory funnel. The phases were separated, and the chloroform layer was washed with water and then evaporated under vacuum to give the free acid (properties are given in Table 2).

Single crystal X-Ray analysis of CP-82,996 free acid monohydrate. A representative crystal, obtained by a slow crystallization from acetonitrile-water, was surveyed and a 1 Å data set (maximum sin $\theta/\lambda = 0.5$) was collected on a Nicolet R3m/μ diffractometer. The diffractometer data were collected at room temperature.

Antimicrobial and anticoccidial assays. MICs were determined as described by Dirlam et al. [6], except that all anaerobes were tested on tryptose agar (Difco) supplemented with 5% bovine blood (TBA) and incubated 48 h at 39 °C in a Coy (Ann Arbor, MI) 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 (BHI) agar (Difco) was used and plates were incubated aerobically at 37 °C for 18–20 h.

The evaluation of drug activity against *Eimeria* coccidial infections in chickens was conducted as described by Chappel et al. [4].

RESULTS AND DISCUSSION

Structural determination

The fast atom bombardment mass spectrum (FAB-MS) of CP-82,996-Na salt showed peaks at 966 (M + Na) and 988 (M + 2Na − H) that were in agreement with the molecular formula $C_{50}H_{85}O_{16}Na$ (965.2), calculated from microanalytical data (assuming a dihydrate; Table 2). As expected, the molecular formula for the free acid of CP-82,996 was $C_{50}H_{86}O_{16}$, calculated from microanalytical data (Table 2).

A comparison of the ^{13}C NMR spectrum of CP-82,996 Na-salt with representative spectra from the over 120 known polyether antibiotics, indicated that CP-82,996 was a "group 3d" ionophore as classified by Seto and Otake [15], of which monensin (Fig. 1) is a prime example. However, in addition to this type of polyether structure that possesses two (hemi)ketal functions, i.e., 98.3 and 106.8 ppm, two anomeric carbons were also observed at 97.2 and 101.3 ppm (Table 3). We suspected the presence of two sugars, and the ^1H NMR spectrum of CP-82,996 Na-salt also showed two methoxy peaks at 3.31 and 3.32 ppm (Table 3). 4-O-Methylamicetose, which is by far the most common sugar found in polyether antibiotics, gives a ^{13}C NMR chemical shift for the methoxy methyl in the range of 56.6–56.9 ppm in a number of ionophore structures [15], and we observed a shift at 56.9 ppm for two coincident carbons. Based on a comparison of the ^{13}C NMR data reported for monensin Na-salt [1] and that determined for CP-82,996 Na-salt, the 3-OMe group (i.e., 57.8 ppm) and the ethyl group at C-16 of monensin were noticeably absent in the new structure (Table 3). Therefore, it seemed reasonable to assume that the two additional sugars in CP-82,996 might be located at these positions.

Attempts to prepare a suitable heavy atom salt of CP-82,996 for X-ray analysis, starting from the free acid using rubidium carbonate were unsuccessful. However, the structure of CP-82,996 was determined by a single crystal X-ray analysis of the corresponding free acid monohydrate (space group $P2_12_12_1$, with a = 9.922 (2) Å, b = 21.320 (4) Å and c = 25.768 (7) Å). As shown in Fig. 1, CP-82,996 is an analog of monensin. The crystal structure of CP-82,996 free acid monohydrate is similar to that of monensin free acid monohydrate as determined by Lutz et al. [11], and the space groups are the same. In both cases, the water molecule is enclosed in the center of the neutral antibiotic, and the conformational arrangement about the water molecule is nearly identical. Although the absolute stereochemistry of CP-82,996 was not determined owing to the fact that a heavy atom salt was not used, it is likely the same as monensin since they exhibit similar optical rotation values [16].

The structure of CP-82,996 (Fig. 1) is unique in that it contains two sugars, whereas monensin has none. Both sugars are identical, i.e., 4-*O*-methylamicetose, and are located at C-3 and at the α-position of the *n*-propyl side-chain at C-16. The ^1H and ^{13}C NMR spectra, including ^{13}C DEPT, COSY and HETCOR experiments, of CP-82,996 Na-salt were most informative, and Table 3 gives the chemical shift assignments. Many of the assignments were made by analogy with the chemical shifts and assignments for monensin Na-salt, since a detailed NMR analysis had already been performed on this ionophore by Robinson et al. [1].

TABLE 4

In vitro antimicrobial activity of CP-82,996

Test organism	Strain	MIC (μg/ml)
Staphylococcus aureus	01A106	>100
Streptococcus pyogenes	02C203	0.78
Escherichia coli	51A538	>100
Salmonella choleraesuis	58B015	>100
Erysipelothrix rhusiopathiae	04A005	<0.20
Pasteurella multocida	59A006	>100
P. haemolytica	59B018	>100
Mycoplasma bovis	93A001	25
Treponema hyodysenteriae	94A007	0.78

Biological activity

There are numerous polyether antibiotics that have one sugar moiety, and many of these ionophores exhibit good in vivo anticoccidial activity [10]. In contrast, only a few possess two sugars. The first monovalent diglycoside polyether antibiotic, K-41B, was described in 1979 [17]. Recently, another one was patented, UK-58,852, which is structurally similar to maduramicin and is an effective anticoccidial agent [5].

No naturally occurring analogs of monensin that contain any sugar groups have been previously isolated by conventional fermentation. However, a patent [3] described the use of a new strain of *Streptomyces candidus* (NRRL 5449), which converted monensin in the presence of an adequate supply of glucose into metabolite A-27106, a glucosyl monensin analog of unknown structure. Unfortunately, A-27106 was only partially effective versus *Eimeria tenella* at levels of 100–200 mg/kg. We felt it would be desirable to screen some additional glycoside analogs of monensin for biological activity, and the isolation of the present diglycoside CP-82,996 afforded an opportunity to do so.

The antibacterial testing results of CP-82,996 are summarized in Table 4. 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* and *Mycoplasma*, including *T. hyodysenteriae*, a causative agent in swine dysentery, is often observed [10]. CP-82,996 is surprisingly weak against *Staphylococcus aureus*, and somewhat weaker than anticipated versus *M. bovis*. However, the expected excellent activity against *S. pyogenes*, *E. rhusiopathiae* and *T. hyodysenteriae* was realized with this new antibiotic.

In contrast to the antibacterial studies with CP-82,996, the effect of the additional two sugar groups on the observed anticoccidial activity was profound (Table 5). In fact, this novel diglycoside polyether antibiotic is 10–20 times more potent than the parent structure, monensin. Control of coccidiosis in poultry comparable to monensin at 100 ppm was demonstrated when birds were given CP-82,996 at 5 to 10 ppm in feed versus *E. tenella*,

TABLE 5

Anticoccidial activity of CP-82,996 and monensin in chickens[a]

Drug	ppm	*E. tenella*		*E. acervulina*		*E. maxima*		*E. necatrix*	
		% WG[b]	% LC[c]	% WG	% LC	% WG	% LC	% WG	% LC
Uninfected, untreated	0	100	100	100	100	100	100	100	100
Infected, untreated	0	42	0	83	0	22	0	46	0
CP-82,996	10	63	95	54	100	73	90	67	100
	5	90	36	96	100	91	87	86	100
	2.5	85	13	100	100	77	68	90	66
	1.25	63	4	98	75	54	42	69	20
Monensin	100	75	61	76	79	88	72	89	67
	50	63	25	63	55	91	58	79	48

[a] Data for CP-82,996 and control data are averages from 2–4 tests. Data for monensin are averages from 8–49 different tests.
[b] WG, weight gain.
[c] LC, lesion control.

E. maxima and *E. necatrix*. *E. acervulina* showed the greatest sensitivity; 1.25 ppm of CP-82,996 controlled this species to the same extent as monensin at 100 ppm, indicating a potency difference of 80 times between the two ionophores. From this study, it appears that CP-82,996 is one of the most potent ionophores known in terms of anticoccidial activity [13].

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REFERENCES

1 Ajaz, A.A., J.A. Robinson and D.L. Turner. 1987. Biosynthesis of the polyether ionophore antibiotic monensin A: assignment of the carbon-13 and proton NMR spectra of monensin A by two-dimensional spectroscopy. Incorporation of oxygen-18 labelled molecular oxygen. J. Chem. Soc. Perkin Trans. I: 27–36.

2 Becker, B., M.P. Lechevalier, R.E. Gordon and H.A. Lechevalier. 1964. Rapid differentiation between *Norcardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. 12: 421–423.

3 Brannon, D.R. and D.R. Horton. 1976. Metabolite A-27106, its preparation and use. U.S. Patent 3,932,619.

4 Chappel, L.R., H.L. Howes and J.E. Lynch. 1974. The site of action of a broad-spectrum aryltriazine anticoccidial, CP-25,415. J. Parasitol. 60: 415–420.

5 Cullen, W.P., H. Maeda, J.C. Ruddock and J. Tone. 1988. Polycyclic ether antibiotic. U.S. Patent 4, 746,650.

6 Dirlam, J.P., L.J. Czuba, B.W. Dominy, R.B. James, R.M. Pezzullo, J.E. Presslitz and W.W. Windisch. 1979. Synthesis and antibacterial activity of 1-hydroxy-1-methyl-1,3-dihydrofuro[3,4-b]quinoxalin-4,9-dioxide and related compounds. J. Med. Chem. 22: 1118–1121.

7 Gordon, R.E., D.A. Barnett, J.E. Handerhan and C.H. Pang. 1974. *Norcardia coeliaca, Norcardia autotrophica*, and the nocardicin strain. Int. J. Syst. Bacteriol. 24: 54–63.

8 Huang, L.H. 1980. *Actinomadura macra* sp. nov., the producer of antibiotics CP-47,433 and CP-47,434. Int. J. Syst. Bacteriol. 30: 565–568.

9 Lechevalier, M.P. 1968. Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934–944.

10 Liu, C. 1982. Microbial aspects of polyether antibiotics: activity, production and biosynthesis. In: Polyether Antibiotics, Vol. 1 (Westley, J.W., ed.), pp. 43–102, Marcel Dekker, New York.

11 Lutz, W.K., F.K. Winkler and J.D. Dunitz. 1971. Crystal structure of the antibiotic monensin. Similarities and differences between free acid and metal complex. Helv. Chim. Acta. 54: 1103–1108.

12 Miyazaki, Y., M. Shibuya, H. Sugawara, O. Kawaguchi, C. Hirose, J. Nagatsu and S. Esumi. 1974. Salinomycin, a new polyether antibiotic. J. Antibiot. 27: 814–821.

13 Pento, J.T. 1987. Maduramicin. Drugs of the Future 12: 756–757.

14 Ruff, M.D. 1982. Veterinary applications. In: Polyether Antibiotics, Vol. 1 (Westley, J.W., ed.), pp. 303–332, Marcel Dekker, New York.

15 Seto H. and N. Otake. 1983. ^{13}C NMR spectra of polyether antibiotics. In: Polyether Antibiotics, Vol. 2 (Westley, J.W., ed.), pp. 335–400, Marcel Dekker, New York.

16 Taylor, R.W., R.F. Kauffman and D.R. Pfeiffer. 1982. Cation complexation and transport by carboxylic acid ionophores. In: Polyether Antibiotics, Vol. 1 (Westley, J.W., ed.), p. 106, Marcel Dekker, New York.

17 Tsuji, N., K. Nagashima, Y. Terui and K. Tori. 1979. Structure of K-41B, a new diglycoside polyether antibiotic. J. Antibiot. 32: 169–172.