CP-54,883 A NOVEL CHLORINE-CONTAINING POLYETHER ANTIBIOTIC PRODUCED BY A NEW SPECIES OF *ACTINOMADURA*: TAXONOMY OF THE PRODUCING CULTURE, FERMENTATION, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES OF THE ANTIBIOTIC

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The novel chlorine-containing acidic polycyclic ether antibiotic CP-54,883 ($C_{41}H_{61}O_{12}Cl_2$) is produced by the fermentation of *Actinomadura routienii* Huang sp. nov. This report presents the taxonomy and the fermentation conditions for the antibiotic-producing culture. The antibiotic is mainly active against Gram-positive bacteria. It protects chickens against *Eimeria* challenge *in vivo* and enhances rumen propionic acid *in vitro*. The physico-chemical properties are also characterized.

Screening soil isolates for culture filtrates with Gram-positive activity has been found to be a useful system for detecting novel metabolites such as polyether antibiotics. CP-54,883 isolated from a number of culture filtrates, was found to be produced by members of the *Actinomadura* genus. They were isolated from soil samples collected in Ibaragi Prefecture and Ueda City, Japan and have been identified as a new species *Actinomadura routienii* Huang sp. nov. CP-54,883 was purified and determined to be a novel polyether antibiotic, the second ionophore[1] reported to contain chlorine. It has weak Gram-positive antibacterial activity and *in vitro* it enhances rumen propionic acid production and shows anti-coccidial activity in chickens. In this paper, we describe the taxonomy, production, isolation, physico-chemical properties and biological activity of CP-54,883. Its chemical structure has been determined and is shown in Fig. 1.

Taxonomy

The microorganisms useful for the production of the antibiotics were isolated from three soil samples collected in Japan and designated as N364-77, N365-41 and N412-19. They are characterized

Fig. 1. Structure of CP-54,883.

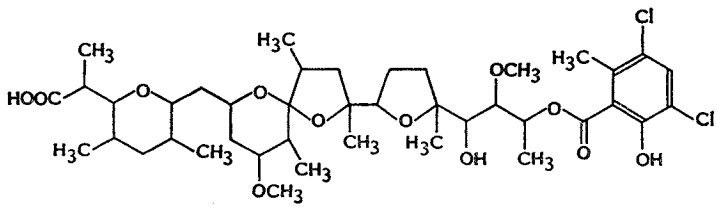


Table 1. Cultural characteristics of *Actinomadura routienii* N365-41.

Agar medium	Amount of growth; texture of color	Color of colony surface; color of aerial mycelium	Color of colony reverse; soluble pigment
Yeast - malt extract (ISP-2)*	Good; raised, granular to roughened	Pale cream to white; white	Pale cream; no soluble pigment
Oatmeal (ISP-3)	Moderate; thin, isolated colonies	Dull white to white; white	Same as surface; no soluble pigment
Inorganic salts - starch (ISP-4)	None	None	None
Glycerol - asparagine (ISP-5)	Moderate; thin, small isolated dots	White; white	Pale cream; no soluble pigment
Gordon - Smith tyrosine	Moderate; small isolated colonies, thin to raised	Pale cream (2ca); none	Same as surface; no soluble pigment
CZAPEK - sucrose	Moderate; thin, small isolated colonies	Dull white to white	Dull white to pale cream; no soluble pigment
Glucose - asparagine	Poor to moderate; small isolated dots	Cream (2ca)	Cream; no soluble pigment
Calcium - malate	Poor; thin, smooth, isolated dots	Colorless to dull white; sparse, white	Dull white; no soluble pigment
Casein	Moderate; small isolated dots, slightly roughened	Pale cream ($1\frac{1}{2}$ca); none	Same as surface; no soluble pigment
Bennett	Moderate to good; roughened or raised isolated colonies	Yellowish (2ea, 2ga to 2ic) with grayish yellow dots (2ie); none	Same as surface; no soluble pigment

The color scheme used was Color Harmony Manual, 4th Ed., Container Corporation of America, Chicago, Ill., U.S.A., 1958.

* Cultural characteristic studied on various media according to WAKSMAN[6], SHIRLING and GOTTLIEB[7].

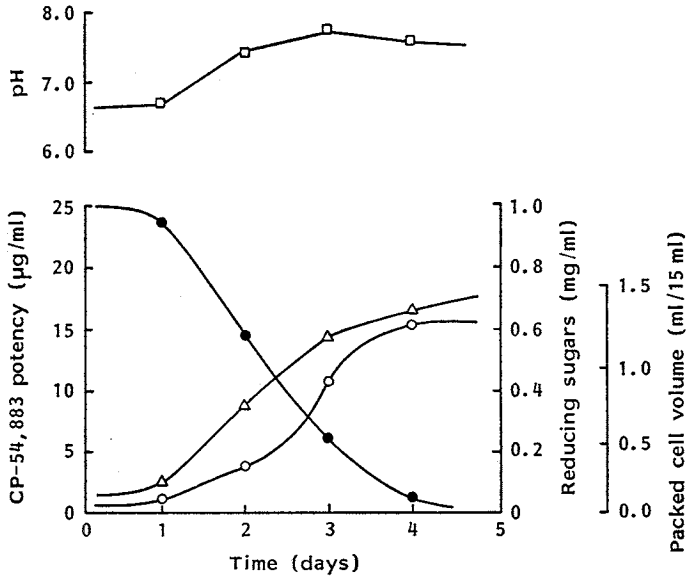
Table 2. Biochemical properties of *Actinomadura routienii*[a] N365-41.

Melanin production	−	Utilization of carbon sources:	
H_2S production	−	Glucose	+
Gelatin liquefaction	+	Arabinose	+
Starch hydrolysis	+	Fructose	−
Nitrate reduction:		Glycerol	−
Organic nitrate	+	Inositol	−
Dextrose nitrate	−	Mannitol	−
Decomposition of cellulose	−	Melibiose	−
Clearing and coagulation of milk	+	Raffinose	−
Casein digestion	+	Rhamnose	+
Tyrosine digestion	−	Sucrose	+
Digestion of calcium malate	−	Xylose	+

[a] For methodology of the biochemical tests, see HUANG[8].

(Tables 1 and 2) by the white aerial mycelium, the smooth spores that are arranged in a straight chain or in a globoid mass, and the presence of *meso*-diaminopimelic acid and madurose in the whole cell hydrolysate[2,3]. The spores were oval, elliptical to rod-shaped and measured $1\sim2\times0.18\sim1.1$ μm. These cultures are similar in morphological and biochemical properties and are considered to be strains of an undescribed species of the genus *Actinomadura*, *A. routienii* Huang sp. nov. with N365-41

Fig. 2. Time course of CP-54,883 fermentation in 4-liter jar fermentors.
□ pH, ● reducing sugars, △ packed cell volume, ○ CP-54,883 potency.

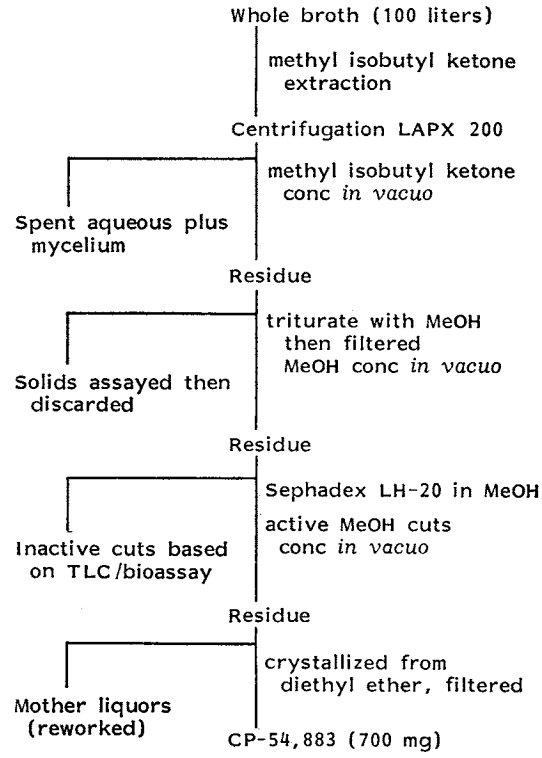


as the type strain.

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 the same JD medium. The fermentation was run at 28°C for 96 to 120 hours (Fig. 2). The antibiotic titers were followed by 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, concentrating the solvent, spotting on a silica gel TLC plate and running in ethyl acetate. The antibiotic could be visualized by spraying with 3% vanillin in 85% phosphoric acid and heating to 80°C; the spot turns green-blue. The polyether can also be visualized with UV light at 254 and 366 nm.

Fig. 3. Isolation and purification of CP-54,883.



The antibiotic was isolated (Fig. 3) from 100 liters of broth by extraction at natural pH into

Table 3. Properties of CP-54,883 Na^+ salt.

	CP-54,883
MP (°C)	330~340
$[\alpha]_D^{25}$ (c 0.2, $CHCl_3$)	+11.9°
UV λ_{max}^{MeOH} nm	315
IR (KBr) (cm^{-1})	3448, 2899, 1689, 1443, 1370, 1220, 1198, 1075, 1036, 980, 905, 709
MW	838
Empirical formula (H^+)	$C_{41}H_{61}O_{12}Cl_2$
(Na^+)	$C_{41}H_{60}O_{12}Cl_2Na$
Elemental *Anal* $C_{41}H_{60}O_{12}Cl_2Na$	
Calcd:	C 58.71, H 7.15, O 22.91, Cl 8.47, Na 2.74
Found:	C 59.26, H 7.50, O —, Cl 7.79, Na —
Color reaction	Vanillin - H_3PO_4, heat to 80°C; green-blue
Solubility Soluble:	Hexane, $CHCl_3$, acetone, MeOH
Insoluble:	H_2O

Fig. 4. UV spectra of CP-54,883.

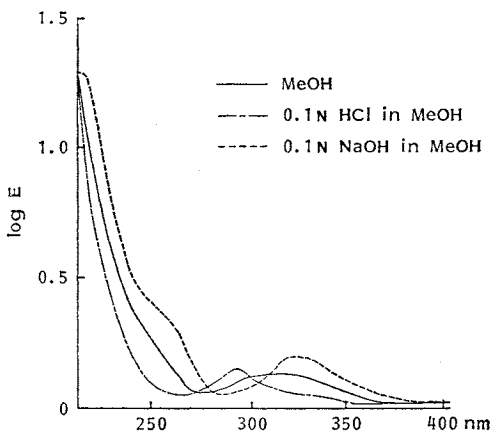


Table 4. Antimicrobial spectrum of CP-54,883.

Test organisms	MIC (μg/ml)
Staphylococcus aureus 01A005	12.5
S. aureus 01A052	12.5
S. aureus 01A100	12.5
S. epidermidis 01B087	6.25
S. epidermidis 01B111	6.25
Streptococcus faecalis 02A006	25.0
S. pyogenes 02C040	12.5
Micrococcus luteus 07A001	12.5
Bacillus subtilis 06A001	6.25
Escherichia coli 51A125	>100
E. coli 51A266	>100
Morganella morganii 57G001	>100
Klebsiella pneumoniae 53A009	>100
Pasteurella multocida 59A001	>100
Saccharomyces cerevisiae FD20117	>100
S. pastorianus FD3737	>100

methyl isobutyl ketone. The extract was clarified, concentrated to a syrup, then triturated with methanol. After trituration, the solvent was filtered, concentrated *in vacuo* and passed down a Sephadex LH-20 column in methanol. Activity was followed by bioassay and TLC. The active cuts were combined and concentrated *in vacuo*. Methanol was used to dissolve the residue from which the antibiotic crystallized on slow evaporation yielding 700 mg of CP-54,883.

Physico-chemical Characterization

CP-54,883 is characterized as a monocarboxylic acid containing chlorine. Elemental analysis suggested a molecular formula of $C_{41}H_{61}O_{12}Cl_2$ for the free acid and $C_{41}H_{60}O_{12}Cl_2Na$ for the sodium salt. Some physico-chemical properties are listed in Table 3. The UV and IR spectrum are shown in Figs. 4 and 5, respectively.

CP-54,883 is a new polyether antibiotic containing a 3,5-dichloro-2-hydroxy-6-methylphenyl

Fig. 5. IR spectrum (KBr) of CP-54,883.

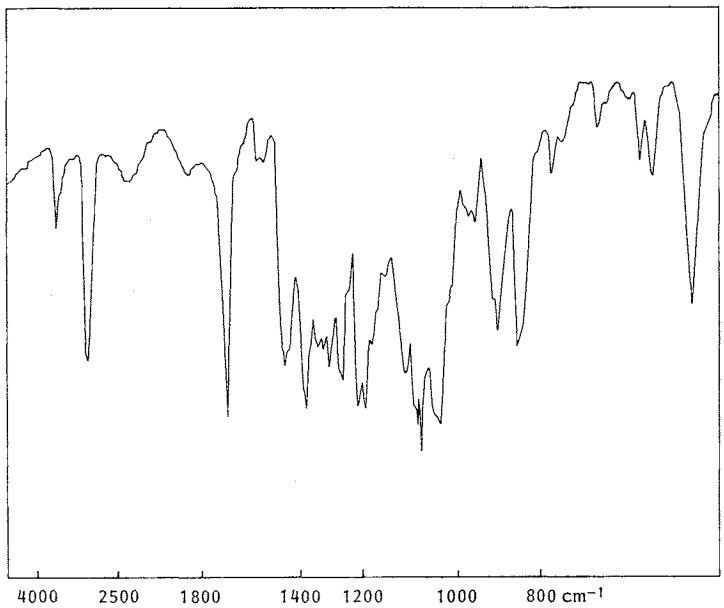


Table 5. Efficacy data for CP-54,883 against coccidial infections in chickens.

Species	Drug	Dose (μg/g of feed)	Weight gain (%)	Lesion control[a] (%)
Eimeria tenella	CP-54,883	20	57.0	100
		15	94.0	100
		10	81.0	10
	Monensin	100	95.0	90
		50	89.0	10
	Infect control	—	19.0	0.0
Eimeria acervulina	CP-54,883	20	53.0	70
		15	66.6	60
		10	68.0	50
	Monensin	100	87.0	90
		50	40.0	30
	Infect control	—	15.0	0.0
Eimeria maxima	CP-54,883	20	80.0	75
		15	59.0	75
		10	53.0	50
	Monensin	100	89.0	100
		50	92.0	87
	Infect control	—	53.0	0.0

[a] Criteria for evaluation, CHAPPEL *et al.*[9].

moiety as the terminal ring. This is the second ionophore reported to contain chlorine, the first being X14766A[4] which is a chlorinated noboritomycin. It contains two methoxy and one spiro-ketal groups and its carbon skeleton appears to be similar to nigericin[5].

Biological Characterization

CP-54,883 as indicated in Table 4 is a weak to moderate narrow spectrum antibiotic exhibiting

activity only against Gram-positive bacteria above 6.25 μg/ml. It was not active against Gram-negative bacteria or yeasts at the levels tested and did not protect mice against *Staphylococcus aureus* when administered by the oral or subcutaneous route.

The polyether antibiotic was active against *Eimeria tenella*, *Eimeria maxima* and *Eimeria acervulina* coccidia when administered in feed at 10 to 20 μg/g (Table 5). Chickens were protected from lesions at the higher levels but suffered from poor weight gains and feed intake.

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. This activity has been implicated as the mechanism for increased feed utilization in rumens by polyether antibiotics and suggests that CP-54,883 is a potential candidate as a ruminant performance enhancer.

Acknowledgments

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