CRISAMICIN A, A NEW ANTIBIOTIC FROM *MICROMONOSPORA*

I. TAXONOMY OF THE PRODUCING STRAIN, FERMENTATION, ISOLATION, PHYSICO-CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES

RICHARD A. NELSON, JOSEPH A. POPE, Jr., GEORGE M. LUEDEMANN, LLOYD E. MCDANIEL and CARL P. SCHAFFNER*

Waksman Institute of Microbiology, Rutgers-The State University of New Jersey, P.O. Box 759, Piscataway, New Jersey 08854, U.S.A.

(Received for publication October 14, 1985)

A microorganism, designated as RV-79-9-101 and now identified as *Micromonospora purpureochromogenes* subsp. *halotolerans*, isolated from a mud sample in the Philippines, has been shown to produce a complex of antibiotics called crisamicins. Thin-layer chromatography and bioautography, employing solvent extracts of whole fermentation broths, revealed a minimum of five antimicrobial components. The major biologically-active component of the antibiotic complex, crisamicin A, was obtained in pure form after preparative silica gel column chromatography followed by crystallization. Based on physico-chemical data crisamicin A has been identified as a novel member of the isochromanequinone group of antibiotics. It exhibits excellent *in vitro* activity against Gram-positive bacteria but little or no activity towards Gram-negative bacteria or fungi.

In the course of screening for novel antimicrobial substances from soil samples in the Philippines, an antibiotic-producing culture designated as RV-79-9-101 was recovered from a mud sample taken in the rice fields of San Crispin, a district of San Pablo City, Luzon[1]. Preliminary taxonomic studies identified this microbial isolate as belonging to the genus *Micromonospora*. Fermentation broths produced by this microorganism contained a complex of biologically-active substances, as demonstrated with solvent extracts of whole broth by thin-layer chromatography and antimicrobial bioautography. The main component of the complex, crisamicin A (CRS-A), was found to be active primarily towards Gram-positive bacteria. An earlier presentation[2] provided the results of preliminary investigations with the crisamicin-producing microorganism. This paper presents data concerning the taxonomy of the producing microorganism, production of CRS-A by fermentation, analytical and isolation procedures employed and the physico-chemical and antimicrobial characterization of CRS-A.

Materials and Methods

Microorganisms

The producing culture, designated RV-79-9-101, was provided through the courtesy of the late Dr. MARTIN S. CELINO, Red V Coconut Products Company, Inc., Lucena City, Luzon, Republic of the Philippines. The type strain, *Micromonospora purpureochromogenes* IMRU 3343 (ATCC 27007), and bacterial strains used to determine the antimicrobial spectrum of activity of CRS-A were obtained from the culture collection of the Waksman Institute of Microbiology. Other microorganisms were from the American Type Culture Collection (ATCC).

Culture Maintenance

The microorganism, RV-79-9-101, was grown in 6B-S medium (ATCC medium 172)[3] consisting

of glucose 1.0%, soluble starch 2.0%, yeast extract 0.5%, NZ-Amine type A (Sheffield Products, Norwich, New York) 0.5% and $CaCO_3$ 0.1% in distilled water. After slant culture inoculation of 300-ml baffled flasks (Bellco, Catalogue No. 2542-00300) containing 50 ml 6B-S medium, RV-79-9-101 was grown for 48 hours at 28°C with agitation at 250 rpm on a Model V rotary shaker (New Brunswick Scientific Company, Edison, New Jersey) with a 2.5-cm circular orbit. Thereafter 1.5 ml aliquots of the vegetative culture were stored in the vapor phase of liquid nitrogen.

Taxonomic Studies

Growth characteristics including carbohydrate utilization, sodium chloride tolerance, growth on potato plugs, biochemical and physiological reactions, and growth on selected media were determined by the methods of LUEDEMANN and BRODSKY[4] and LUEDEMANN[5]. Nitrate reduction was tested by the method of NEYRA *et al.*[6].

Micromorphology was determined with cultures grown on medium A[5] consisting of yeast extract 0.1%, soluble starch 0.1%, glucose 0.1%, $CaCO_3$ 0.1% and supplemented with vitamin B_{12}, 100 μg in 1 liter medium or on medium D[5] consisting of yeast extract 0.5%, glucose 1%, $CaCO_3$ 0.1% and supplemented with vitamin B_{12}, 100 μg in 1 liter medium. The cultures in 250-ml Erlenmeyer flasks containing 50 ml medium were incubated at 28°C on a Model V rotary shaker at 250 rpm with a 2.5-cm circular orbit. The 48 hour-old cultures were fixed, and stained with a 10% Gram's crystal violet stain, followed by examination by phase contrast microscopy under oil immersion at 1,000× magnification.

Oxygen requirements for growth were determined at 28°C by incubating freshly inoculated cultures on agar slants of 6B-M medium, consisting of potato dextrin 2.0%, Cerelose (CPC International, Englewood Cliffs, New Jersey) 1.0%, NZ-Amine type A 0.5%, Ardamine Z (Yeast Products, Inc., Clifton, New Jersey) 0.5%, agar 1.5% and $CaCO_3$ 0.1%, in the open air or in an anaerobic Gas Pak jar (BBL Microbiological Systems). The growth was examined after 2 weeks.

Optimum growth temperatures were determined by culturing the organism on slants of 6B-S medium containing 2% agar at 10, 23, 27, 42, 47 and 52°C. Growth was determined after 1 month for cultures grown at 10°C and after 2 weeks for cultures grown at the higher temperatures.

Whole cell hydrolysis and analyses of whole cell diaminopimelic acid (DAP) type and whole cell sugars were carried out by the method of LECHEVALIER and LECHEVALIER[7].

Color names and numbers were assigned according to the ISCC-NBS Centroid Color Charts, standard sample No. 2106[8].

Shake Flask Fermentations

Frozen vials of the stock culture were thawed at room temperature and the contents served as a primary inoculum for 300-ml baffled flasks containing 50 ml 6B-S medium to give the G1 stage of inoculum. After 48 hours at 28°C with rotary agitation at 250 rpm the G1 culture served as inoculum (5%) for additional 300-ml baffled flasks containing 50 ml 6B-S medium. After 48 hours at 28°C with similar agitation these G2 cultures served as inoculum (5%) for other shake flask studies.

Fermentors

For the production of crisamicin complex in fermentors, 2-liter baffled flasks (Bellco, Catalogue No. 2542-02000) containing 500 ml 6B-S medium were inoculated (5%) with 48 hour-old G2 cultures. Agitation at 28°C for 48 hours on a rotary shaker at 200 rpm with a 5-cm circular orbit produced the fermentor inoculum (5%). A 50-liter Fermacell Model FS150 fermentor (New Brunswick Scientific) with a working volume of 30 liters was utilized with 6B-P medium consisting of glucose 10% (sterilized separately), potato dextrin 2.0%, NZ-Amine type A 1.0%, Ardamine Z 0.5% and $CaCO_3$ 0.1% in distilled water. Agitator speed was 250 rpm and air flow, 15 liters/minute. Dow Corning B, a silicone antifoaming agent, was used at a 1 to 10 dilution. The incubation temperature was 28°C.

DNA, Packed Cell Volume and Glucose Determination

DNA determinations were conducted using the colorimetric assay of BURTON[9]. Packed cell volumes were estimated by centrifuging 10 ml aliquots of the whole fermentation broth in graduated test tubes at high speed in a clinical centrifuge for 20 minutes. Glucose levels were monitored using

a YSI Model 27 Industrial Analyzer (Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio).

Thin-layer Chromatography/Bioautography

Antibiotic preparations were dissolved in $CHCl_3$ or EtOAc at a concentration of 0.5 to 1.0 mg/ml. For chromatography 10 μl of each sample was applied to pre-coated Silica Gel 60 F254 plates with a thickness of 250 μm (E. Merck, Darmstadt, F.R.G.). The developing solvent system consisted of $CHCl_3$ - MeOH - AcOH (94: 5: 1).

Antimicrobial activity was detected by bioautography against *Staphylococcus aureus* ATCC 6538P. Antibiotic preparations or fermentation extract material were applied to Gelman instant thin-layer chromatography (ITLC) strips (Gelman Instrument Co., Ann Arbor, Michigan) and developed with the solvent systems consisting of $CHCl_3$ - MeOH (99: 1) or $CHCl_3$ - MeOH (98: 2).

High Performance Liquid Chromatography (HPLC)

A Varian 5000 Liquid Chromatograph equipped with a Spectra Physics SP4100 computing integrator and a Laboratory Data Control Spectromonitor II Model 1202 UV-visible detector was employed for this study. A prepacked Lichrosorb RP-18 column (10 μm, particle size, 250 mm $\times$ 4.6 mm (ID), E. Merck, Darmstadt, F.R.G.) was used with the solvent system, CH_3CN - H_2O (60: 40) at a flow rate of 2 ml/minute (5 minutes run time). Antibiotic components were detected by absorbance at 254 nm.

Generally whole fermentation broth samples were diluted (1: 5) with 0.1 M phosphate buffer, pH 7.0, and then extracted with an equal volume of EtOAc for 1 hour. Aliquots of the EtOAc extract were taken to dryness under nitrogen and the residue redissolved in CH_3CN for HPLC analysis.

Antimicrobial Activity

The antimicrobial spectrum of activity for purified antibiotic preparations of CRS-A was determined by the agar dilution method. Due to the poor solubility in H_2O, CRS-A was initially dissolved in DMF and subsequently diluted with medium. The final concentration of DMF was 1.0%.

Results and Discussion

Taxonomy

Cultural Characteristics

Culture RV-79-9-101 grew on a variety of complex and synthetic media as presented in Table 1. Differences were seen in the degree of colony darkening, gross colony morphology and the amount of soluble pigment produced. In contrast to the overall good growth of the culture on media containing complex nitrogen sources, little or no growth was seen on media containing inorganic nitrogen or amino acids. When grown on solid media containing organic nitrogen sources, RV-79-9-101 initially appeared as bright orange colonies raised from the surrounding agar with wrinkled and folded growth. The culture does not spread readily over the surface of the agar. In general, aerial mycelia were not observed; however, at times scant areas of aerial mycelia were noted. Within four to five

Table 1. Growth of RV-79-9-101 on various media.

Medium	Growth, appearance; color	Diffusible pigment (amount, color)
BENNETT'S	Good, rough; 62 dark grayish-brown	Heavy, brown
EMERSON'S	Good, wrinkled; 65 brownish-black	Heavy, dark brown
Yeast extract - dextrose	Good, rough; 50 strong orange	Slight
Glucose - asparagine	No growth	
Tomato paste - oatmeal	Good, rough; 72 dark orange-yellow	Absent
3% NZ-Amine	Good, rough; 51 deep orange	Absent
CZAPEK'S	Growth very light; 53 moderate orange	Absent

Fig. 1. Electron micrograph of strain RV-79-9-101 from a 10-day culture grown on 6B-S slant.

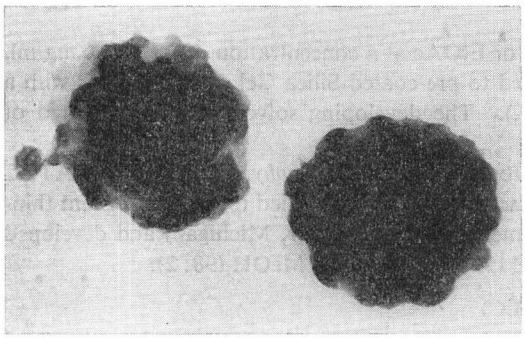
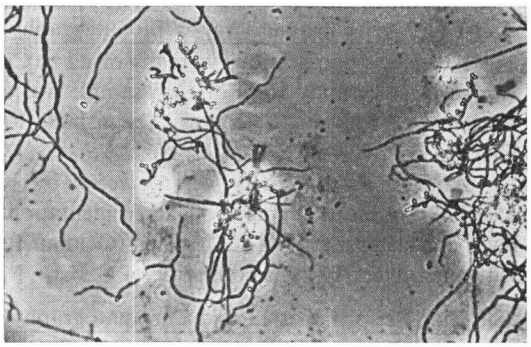


Fig. 2. Sporophore morphology of strain RV-79-9-101 from a 2-day culture grown in medium A.



days a brownish surface pigment developed which later turned dark brown to black as sporulation occurred. Concurrently with the darkening of the colony surface, production of a dark brown diffusible pigment began. When first observed this pigment was purple in color but after two or three days the agar surrounding the colonies became yellowish-brown to dark brown.

The culture RV-79-9-101 grew well at temperatures ranging from 23 to 42°C but did not grow at 47°C and grew slowly at 10°C. Growth was observed only under aerobic conditions.

Morphological Characteristics

Examination of culture RV-79-9-101 by light microscopy after growth in medium A or D revealed the presence of single spores on short sporophores. Electron microscopy revealed the presence of individual spores approximately 0.7 to 0.8 μm in diameter with a rough surface as shown in Fig. 1. Sporophores were monopodially branched and occurred either singly or in dense clusters as shown in the phase contrast photomicrograph in Fig. 2.

The general growth habit of the microorganism, lack of aerial mycelium, production of single spores on short sporophores, morphology and structure of spores, and production of a diffusible dark brown pigment suggested that culture RV-79-9-101 may be a strain of *Micromonospora purpureochromogenes*. The type strain of this species (IMRU 3343) was selected for comparative studies.

Physiological Characteristics

Both RV-79-9-101 and IMRU 3343 grew well on potato plugs in the presence and absence of $CaCO_3$ indicating a tolerance to acidic conditions. While IMRU 3343 tolerated only low levels of NaCl (no growth was observed on agar medium containing 1.5% NaCl), culture RV-79-9-101 grew well and produced normal quantities of pigment on media containing up to 4.0% NaCl. Good growth but no pigment production was observed at 5.0% whereas no growth occurred at 7.0% NaCl with RV-79-9-101.

The physiological characteristics of RV-79-9-101 and IMRU 3343 are summarized in Table 2. Gelatin and starch were readily hydrolyzed by both cultures. Milk hydrolysis was slow with the type culture whereas with RV-79-9-101 the hydrolysis was very rapid. Neither strain hydrolyzed cellulose. Nitrate was reduced by RV-79-9-101 in CZAPEK's broth, but not in a medium containing yeast extract whereas IMRU 3343 did not reduce nitrate. Hydrogen sulfide was not produced by either culture. While RV-79-9-101 was strongly positive for tyrosinase, IMRU 3343 was marginal.

Table 2. Physiological reactions of RV-79-9-101 and IMRU 3343.

Reaction	RV-79-9-101	IMRU 3343
Starch hydrolysis	+	+
Milk hydrolysis	+	±
Gelatin hydrolysis	+	+
Cellulose hydrolysis	−	−
Nitrate reduction	±	−
Tyrosinase	+	±
H_2S production	−	−

+ Positive, ± doubtful, − negative.

Table 3. Carbohydrate utilization patterns.

Carbohydrate	Culture	
	RV-79-9-101	IMRU 3343
D-Glucose	++	++
L-Arabinose	++	−
Sucrose	++	++
D-Xylose	++	++
D-Fructose	++	++
Raffinose	−	+
Glycerol	±	+
i-Inositol	−	−
D-Mannitol	−	−
Rhamnose	−	−
Cellulose	−	−

++ Growth better than glucose, + growth equal to glucose, ± growth less than glucose but better than on basal medium, − growth equal to or less than basal medium.

The results of studies on carbohydrate utilization are presented in Table 3. The patterns are similar for both cultures with a few exceptions. Raffinose utilization was positive for IMRU 3343 and negative in two out of three trials for RV-79-9-101. Utilization of L-arabinose was negative for IMRU 3343 and strongly positive for RV-79-9-101, with luxuriant growth and heavy pigment production. Growth on glycerol was positive for IMRU 3343 but negative for RV-79-9-101.

Whole Cell Chemical Analysis

Chemical analysis of whole cell hydrolysates of RV-79-9-101 and IMRU 3343 demonstrated the presence of *meso*-DAP and LL-DAP in a ratio of 9 to 1. Arabinose and xylose were found as whole cell sugars in both cultures. This whole cell composition is consistent with the placement of RV-79-9-101 in the genus *Micromonospora*.

Classification of RV-79-9-101

The observation of single spores and the detection of *meso*-DAP in whole cell hydrolysates, as well as the general macroscopic appearance of colonies of RV-79-9-101 on agar, place it in the genus *Micromonospora*. Placement in the species *purpureochromogenes* is dictated by the production of a dark-brown diffusible pigment as discussed by LUEDEMANN[5]. At present, all *Micromonospora* producing this type of pigment are placed in this taxon. Two of the published keys for the genus *Micromonospora*[10,11] have used this rare trait to place isolates within a unique species. In addition, the formation of monopodially-branched sporophores, singly or in clusters, supports this assignment. The most probable classification at the present time would be *M. purpureochromogenes* subsp. *halotolerans*. The subspecies epithet is derived from the NaCl tolerance exhibited by RV-79-9-101.

RV-79-9-101 is unique among the known species of *M. purpureochromogenes*. It is relatively tolerant of sodium chloride giving good growth at concentrations up to 5%. The upper limit, determined from the literature, for all other strains is 2%. All other strains are able to utilize glycerol as the sole source of carbon, and do not grow on L-arabinose. In contrast, RV-79-9-101 gives excellent growth on L-arabinose but is unable to grow on glycerol. Finally, RV-79-9-101 readily digests milk, whereas the other strains digest milk only slowly.

Fermentation

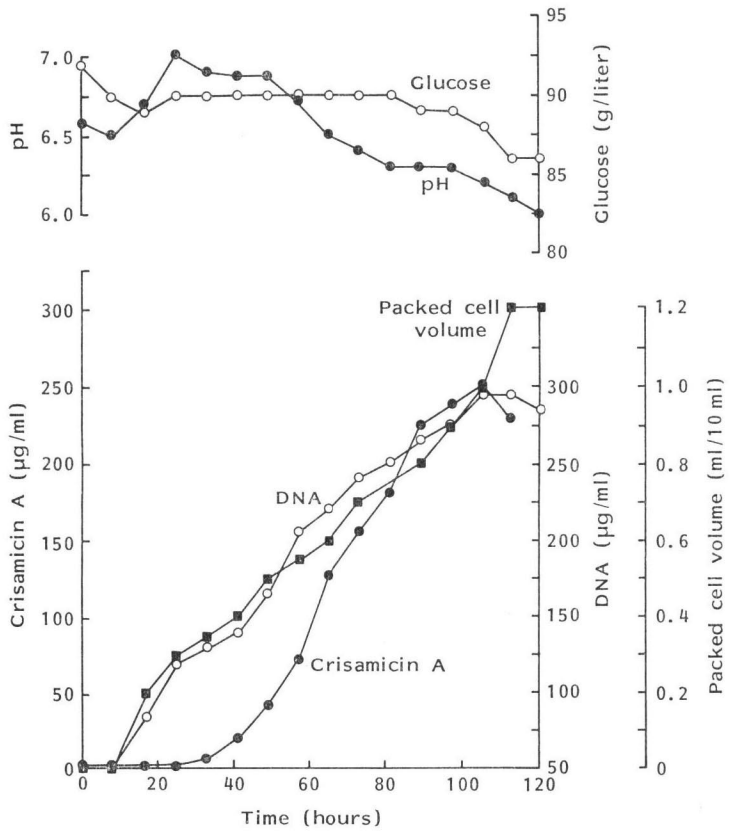
Production of CRS-A

Fig. 3 presents the data from a typical 30-liter fermentation regarding CRS-A antibiotic production, DNA content, pH, packed cell volume and glucose levels. The pH of the fermentation broth initially increases from 6.6 to around 7.0, levels off and after 48 hours begins to decrease corresponding to the onset of antibiotic production.

Although the 6B-P medium contains a high glucose concentration (10%, w/v), most of this glucose is not consumed during the course of the fermentation. During the early stages of the fermentation (18~20 hours) some glucose is metabolized. However, during the time period of 20 to 80 hours glucose levels remain essentially unchanged. It is not until the later stages of the fermentation (after 80 hours) that glucose utilization resumes. Presumably during this middle period of no glucose utilization, starch is the carbon source for the fermentation.

CRS-A production begins after about 40 hours and reaches a maximum of 250~300 μg/ml by 100 hours and then declines slightly. Antibiotic production was monitored by HPLC, and DNA levels and packed cell volumes were used as indicators of growth. Correlating the time course of crisamicin A production with the growth of RV-79-9-101 indicates that crisamicin A is a growth associated compound.

Fig. 3. Typical 30-liter crisamicin fermentation.



Isolation of Crisamicin A

CRS-A is a solvent extractable antibiotic recoverable from the whole fermentation broth with $CHCl_3$, BuOH or EtOAc. EtOAc is the solvent of choice as it produces less emulsion and more CRS-A is extracted than with the other solvents tested.

The extractability of CRS-A into EtOAc from the whole fermentation broth at various buffered pH's ranging from 4 to 10 was examined. The efficiency of extraction reaches a maximum between pH 7.0 and 8.0. Whole broth extracts were generally between 45~60% crisamicin A by weight. Using a single extraction of equal volumes, approximately 85% of the CRS-A present in the fermentation broth was recovered. Extraction and purification procedures were monitored by HPLC and TLC.

Fig. 4 shows a representative example of the biologically active components of an EtOAc crude extract from an RV-79-9-101 whole fermentation broth. When chromatographed by ITLC with $CHCl_3$ - MeOH (99: 1) as the solvent system, the crude material separates into 4 biologically active compounds as determined by bioautography employing *S. aureus* ATCC 6538P. With a slightly more polar solvent system ($CHCl_3$ - MeOH, 98: 2) the active material near the origin separates into 2 biologically active components indicating that the crude extract contains a mixture of at least 5 antibiotic compounds.

The main component in the crude extract with the fastest mobility on TLC and activity against *S. aureus* was designated as CRS-A. The active compound following closely behind CRS-A on TLC was referred to as crisamicin B. Other biologically active components were not specifically designated as crisamicin type compounds since evidence for their structural relationship to

Fig. 4. TLC bioautography of crisamicin complex against *Staphylococcus aureus*.

1, Crisamicin A; 2, crude crisamicin extract; 3, crisamicins A and B.

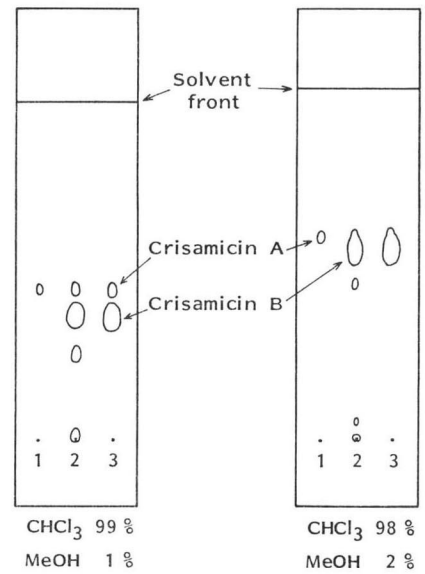


Table 4. Mobility of crisamicins A and B using various TLC systems.

TLC plate	Solvent system	Compound	Solvent front (cm)	Rf
A	1	CRS-A	15.0	0.50
		CRS-B	15.0	0.46
A	2	CRS-A	13.5	0.43
		CRS-B	13.5	0.35
B	3	CRS-A	13.6	0.45
		CRS-B	13.6	0.36
B	4	CRS-A	13.9	0.59
		CRS-B	13.9	0.55

TLC plates: A, Silica Gel 60 F254; B, ITLC.

Solvent systems: 1, $CHCl_3$ - MeOH - AcOH (94: 5: 1); 2, $CHCl_3$ - AcOH (92.5: 7.5); 3, $CHCl_3$ - MeOH (99: 1); 4, $CHCl_3$ - MeOH (98: 2).

Compounds: CRS-A, crisamicin A; CRS-B, crisamicin B.

crisamicin A or B was not obtained.

As shown in Fig. 4, lane 1 contains CRS-A purified by silica gel column chromatography and represents a single biologically active compound. Lane 2 is the crude EtOAc extract of the whole fermentation broth. Lane 3 contains a partially purified mixture of crisamicins A and B in the ratio of approximately 4:1, w/w. Table 4 gives the Rf values for crisamicins A and B in several TLC systems.

Fig. 5 outlines the isolation scheme for CRS-A, from the solvent extraction of the fermentation broth to the crystalline product.

Fig. 5. Isolation scheme for crisamicin A (CRS-A).

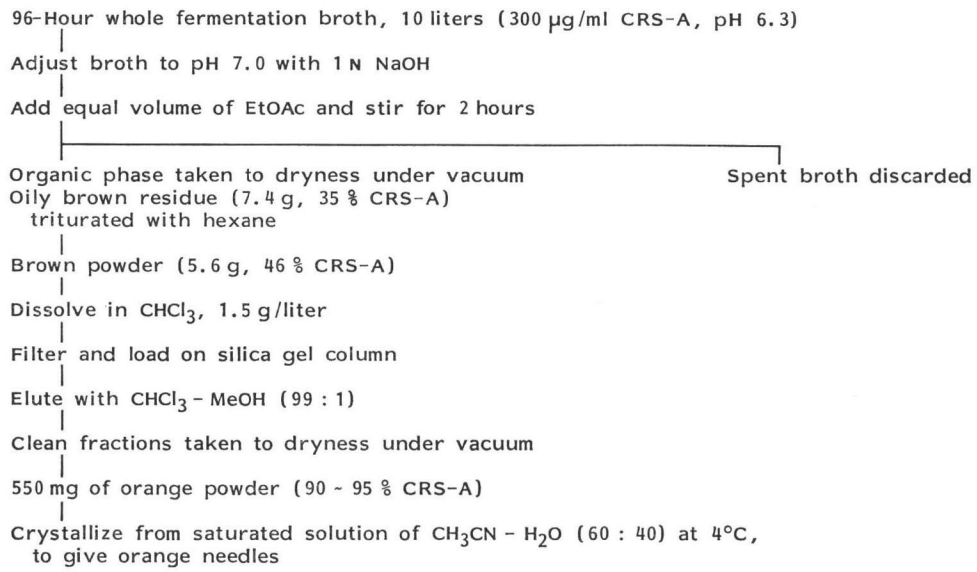


Table 5. Antimicrobial spectrum of CRS-A.

Test organism	Medium	Minimal inhibitory concentration (μg/ml)
Bacillus licheniformis ATCC 14580	N	1.0
B. subtilis ATCC 7972	N	0.5
Escherichia coli PP 01	N	>100.0
Mycobacterium smegmatis IMRU 24	N	>100.0
Nocardia rhodochrous IMRU 21	N	0.5
Pseudomonas aeruginosa NC 2	N	>100.0
Micrococcus luteus IMRU 14	N	0.2
Serratia marcescens IMRU 70	N	>100.0
Staphylococcus aureus ATCC 6538P	N	1.0
Enterococcus faecalis ATCC 14506	N	10.0
Candida albicans ATCC 18527	S	>100.0
C. lipolytica MF 47	S	>100.0
Saccharomyces cerevisiae MY 306	S	>100.0
Aspergillus niger MF 29	S	>100.0
A. sulphureus PP 17	S	>100.0
Mucor rouxii IMRU 80	S	>100.0
Penicillium terrestre MF 84	S	>100.0

N: Nutrient agar, S: Sabouraud dextrose agar.

Table 6. Physico-chemical properties of CRS-A.

MW[13]	598.1124
Molecular formula[13]	$C_{32}H_{22}O_{12}$
UV λ_{max} nm ($E^{1\%}_{1cm}$)	435 (234), 268 (659), 232 (767)
MP	Gradually decomposes and turns brown, no sharp melting point
Solubility	
Very soluble:	*N,N*-Dimethylacetamide, DMF, DMSO
Moderatly soluble:	CH_3CN, EtOAc, $CHCl_3$, AcOH, acetone
Poorly soluble:	H_2O, hexane, MeOH
Acid-base indicator	Acid - yellow
	Base - purple
Oxidation-reduction	Sodium dithionate, colorless; Air oxidation, yellow
Physical appearance	May be isolated as an orange amorphous powder or crystalline material

Antimicrobial Spectrum

The antimicrobial spectrum of CRS-A when tested against Gram-positive and Gram-negative bacteria, yeast and filamentous fungi by the agar dilution method is presented in Table 5. CRS-A exhibits good activity against Gram-positive bacteria with minimal inhibitory concentrations ranging from 0.2 to 10 μg/ml against the organisms tested. In general no activity was observed against the Gram-negative bacteria, fungi or the acid-fast *Mycobacterium smegmatis*.

Chemical Characterization

Physico-chemical Properties

CRS-A may be isolated as an amorphous orange powder or as orange crystalline needles with no sharp melting point. This compound is most soluble in DMF, *N,N*-dimethylacetamide and DMSO, moderately soluble in CH_3CN, EtOAc, $CHCl_3$, AcOH and acetone and nearly insoluble in H_2O, hexane and MeOH.

Based on high resolution field desorption (HRFD) mass spectrometry the molecular weight of CRS-A was determined to be 598.1124 and a molecular formula of $C_{32}H_{22}O_{12}$ was assigned[12].

CRS-A exhibits acid-base indicator properties being yellow in acid and purple at alkaline pH. Upon treatment with sodium dithionate CRS-A gives a colorless product which reverts to the original yellow color after air oxidation.

The UV-visible spectrum of CRS-A in CH_3CN exhibits maxima at 435, 268 and 232 nm. The physico-chemical properties of crisamicin A are summarized in Table 6.

Based on structure determination of LING *et al.*[13] CRS-A is a novel member of the dimeric isochromanequinone group of antibiotics[14]. Other members of this dimeric class of antibiotics include actinorhodin[15], phenocyclinone[16] and naphthocyclinone[17]. A quinoid antibiotic complex, M-92[18] has been isolated from *Micromonospora* and is thought to be related to actinorhodin. With the possible exception of M-92, the antibiotic isochromanequinones are *Streptomyces* metabolites, while CRS-A is a dimeric isochromanequinone produced by *Micromonospora*. Studies are underway to isolate and identify the other components of crisamicin complex.

Acknowledgments

We wish to thank MAGDA GAGLIARDI for her assistance with the whole cell chemical analysis and MARY P. LECHEVALIER for her many helpful suggestions regarding taxonomic procedures. LEE SIMON is also thanked

for his help with the electron microscopy of RV-79-9-101 spores.

This work was supported in part by the Charles and Johanna Busch Memorial Fund.

References

1) CELINO, M. S. (Red V Co-conut Products Co., Inc.): Novel antibiotic crisamicin A and compositions thereof. U.S. Pat. Appl. 603,621, Apr. 24, 1984
2) NELSON, R. A.; J. A. POPE, Jr., G. M. LUEDEMANN, L. E. MCDANIEL & C. P. SCHAFFNER: Crisamicin, a new quinone-type antibiotic complex from *Micromonospora*: Taxonomy of producing organism, fermentation, and isolation. Program and Abstracts of the 24th Intersci. Conf. Antimicrob. Agents Chemother., No. 791, Washington, D.C., 1984
3) American Type Culture Collection: Catalogue of Strains. I. 15th Ed., American Type Culture Collection, Rockville, Maryland, 1982
4) LUEDEMANN, G. M. & B. C. BRODSKY: Taxonomy of gentamicin-producing *Micromonospora*. Antimicrob. Agents Chemother. -1963: 116~124, 1964
5) LUEDEMANN, G.: *Micromonospora purpureochromogenes* (Waksman and Curtis 1916) comb. nov. (Subjective synonym: *Micromonospora fusca* Jensen, 1932). Int. J. Syst. Bacteriol. 21: 240~247, 1971
6) NEYRA, C. A.; J. DÖBEREINER, R. LALANDE & R. KNOWLES: Denitrification by N_2-fixing *Spirillum lipoferum*. Can. J. Microbiol. 23: 300~305, 1977
7) LECHEVALIER, M. P. & H. A. LECHEVALIER: The chemotaxonomy of actinomycetes. *In Actinomycete* Taxonomy. *Eds.*, A. DIETZ & D. W. THAYER, pp. 227~291, SIM Special Publication No. 6, Soc. Indust. Microbiol., Arlington, 1980
8) U.S. Department of Commerce. National Bureau of Standards. ISCC-NBS Centroid Color Charts Standard Sample No. 2106
9) BURTON, D.: Determination of DNA concentration with diphenylamine. Methods Enzymol. 12: 163~165, 1968
10) LUEDEMANN, G. M.: Genus I. *Micromonospora* Ørskov 1923, 147. *In* Bergey's Manual of Determinative Bacteriology. 8th Ed., *Eds.*, R. E. BUCHANAN & N. E. GIBBONS, pp. 846~855, Williams & Wilkins, Baltimore, 1974
11) SVESHNIKOVA, M.; T. MAXIMOVA & E. KUDRINA: Species of the genus *Micromonospora* Ørskov 1923 and their taxonomy. *In* The Actinomycetales. *Ed.*, H. PRAUSER, pp. 187~197, The Jena International Symposium on Taxonomy. Veb Gustav Fischer Verlag, Jena, 1970
12) LING, D. & K. L. RINEHART, Jr.: The structure of crisamicin. Program and Abstracts of the 24th Intersci. Conf. Antimicrob. Agents Chemother., No. 792, Washington, D.C., 1984
13) LING, D.; L. S. SHIELD & K. L. RINEHART, Jr.: Isolation and structure determination of crisamicin A, a new antibiotic from *Micromonospora purpureochromogenes* subsp. *halotolerans*. J. Antibiotics 39: 345~353, 1986
14) FLOSS, H. G.: Biosynthesis of isochromanequinone antibiotics. *In* Antibiotics. Vol. 4. Biosynthesis. *Ed.* J. CORCORAN, pp. 215~235, Springer-Verlag, New York, 1981
15) BROCKMANN, H.; A. ZEECK, K. VAN DER MERWE & W. MULLER: Über Actinomycetenfarbstoffe VIII. Die Konstitution des Actinorhodins. Justus Liebigs Ann. Chem. 698: 209~229, 1966
16) BROCKMANN, H. & P. CHRISTIANSEN: Dyes from Actinomycetes. XI. Phenocyclinone. Chem. Ber. 103: 708~717, 1970
17) ZEECK, A.; H. ZÄHNER & M. MARDIN: Metabolic products of microorganisms. 129—Isolation and structure of the isochromanequinone antibiotics β- and γ-naphthocyclinone. Liebigs Ann. Chem. 1974: 1100~1125, 1974
18) TANI, K. & T. TAKAISHI: Studies on a new antibiotic M-92 produced by *Micromonospora*. II. Isolation and physicochemical properties of M-92 and its components. J. Antibiotics 35: 1437~1440, 1982