PRODUCTION AND BIOLOGICAL ACTIVITY OF REBECCAMYCIN, A NOVEL ANTITUMOR AGENT

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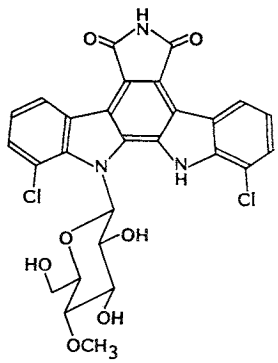
An actinomycete, strain C-38,383, was selected in a screening program for the isolation of novel antitumor agents. A yellow crystalline product, named rebeccamycin, was isolated from the mycelium and was found to have activity against P388 leukemia, L1210 leukemia and B16 melanoma implanted in mice. Rebeccamycin inhibits the growth of human lung adenocarcinoma cells (A549) and produces single-strand breaks in the DNA of these cells. No DNA-protein cross-links were detected. A related antibiotic, staurosporine, is produced by *Streptomyces staurosporeus* and *Streptomyces actuosus*. Strain C-38,383 was found to resemble closely strains of *Nocardia aerocolonigenes* recently renamed *Saccharothrix aerocolonigenes*. A strain selection isolate without aerial mycelium, C-38,383-RK-1, failed to produce rebeccamycin while a strain with aerial mycelium, C-38,383-RK-2, was found to be a suitable strain for production. A description of the producing strain is presented and its taxonomic position is reviewed. A fermentor containing 37 liters of production medium gave a rebeccamycin yield of 663 mg/liter after 204 hours of incubation with strain C-38,383-RK-2.

Strain C-38,383, an actinomycete isolated from a soil sample collected in Panama, was selected for further study since clarified culture fluid inhibited KB cell culture growth.[1] A product, crystallizing as fine yellow needles, was isolated from the mycelium of strain C-38,383 grown in submerged culture. This compound was found to be soluble in dimethyl sulfoxide and tetrahydrofuran, poorly soluble in commonly used organic solvents and insoluble in water. Significant *in vivo* antitumor activity was obtained with murine tumor tests. The active compound, a novel *N*-glycoside containing a symmetric heterocyclic aglycone with two chlorine atoms, was named rebeccamycin (Fig. 1). Isolation procedures, physico-chemical data, structure determination and total synthesis are presented in companion publications.[2,3] A related antibiotic without chlorine, staurosporine, produced by *Streptomyces staurosporeus*, was reported to have antifungal and hypotensive activity.[4,5] Staurosporine, isolated from *Streptomyces actuosus*, induces differentiation in human neuroblastoma cells (NB-1) and is cytotoxic to this cell line.[6]

Fig. 1. Structure of rebeccamycin.



Materials and Methods

The procedures of BECKER *et al.*[7] and YAMAGUCHI[8] were used for amino acid analysis of cell-wall preparations. The sugar composition of whole-cell hydrolysates was determined with the procedures of LECHEVALIER and LECHEVALIER.[9] The methods of MINNIKIN *et al.*[10] were used for detection of nocardomycolic acids in cell-wall preparations; however, the thin-layer chromatographic system was changed to hexane - diethyl ether (80 : 20). The determination of *N*-acyl type of cell-wall glycan was performed as described by UCHIDA and AIDA.[11] *Nocardia aerocolonigenes* JCM 4150, *Nocardia asteroides* CS-45 and *Nocardia corallina* NIHJ301 were included for comparison. Tests for utilization of carbon sources were done with PRIDHAM-GOTTLIEB's inorganic medium.[12] Observations were made after incubation at 37°C for 21 days.

Tests for inhibition of L1210 leukemia, P388 leukemia and B16 melanoma in mice were performed with procedures described previously.[13~15] For biological testing rebeccamycin was dissolved in DMSO and stored at −20°C as 10 mM or 1 mg/ml aqueous solutions for no longer than 2 weeks.

The effect of rebeccamycin on the viability of human lung adenocarcinoma A549 cells[16] was determined as described previously.[17] Briefly, 50,000 cells were added to 25 square-cm flasks and incubated at 37°C in McCOY's medium (Gibco Diagnostics 430-1500, Madison, Wisconsin, U.S.A.) for 24 hours, incubated with serial dilutions of the drug for 1 hour and then in the absence of drug for 5 days. At this time cells were suspended with trypsin - EDTA, treated with trypan blue and counted with a hemocytometer.

For measurement of inhibition of macromolecular biosynthesis A549 cells were harvested, washed in culture medium (37°C) and distributed as 1.0 ml aliquots of 1×10^6 cells/ml in plastic test tubes. The cells were preincubated for 15 minutes at 37°C in the presence of different dilutions of drug then incubated for 2 hours after addition of 10 μCi of [*methyl*-^{3}H]thymidine to assess inhibition of DNA synthesis; for 1 hour after addition of 6.25 μCi of [5,6-^{3}H]uridine and 20 μm unlabeled uridine to assess inhibition of RNA synthesis; and for 1 hour after addition of 6.25 μCi of [4,5-^{3}H]leucine to assess inhibition of protein synthesis. The samples were adjusted to a final concentration of 10% TCA, cooled on ice and filtered through glass fibers (GF/A, Whatman). Tubes containing radioactive leucine were heated for 20 minutes at 70°C and placed in an ice bath for 1 hour. The filters were washed with 15 ml of 5% TCA solution, dried and placed in scintillation fluid (Insta-Fluor, Packard) for radioactive counting.[18]

Experimental cultures intended for alkaline elution were plated in 60 mm tissue culture dishes 3 days before the experiment and 0.01 μCi/ml [^{14}C]thymidine was added 48 hours prior to addition of the drug. Reference cells were also grown for 3 days in 75 cm^2 flasks and labeled with 0.1 μCi/ml [^{3}H]thymidine 2 days before the experiment. Cells containing ^{14}C-DNA were assayed for single-strand break formation immediately following 1 hour incubation with drug in tissue culture medium with serum. The dishes were placed immediately on ice and the medium was exchanged for trypsin - EDTA to release the cells. The drug-treated cells (5×10^5) and γ irradiated cells containing ^{3}H-DNA added as an internal standard (5×10^5) were layered over polycarbonate filters (2.0 μm pores, 25 mm diameter from Nucleopore) and lysed in the presence of proteinase K and sodium dodecyl sulfate and the retained DNA was slowly eluted at pH 12.1 as described by KOHN *et al.*[19,20] Additional details of the procedure are provided elsewhere.[21,22]

The resulting ^{14}C and ^{3}H cpm of each eluted fraction were expressed as percents of total cpm obtained from the sum of the respective radioactivities in the filter, tubing, lysis and eluted fractions. Log percent values for ^{14}C cpm of each eluted fraction were plotted against the log percent of ^{3}H cpm of the same fraction and yielded elution curves that were linear with correlation coefficients >0.99 for controls and low concentration of drug. Slopes of elution curves from drug-treated cells were compared with slopes of elution curves from cells exposed to varying doses of ionizing radiation to obtain rad equivalents,[19] which are related to DNA break frequencies by the equation: 1 rad equivalent $=9\times10^{-10}$ single-strand DNA breaks per nucleotide[23] as previously described.[21]

Cell lines of murine or human origin (Table 9) were maintained in continuous logarithmic culture in MEM (EAGLE) with EARL's salts (Gibco Diagnostics 320-1090, Madison, Wisconsin, U.S.A.)

Table 1. Media for production of rebeccamycin.

	Medium H34	Medium H95	Medium H96	Medium G134
Glucose (g/liter)	60.0	20.0	10.0	10.0
Corn starch (g/liter)	—	50.0	60.0	75.0
Soy flour (g/liter)	10.0	5.0	—	—
Linseed meal (g/liter)	10.0	—	15.0	15.0
Cottonseed embryo meal (g/liter)	—	5.0	—	—
Dried beef blood (g/liter)	—	10.0	—	—
Autolyzed yeast (g/liter)	—	—	5.0	5.0
$FeSO_4 \cdot 7H_2O$ (g/liter)	0.5	—	1.0	1.0
$(NH_4)_2SO_4$ (g/liter)	—	—	1.0	1.0
$NH_4H_2PO_4$ (g/liter)	1.0	—	1.0	1.0
$CaCO_3$ (g/liter)	10.0	10.0	10.0	10.0

Erlenmeyer flasks (500 ml) with 100 ml of medium.
Rotary shaker, 250 rpm, 27°C.

containing 2 mM L-glutamine, benzylpenicillin (10 U/ml), streptomycin (10 μg/ml), 3 mM MEM nonessential amino acids (100X, Gibco Diagnostics 320-1140) and 10% fetal bovine serum (enriched MEM). Cells were incubated at 37°C in a 5% CO_2, balance air, high humidity incubator. Cytotoxicity testing was performed as previously described[24] with 48-hour continuous drug exposure. Cells were fixed with formalin (10%) in phosphate buffered saline for 10 minutes, air dried and stained with 0.09% crystal violet for 15 minutes. The stain was solubilized with 0.2 ml of 0.1 M AcOH - EtOH (1 : 1) and optical densities determined at 590 nm with a Dynatech MR 600 microtiter plate reader. IC_{50} values were calculated by linear regression analysis of absorption data.

To obtain the antimicrobial spectrum of rebeccamycin Mueller-Hinton Agar (BBL) was used for tests with bacteria and SABOURAUD Liquid Broth Modified (BBL) plus agar (10 g/liter) was used for tests with fungi.

The analysis of rebeccamycin in fermentation extracts was performed with HPLC on the RCM-100 (Waters Associates, Milford, MA, U.S.A.) system, including a radial pack silica cartridge (8 mm internal diameter ×10 cm, 10 μm packing). The solvent system was 2% MeOH in $CHCl_3$, the flow rate was 3 ml/minute and the detector wavelength was set at 313 nm.

Strain C-38,383 was grown in test tubes on agar slants of yeast - malt extract agar. This medium consisted of glucose 4.0 g, yeast extract 4.0 g, malt extract 10 g and agar 20 g in 1 liter distilled water. The culture was incubated for 7 days at 27°C. To prepare an inoculum for the production phase the surface growth from the slant culture was transferred to a 500-ml Erlenmeyer flask containing 100 ml of medium consisting of glucose 30 g, soy flour 10 g, cottonseed embryo meal 10 g and $CaCO_3$ 3 g in 1 liter distilled water. This vegetative culture was incubated at 27°C for 48 hours on a Gyrotory tier shaker (Model G53, New Brunswick Scientific Co., Inc., New Brunswick, N.J., U.S.A.) set at 210 rpm. Four ml of vegetative culture was transferred to a 500-ml Erlenmeyer flask containing 100 ml of production medium, some of which are listed in Table 1. The production culture was incubated at 27°C on the same shaker. The agitation rate was 250 rpm. *Nocardia aerocolonigenes* OEU 701 (ATCC 23890) obtained from the American Type Culture Collection was included in flask fermentation studies. For production in a fermentor 2 liters of seed culture was transferred to 30 liters of production medium G134 (Table 1). The incubation temperature was 27°C, the agitation rate was 375 rpm, the air flow was 60 liters/minute and the back pressure was 0.3 atm.

Results

Strain C-38,383 forms branching hyphae which develop into substrate mycelium and aerial mycelium (0.5 μm in width). These mycelia are long, well branched and do not fragment into filaments. Arthrospores, which are continuous chains or are intercallated with empty hyphae, are formed throughout the aerial mycelium. The aerial hyphae are divided into long segments which subdivide

Table 2. Cultural characteristics of strain C-38,383.[a]

Medium	Growth and aerial mycelium[b]	Substrate mycelium	Diffusible pigment
Tryptone - yeast extract broth (ISP No. 1)	Floccose pale yellow	Yellow	None
Sucrose - nitrate (CZAPEK's) agar	Yellowish white (92) to pale yellow (89)	Strong yellow (84) to vivid yellow (82)	Dark grayish yellow to light olive brown (94)
Glucose - asparagine agar	Scant, yellowish white (92) to pale yellow (89)	Yellowish white (263)	None
Glycerol - asparagine agar (ISP No. 5)	Abundant, pale yellow (89) to light yellow (86)	Brilliant yellow (83) to strong yellow (84)	Yellow gray (93) to grayish yellow (90)
Inorganic salts - starch agar (ISP No. 4)	Abundant, white (263) to yellowish white (92)	Pale yellow (89) to strong yellow (84)	None
Tyrosine agar (ISP No. 2)	Pale yellow (89) to light yellow (86)	Brilliant yellow (83) to strong yellow (84)	Pale yellow (89)
Nutrient agar	White (263)	Yellowish white (92) to pale yellow (89)	None
Yeast extract - malt extract agar (ISP No. 2)	Yellowish white (92) to pale yellow (89)	Brilliant orange yellow (67) to strong orange yellow (68)	Dark orange yellow (72) to moderate yellowish brown (77)
Oatmeal agar (ISP No. 3)	Moderate, yellowish white (92) to pale yellow	Light yellow (86) to brilliant yellow (83)	None
BENNETT's agar	Abundant, yellowish white (92) to pale yellow (89)	Brilliant yellow (83) to strong yellow (84)	Vivid yellow (82)
Peptone - yeast extract - iron agar (ISP No. 6)	Moderate, white (263) to yellowish white (92)	Pale yellow (89) to light yellow (86)	None

[a] Observations were made after 3 weeks of incubation at 28°C.
[b] Color and number follow the ISCC-NBC designation.

into spores of irregular size. Chains of intercalary or continuous spores are straight or flexuous. Some of these chains range in length from 50 to 100 spores and other chains are shorter in length. These spores are non-motile, have a smooth surface and are cylindrical in shape ($0.5 \sim 0.7 \times 0.7 \sim 5.0$ μm). Sclerotia are formed on the aerial mycelium but no sporangia or whorls are evident. When strain C-38,383 is grown on agar media, colonies with and without aerial mycelium are evident. The formation of aerial mycelium is maintained when the culture is lyophilized and recovered.

The cell-wall of strain C-38,383 contains *meso*-diaminopimelic acid but lacks glycine. Whole-cell hydrolysates show glucose, galactose, mannose and rhamnose. No nocardomycolic acids were observed with strain C-38,383 or *Nocardia aerocolonigenes* JCM 4150 and the *N*-acyl type of cell-wall glycan was found to be acetyl. On the other hand, nocardomycolic acids were observed with *Nocardia asteroides* CS-45 and *Nocardia corallina* NIHJ301 and the *N*-acyl type of cell-wall glycan was found to be glycolyl.

Strain C-38,383 utilizes glycerol, D-(−)-arabinose, L-(+)-arabinose, D-xylose, D-ribose, L-rhamnose, D-glucose, D-galactose, D-fructose, D-mannose, sucrose, lactose, melibiose, trehalose, raf-

Table 3. Comparison of diagnostic physiological properties of strains C-38,383 with *Nocardia aerocolonigenes* and *Nocardiopsis dassonvillei* strains.

	Strain C-38,383	*Nocardia aerocolonigenes* 14 strains[a]	*Nocardiopsis dassonvillei* 31 strains[a]
Decomposition of			
Adenine	−	−	+
Casein	+	+	+
Hypoxanthine	+	+	+
Tyrosine	+	+	+
Urea	−	+	−
Xanthine	−	−	+
Resistance to			
Lysozyme	+	+	−
Rifampicin	−	−	−
Hydrolysis of			
Aesculin	+	+	−
Hippurate	−	V	+
Starch	+	+	+
Acid from			
Inositol	+	+	−
Lactose	+	+	−
Melibiose	+	+	−
Raffinose	+	V	−
Utilization of			
Benzoate	−	−	−
Citrate	+	+	+
Succinate	+	+	+
Tartrate	−	−	−
Nitrite from			
Nitrate	+	V	+
Survival at 50°C for 8 hours	−	V	+

[a] Data from GORDON *et al.*[25]
V: Variable.

finose, soluble starch, cellulose, inositol, D-mannitol and salicin. This strain does not utilize L-(−)-sorbose, D-(+)-melezitose, dulcitol and D-sorbitol.

Cultural characteristics are given in Table 2. The aerial mycelium, when fully developed, is light yellow. No melanin pigment is produced in peptone - yeast extract - iron agar, Tryptone - yeast extract agar or tyrosine agar. The optimal growth range is 28~37°C. There is moderate growth at 20°C and 41°C with no growth at 7°C or at 45°C. There is no growth on yeast extract - malt extract agar at pH 4.5 but there is growth at pH 5.0. Growth occurs with the medium containing 7% NaCl, but no growth is possible with 8% NaCl. Strain C-38,383 tolerates 0.01% lysozyme in Trypticase soy broth plus 1.5% agar. Gelatin is liquefied, starch is hydrolyzed and nitrate is reduced. In Difco skimmed milk medium there is peptonization without coagulation. A comparison of strain C-38,383 with published data[25] on strains of *N. aerocolonigenes* and *Nocardiopsis dassonvillei* is given in Table 3.

Prolongation of survival of leukemic mice was observed at dose levels ranging from 8 to 256 mg/kg with P388 leukemia (Table 4) and L1210 leukemia (Table 5). The effect of this drug on B16 melanoma is given in Table 6. At a concentration of 6.0 μg/ml rebeccamycin gave a 50% inhibition (IC_{50}) of human lung adenocarcinoma cells (Fig. 2). In the same test procedure etoposide (VP-16-213), teni-

Table 4. Effect of rebeccamycin on P388 leukemia.

Treatment schedule	Dose (mg/kg/injection)	Effect MST % T/C
Day 1	512	155
	256	136
	128	132
	64	136
	32	118
	16	136
Q4D, days 1, 5, 9	256	141
	128	145
	64	150
	32	136
	16	123
	8	141
QD 1 to 5 days	256	150
	128	168
	64	127
	32	145
	16	145
	8	141

Tumor inoculum: 10^6 ascites cells, ip.
Host: CDF_1 mice.
Evaluation: MST=median survival time.
Effect: % T/C=(MST treated/MST control)×100.
Criteria: % T/C ≥125 considered significant antitumor activity.

Table 5. Effect of rebeccamycin on L1210 leukemia. Treatment schedule; ip, QD 1 to 9 days.

Dose (mg/kg/injection)	Effect MST % T/C
256	175
128	167
64	142
32	133
16	133
8	125
4	117
2	100

Tumor inoculum: 10^6 ascites cells, ip.
Host: CDF_1 mice.
Evaluation: MST=median survival time.
Effect: % T/C=(MST treated/MST control)×100.
Criteria: % T/C ≥125 considered significant antitumor activity.

Table 6. Effect of rebeccamycin on B16 melanoma. Treatment schedule: ip, QD 1 to 9 days.

Dose (mg/kg/injection)	Effect MST % T/C
256	150
128	150
64	147
32	133
16	131
8	114
4	111
2	106

Tumor inoculum: 10^6 ascites cells, ip.
Host: CDF_1 mice.
Evaluation: MST=median survival time.
Effect: % T/C=(MST treated/MST control)×100.
Criteria: % T/C ≥140 considered significant antitumor activity.

poside (VM-26) and mitomycin C gave IC_{50} values of 2.6, 0.5 and 0.2 μg/ml, respectively. In Table 7 data on the inhibition of macromolecular biosynthesis are presented. At 100 μg/ml rebeccamycin had limited effect on RNA and protein synthesis. On the other hand there was a 43% inhibition of DNA synthesis at 100 μg/ml. Fig. 3 presents typical alkaline elution curves resulting from 1 hour exposure of cells to increasing concentrations of rebeccamycin. DNA breakage was observed at 0.15~5.0 μg/ml. The elution curve for 0.5 μg/ml is almost linear. In Table 8 rebeccamycin shows an accelerated rate of DNA breakage with increasing concentration. Rebeccamycin, mitomycin C and cisplatin gave a positive response in the microtiter mammalian cell adhesion test (Table 9). The antimicrobial spectrum of rebeccamycin (Table 10) indicates that *Staphylococcus aureus* is sensitive at 1 μg/ml and *Streptococcus faecalis* is sensitive at 8 μg/ml.

On agar media strain C-38,383 gave rise to colonies with and without aerial mycelium. Strain RK-1 without aerial mycelium and strain RK-2 with aerial mycelium were selected and tested for production of rebeccamycin. Strain RK-1 failed to produce detectible levels of antibiotic in 3 media (<0.05 mg/liter) while strain RK-2 gave 27.1 mg/liter in medium H34, 71.3 mg/liter in medium H95 and 160.8 mg/liter in medium H96 (Table 1) after 168 hours of incubation. *N. aerocolonigenes*

Fig. 2. Effect of rebeccamycin on the viability of human lung adenocarcinoma cells (A549).

Cells were incubated with different concentrations of rebeccamycin for 1 hour at 37°C then incubated in drug-free medium for 5 days. Trypsinized cells were counted and the cell numbers from drug-treated cultures were expressed as percents of control cultures. Averages of at least four different values for each drug concentration were converted to probit units and plotted against respective drug concentrations.[18]

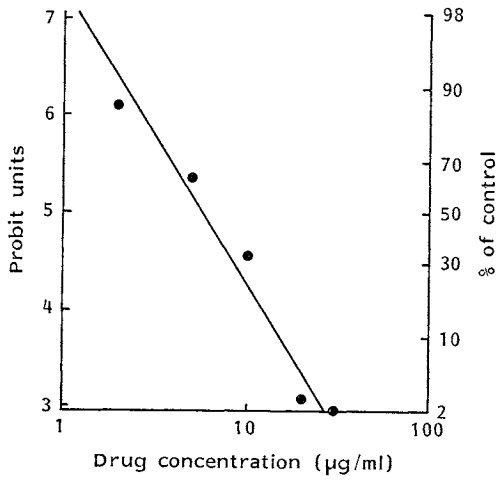


Fig. 3. Representative pH 12.1 elution curves of DNA from A549 cells incubated with rebeccamycin.

Cells containing ^{14}C-DNA were incubated with drug for 1 hour in dishes. Lysed cells on polycarbonate filters were treated with proteinase K and the eluted ^{14}C radioactivity was plotted against the elution of ^{3}H-DNA from added internal standard cells irradiated with 300 rads.

● No rebeccamycin, ○ 0.05 μg/ml, ■ 0.15 μg/ml, □ 0.5 μg/ml, ▲ 1.5 μg/ml and △ 5.0 μg/ml.

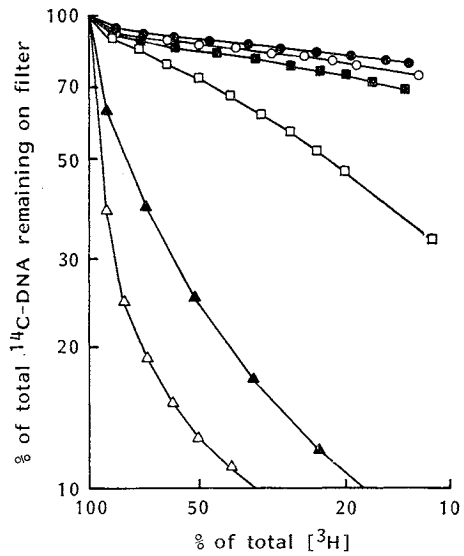


Table 7. Inhibitory effect of rebeccamycin on DNA, RNA and protein biosynthesis *in vivo*.

Drug concentration (μg/ml)	% of control		
	DNA	RNA	Protein
1	96	95	87
10	87	88	76
100	57	84	87

Table 8. Calculated frequency for single-strand DNA breakage produced by different concentrations of rebeccamycin.

Drug concentration (μg/ml)	Breaks/10^8 nucleotides
0.1	0.5
0.3	1.8
1.0	11.9
3.0	74.0
10.0	316.4

OEU 701 (ATCC 23870) has no aerial mycelium and failed to produce rebeccamycin in fermentation trials. In a fermentor with 30 liters of an improved production medium G134 (Table 1), the yield at 204 hours was 663 mg/liter. A successful tank fermentation with 3,000 liters was completed for the isolation of rebeccamycin in sufficient yield for chemical studies and biological evaluation.

Discussion

Strain C-38,383 was compared with ten genera of Order Actinomycetales, including *Nocardia*, *Actinopolyspora*, *Micropolyspora*, *Nocardiopsis*, *Saccharomonospora*, *Saccharopolyspora*, *Pseudonocardia*, *Actinosynnema*, *Streptoalloteichus* and *Saccharothrix*[28] all of which produce spore chains on aerial mycelium and contain *meso*-diaminopimelic acid in the cell-wall. Strains in the genus *Nocardiopsis* are similar to strain C-38,383 with regard to spore-chain and spore morphology, but differ from strain

Table 9. Effect of rebeccamycin, cisplatin and mitomycin C on adherent mammalian cells in microtiter plates.

Compound	IC_{50}					
	A549	B16-F10	HCT-116	KB	MOSER	M109
Rebeccamycin DMSO* μg/ml	1.2	0.48	0.41	16.4	98.0	27.9
DMSO % (vol/vol) at end point	(0.015)	(0.006)	(0.005)	(0.2)	(1.2)	(0.35)
DMSO % (vol/vol) control	2.3	4.0	3.8	4.7	3.4	3.8
Cisplatin, saline μg/ml	8.1	8.0	8.2	2.6	9.6	6.6
Mitomycin C DMSO/saline μg/ml	0.72	2.6	0.8	0.65	6.0	1.7

Cell lines: A549 – human lung, B16-F10 – murine melanoma, HCT-116 – human colon, KB – human nasopharyngeal, MOSER – human colon, M109 – murine lung.

Average values of at least two trials.

* Starting solution was 8 mg/ml in DMSO diluted serially with McCOY's 5A medium.

Table 10. Antimicrobial spectrum of rebeccamycin.

Test organism	MIC (μg/ml)		
	Rebeccamycin	Ampicillin	Amphotericin
Staphylococcus aureus A9537	1	0.03	—
Streptococcus faecalis A20688	8	0.5	—
Escherichia coli A15119	>125	1.0	—
Klebsiella pneumoniae A9664	>125	63.0	—
Pseudomonas aeruginosa A9843a	>125	>125	—
Proteus mirabilis A9900	>125	0.13	—
Candida albicans A9540	>32	—	0.5
C. albicans A15049	>32	—	0.5
C. krusei A15052	>32	—	1.0
C. krusei A22492	>32	—	1.0
C. tropicalis A15051	>32	—	1.0
C. tropicalis A22493	>32	—	0.25
Trichophyton tonsurans A22833	>32	—	1.0
T. rubrum A22789	>32	—	0.5
Microsporum gypseum A22810	>32	—	4.0
M. canis A9872	>32	—	4.9
M. canis A22494	>32	—	2.0

C-38,383 in lacking galactose and mannose in the whole-cell sugar pattern. As seen in Table 2, strain C-38,383 shows more similarity to *Nocardia aerocolonigenes* (Shinobu and Kawato) Pridham[27~29] than *Nocardiopsis dassonvillei*.[30,31] GORDON *et al.*[25] reported that 8 of 14 strains of *Nocardia aerocolonigenes* formed aerial mycelium on initial examination but lost the ability to form aerial mycelium on continued transfer. Only 1 of the 14 strains formed aerial mycelium with conidia. The cell-wall of these 14 strains contain galactose but no arabinose. Four strains were reported to contain madurose and all were reported to be non-acid fast when grown on glycerol agar.[25,32] This pattern of cell-wall and whole-cell components which includes *meso*-diaminopimelic acid, galactose with no arabinose or madurose is type IIIC.[9] While the sporulation of strain C-38,383 resembles *N. dassonvillei*, strain C-38,383 resembles closely strains of the atypical *Nocardia* species, *N. aerocolonigenes*.

All strains of *N. aerocolonigenes* including strain C-38,383 lack mycolic acids and strains IMRU 983, IMRU 1663 and IMRU 1432 were found to be resistant to phages from *Nocardia* strains with

mycolic acids.[33,34] PRIDHAM and LYONS[28] reported that traces of arabinose were present in whole-cell hydrolysates of the type strain of *N. aerocolonigenes* strain OEU 701, ISP 5034, ATCC 23870. GOODFELLOW and CROSS[35] accordingly considered that taxon to have cell-wall type IV and they proposed to place strains designated *N. aerocolonigenes* in the group with the epithet 'Micropolysporas' along with *Actinopolyspora*, *Pseudonocardia*, *Saccharomonospora* and *Saccharopolyspora*. Recently, LABEDA proposed the placement of "*Nocardia aerocolonigenes*" in the genus *Saccharothrix* as *Saccharothrix aerocolonigenes*.[36] The chemotaxonomic properties characteristic of the genus *Saccharothrix* are such that whole-cell hydrolysates contain the *meso* isomer of diaminopimelic acid. Rhamnose and galactose are the characteristic sugars and mycolic acids are absent.

It is expected that continued study of non-*Streptomyces* sporogenic actinomycetes will results in additional changes in classification of these diverse strains. For the present time, strain C-38,383 is considered to be a member of the species *Saccharothrix aerocolonigenes*. Strain C-38,383-RK-2 has been deposited with the American Type Culture Collection as ATCC 39243. This organism gives rise to mutants without aerial mycelium. If production of aerial mycelium is to be maintained, serial passage of the culture should be kept to a minimum.

Evidence has been presented showing that rebeccamycin introduces breaks in eukaryotic DNA. These breaks appear to be random since rebeccamycin at 0.5 μg/ml gave an approximately linear elution curve. It is possible that the planar ring structure may favor intercalation into DNA comparable to 4'-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA), doxorubicin and ellipticine.[37] Recent evidence suggests that some DNA-intercalating drugs produce DNA breakage by a direct or indirect action upon Type II topoisomerase.[38,39] However, recent studies utilizing alkaline elution techniques have failed to demonstrate the presence of either DNA - protein cross-links or double-strand DNA breaks following exposure of A549 cells to rebeccamycin (B. H. LONG, unpublished results). These results argue against a mechanism of action involving either direct or indirect involvement of type II topoisomerase, but do not exclude free radical or alkylation mechanisms. Further studies are being conducted to explore these possibilities. The level of rebeccamycin required to give 43% inhibition of DNA synthesis is over 16-fold higher than the concentration needed to give a 50% inhibition of cell growth (6.0 μg/ml). Most likely the primary effect of rebeccamycin is extensive fragmentation of DNA from high doses of the drug rather than inhibition of DNA synthesis. In the microtiter mammalian cell adhesion test A549, B16-F10 and HCT-116 cell lines are more sensitive that KB, MOSER and M109 cell lines while cisplatin and mitomycin C do not show such differential effects. Other studies have shown the usefulness of the microtiter test for the evaluation of anticancer compounds.[40,41] Rebeccamycin has a limited antimicrobial spectrum which shows inhibition of *Staphylococcus aureus* at 1 μg/ml and *Streptococcus faecalis* at 8 μg/ml. The related antibiotic staurosporine is not active against these organisms at 200 μg/ml. Staurosporine is active against yeasts and filamentous fungi[6] while rebeccamycin is inactive at 32 μg/ml. The cytotoxicity of staurosporine and its ability to induce differentiation in human neuroblastoma cells (NB-1) is noteworthy.

Rebeccamycin has a structure different from the drugs used thus far for clinical anticancer therapy. There is a broad therapeutic range with rodent tumor models and promising effects with human lung adenocarcinoma cells have been observed. Therefore, rebeccamycin is considered a possible candidate for further development toward clinical trial.

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