THE SORANGICINS, NOVEL AND POWERFUL INHIBITORS OF EUBACTERIAL RNA POLYMERASE ISOLATED FROM MYXOBACTERIA†

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A new antibiotic, sorangicin, was isolated from the culture supernatant of the myxobacterium, *Sorangium* (*Polyangium*) *cellulosum* strain So ce12. It is a macrocyclic lactone carbonic acid and is produced in two structural variants, sorangicins A and B. In addition small quantities of the respective glycosides, sorangiosids A and B, may be found. The antibiotic acts mainly against Gram-positive bacteria, including mycobacteria, with MIC values between 0.01 and 0.1 μg/ml, but at higher concentrations (MIC 3~30 μg/ml) Gram-negatives are also inhibited. Yeasts and molds are completely resistant. The new antibiotic is a specific inhibitor of eubacterial RNA polymerase which it blocks, however, only if added before RNA polymerization has started.

During our screening program for antibiotics from myxobacteria the culture supernatant of a strain of *Sorangium cellulosum* showed antibiotic activity against Gram-positive bacteria. When isolated and chemically characterized, the active complex turned out to consist of 4 compounds of a novel structure, macrocyclic lactone carbonic acids (Fig. 1). They were named sorangicins A and B, and sorangiosids A and B; the latter are glycosides of the former. In this paper we describe the production and some chemical and biological properties of the new antibiotics, while the structure elucidation is described elsewhere[1,2].

Fermentation of the Antibiotics

The producing organism, *Sorangium* (*Polyangium*) *cellulosum* strain So ce12, was isolated in 1978 from a soil sample from Xcaret, Mexico. It could be cultivated in MD1 liquid medium (MD1 l.m.)[3]: peptone from casein, tryptically digested 0.3%, $MgSO_4 \cdot 7H_2O$ 0.2%, $CaCl_2 \cdot 2H_2O$ 0.05%, pH 7.2, provided a suitable carbohydrate, *e.g.* 0.1% glucose or starch was added. Another useful medium was CK6 liquid medium (CK6 l.m.)[3]: $MgSO_4 \cdot 7H_2O$ 0.15%, $CaCl_2 \cdot 2H_2O$ 0.15%, $FeCl_3 \cdot 7H_2O$ 8 mg/liter, KNO_3 0.2%, K_2HPO_4 0.025%, glucose 0.5%.

In MD1 l.m., So ce12 grew homogeneously, whereas in CK6 l.m. there were always lumps besides single cells in the culture liquid. Addition of peptone in low concentrations (0.05%) improved homogeneous growth in CK6 l.m.

The influence of the peptone concentration on growth and antibiotic production, as determined from the diameter of the inhibition zone with *Staphylococcus aureus* as an indicator organism, is shown in Table 1. Best production occurred at low peptone concentrations. An increase in the glucose content led to a rapid drop of the pH to below 6, and growth and production became inhibited.

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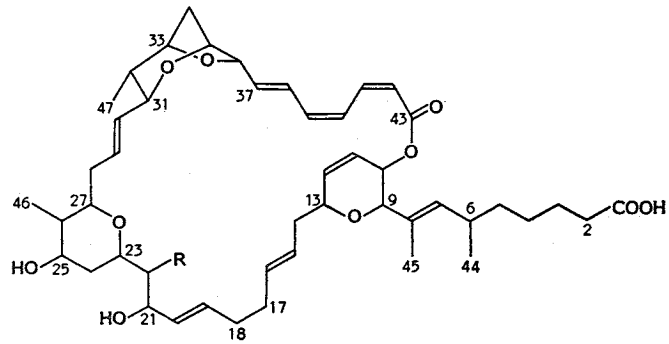
Fig. 1. The chemical structures of sorangicins A and B[1,2].

Sorangiosids A and B are the corresponding glycosides with a D-glucose attached to the OH-group at C-21.



Sorangicin A R = OH

Sorangicin B R = H

Table 1. Influence of the peptone concentration of the medium on growth and antibiotic production of *Sorangium cellulosum* strain So ce12[a].

Peptone concentration (%)	Maximal cell density OD_{623}	Final pH	Diameter of inhibition zone[b] (mm)
0	0.04	5.3	9
0.02	0.16	5.3	11
0.05	0.56	5.5	14
0.1	1.2	6.0	16
0.2	1.5	6.6	16
0.3	1.7	7.1	15
0.5	2.0	7.4	13
0.7	2.2	7.3	11

[a] MD1 l.m. with 0.1% glucose and varying peptone concentrations.

[b] Determined with paper discs of 6 mm diameter and *Staphylococcus aureus* as indicator organism. The results given are the maximum values observed in the course of the culture.

Fermentations were consequently performed in CK6 l.m. supplemented with 0.05% peptone. Bioreactors from Giovanola Frères, Manthey, Switzerland, with a volume of 700 liters were used. They were equipped with turbine impellers and inoculated with 10% from an earlier fermentor culture. The aeration and stirring rates were adjusted to 0.012 vol/vol/minute and 100 rpm, respectively. The pO_2 decreased slowly to about 20% during the first 50 hours of fermentation. It was kept at this value during the next 40 hours. The pH was not regulated and decreased from 7.3 at the beginning to 6.7 after 35 hours. Then it rose again to 7.3 at the end of the fermentation. At harvest time, the cells were separated from the culture broth by centrifugation. The antibiotic yield of the first fermentations was only about 1 mg per liter.

Isolation of the Antibiotics

The cell free supernatant (700 liters) was extracted in a counter current device with ethyl acetate. Vacuum evaporation of the solvent resulted in an oily residue (13.5 g) containing approximately 700 mg of sorangicin A. By distribution between methanol and heptane, the antibiotic was concentrated

Fig. 2. IR spectrum of sorangicin A in KBr.

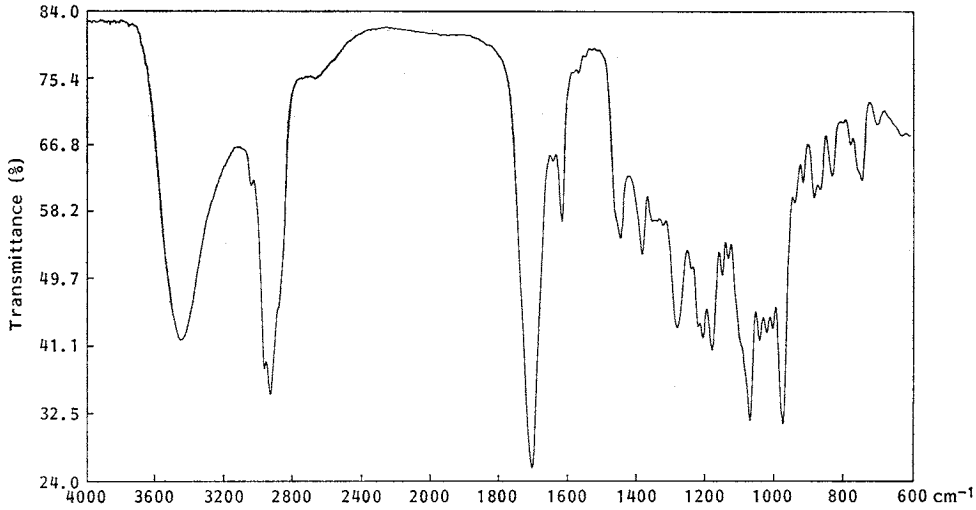


Fig. 3. ^{1}H NMR spectrum of sorangicin A in deuterated methanol, recorded with a Bruker WM 400 spectrometer.

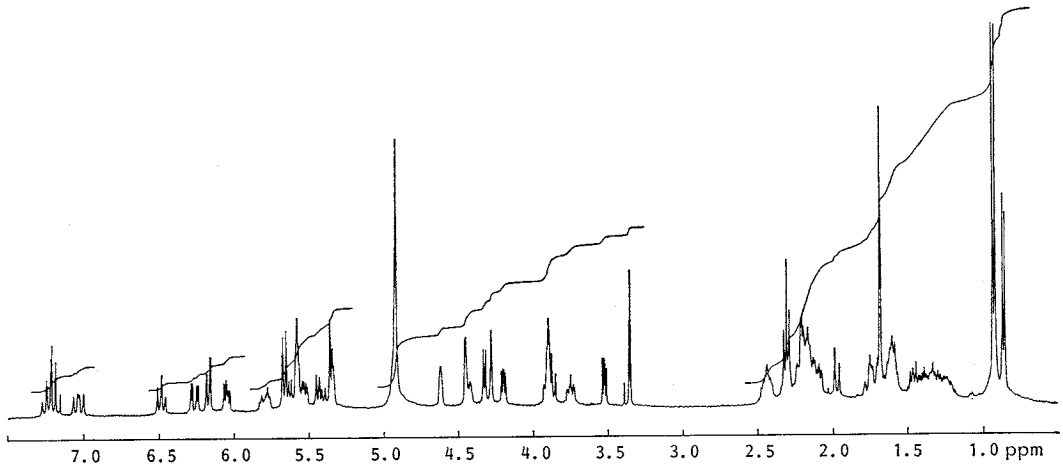
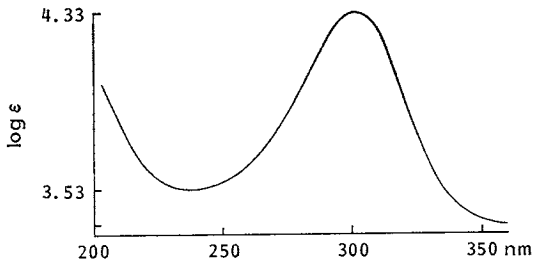


Fig. 4. Electronic absorption spectrum of sorangicin A in methanol.



2-fold in the methanolic phase. This material was partitioned between ethyl ether and 12% aqueous ammonia. After evaporation of the ammonia from the aqueous phase and acidification, the crude antibiotic (2.7 g) could be re-extracted with dichloromethane. Final fractionation was done by reversed phase, medium pressure chromatography with methanol - 1% aqueous triethylammonium formate, pH 7 (75:25) as the eluant, using a 670×37 mm column (Labomatic, Sinsheim) with LiChroprep RP-18 (25~40 μm, Merck, Darmstadt). Fractions were collected according to peaks at 313 nm and evaporated. The residues were dissolved in dichloro-

Table 2. The antibiotic spectrum of the sorangicins.

Test organism	Diameter of the inhibition zones (mm)					
	Sorangicin A[a]		Sorangicin B[a]		Sorangiosid A[a]	Sorangiosid B[a]
	0.5	5	0.5	5	5	5
Bacillus polymyxa	14	22	9	18		
Micrococcus luteus	25	28	23	27	0	14
M. lysodeikticus	25	31	30	35		
Corynebacterium mediolanum	25	32	30	39	0	12
Nocardia flava	22	28	23	30		
Arthrobacter rubellus	20	25	19	25		
A. simplex	9	14	9	14		
Brevibacterium ammoniagenes	20	23	20	23		
Klebsiella sp.	0	10	0	10		
Salmonella typhimurium	0	9	0	9	0	0
Rhizobium meliloti	14	24	10	18		
Candida albicans		0		0	0	0
Hansenula anomala		0		0	0	0
Nematospora oryzae		0		0	0	0
Mucor hiemalis		0		0	0	0
Polysticus sp.		0		0	0	0
Polyporus sp.		0		0	0	0

[a] μg per disc. Tested with paper discs of 6 mm diameter.

Table 3. Minimal inhibitory concentrations (MIC) of the sorangicins.

Test organism	MIC (μg/ml)			
	Sorangicin A	Sorangicin B	Sorangiosid A	Sorangiosid B
Staphylococcus aureus	0.03	0.008	125	6
Bacillus subtilis	0.3	0.06	>125	31
B. megaterium	0.3			
Nocardia corallina	0.01		15	
Mycobacterium phlei	0.08			
M. chitae	0.125	0.08		
Pseudomonas acidovorans	6	3		
P. aeruginosa	35			
Morganella morganii	6	6		
Serratia marcescens	25			
Escherichia coli	12	6		
E. coli tol C[a]	6	3		
Myxococcus virescens	0.4			
M. fulvus	0.1			
Sorangium cellulosum strain So ce14	3			
S. cellulosum strain So ce12	50			
Saccharomyces cerevisiae	>50			

[a] Mutant strain with increased permeability.

methane, then the buffer was removed by washing with 1% formic acid. The first two fractions contained small amounts of the glycosides, sorangiosids A and B[2]. Fraction 3 yielded 400 mg of crystalline sorangicin A[1,2] (Fig. 1), and the last fraction 43 mg of sorangicin B as a syrup[2] (Fig. 1).

As in the example above, sorangicin A also was the main component in all other fermentations,

Fig. 5. Effect of sorangicin A on DNA synthesis in *Staphylococcus aureus*, measured as incorporation of [*U*-^{14}C]thymidine (specific activity 52.5 Ci/mol) into perchloric acid insoluble material.

At the time indicated by the arrow the antibiotic (0.05 μg/ml) was added to the experimental culture (○). Control (●) without sorangicin A.

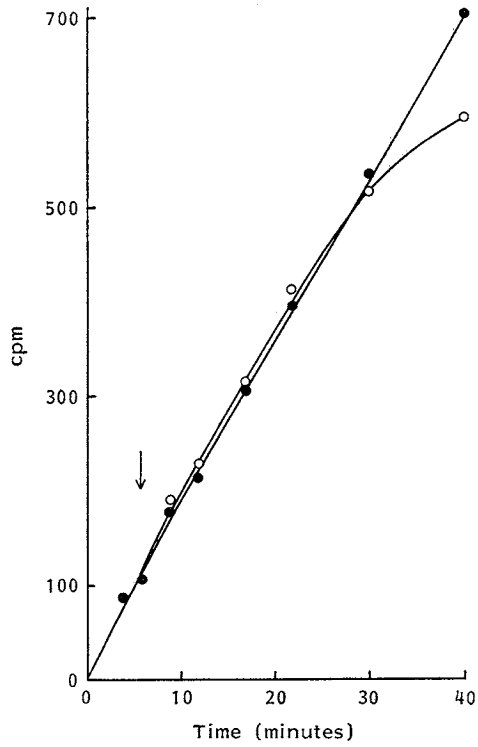
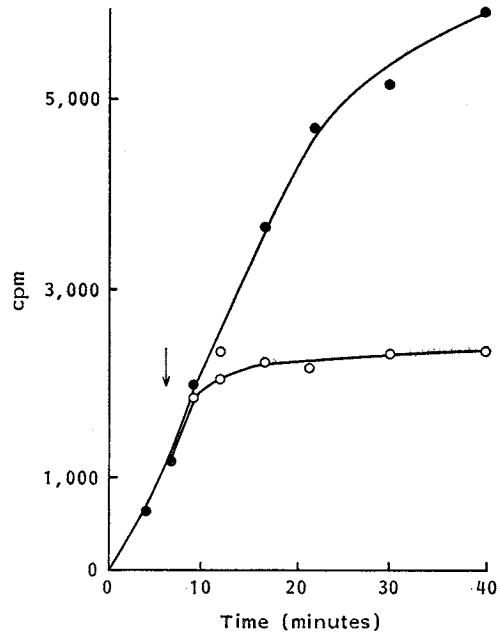


Fig. 6. Effect of sorangicin A (0.05 μg/ml) on RNA synthesis in *Staphylococcus aureus*, measured as incorporation of [2-^{14}C]uracil (specific activity 58 Ci/mol) into perchloric acid insoluble material.

Symbols as in Fig. 5.



while sorangicin B constituted about 10% of the total, and sorangiosids A and B were found always in small quantities, and only in some fermentations.

Physico-chemical Properties

Sorangicin A crystallized from ethyl acetate (mp 105~107°C).

IR, ^{1}H NMR and UV spectra of the main component, sorangicin A, are shown in Figs. 2, 3 and 4.

Biological Activity

Sorangicins A and B were active mainly against Gram-positive bacteria, although at higher concentrations they also acted against Gram-negatives (Tables 2 and 3). Yeasts and fungi were completely resistant. The glycosides of the sorangicins, the sorangiosids, had only poor activities. Curiously, several myxobacteria including strains of *S. cellulosum* were very sensitive to sorangicin A, but the producing strain was influenced by the antibiotic only at very high concentrations (50 μg/ml).

The effect of sorangicin A on the syntheses of biomacromolecules was tested with *S. aureus* as described recently[4]. RNA and protein synthesis were inhibited 3 and 10 minutes, respectively, after the addition of 0.05 μg/ml sorangicin A, and thymidine incorporation into DNA not before 25 minutes (Figs. 5, 6 and 7). These data suggested that RNA synthesis was the primary target of sorangicin A. This was confirmed by *in vitro* experiments with RNA polymerase from *Escherichia coli* (E.C. 2.7.7.6.; Boehringer, Mannheim). With 0.02 μg/ml sorangicin A, a 50% inhibition of the enzyme activity

Fig. 7. Effect of sorangicin A (0.05 μg/ml) on protein synthesis in *Staphylococcus aureus*, measured as incorporation of [*U*-^{14}C]isoleucine (specific activity 339 Ci/mol) into perchloric acid insoluble material.

Symbols as in Fig. 5.

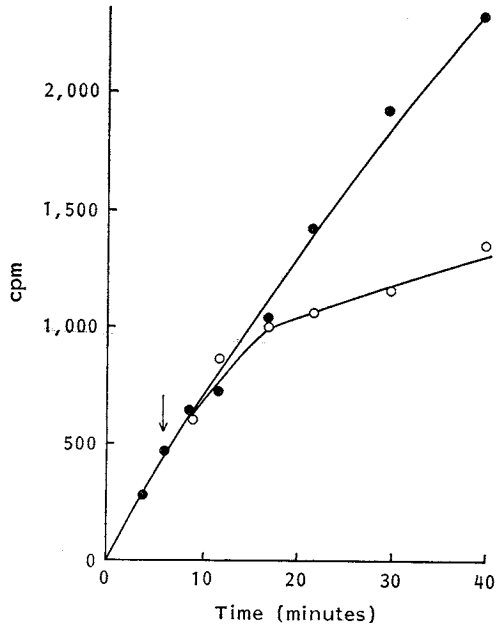


Fig. 8. Inhibition of DNA-dependent RNA polymerase from *Escherichia coli* by sorangicin A, dose response curve.

The incorporation of [4-^{14}C]UTP into perchloric acid insoluble material was measured[7].

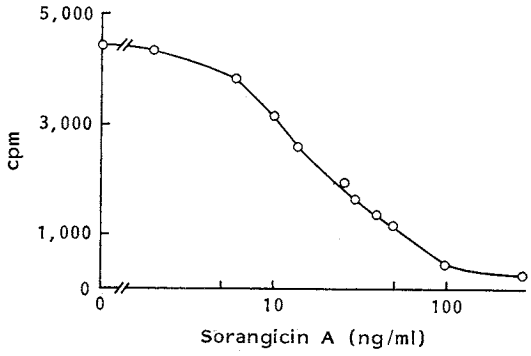
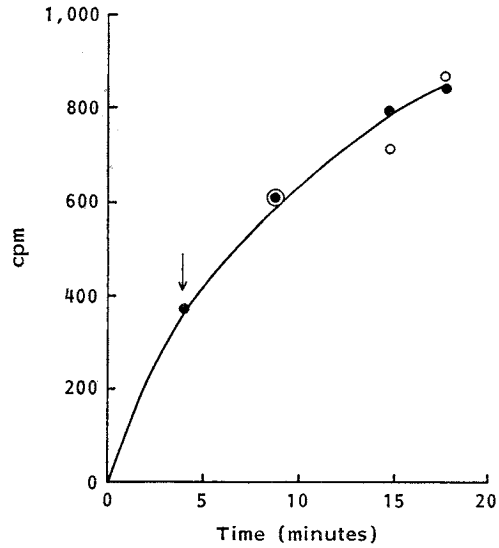


Fig. 9. Kinetics of RNA synthesis by *Escherichia coli* RNA polymerase in the presence and absence of sorangicin A with calf thymus DNA as the template[7].

The antibiotic was added 4 minutes after the polymerization reaction had been started (arrow). For details, see text.

○; Experimental culture with 0.1 μg/ml sorangicin A, ●; control without the antibiotic.



was observed, and total inhibition occurred with 0.1 μg/ml (Fig. 8).

In contrast, RNA polymerase II from wheat germ (Miles Laboratories, Elkart, Indiana) was not inhibited by 50 μg/ml sorangicin A.

The kinetics of uridine incorporation into RNA by *E. coli* polymerase[4] is shown in Fig. 9. The mixture was divided 4 minutes after the reaction had been started. To one half, 0.1 μg/ml sorangicin A was added, and to the other an equivalent volume of methanol. As can be seen, the RNA polymerization was no longer inhibited when sorangicin A was added after the reaction had been initiated.

Discussion

The sorangicins are macrocyclic antibiotics of a novel type (Fig. 1). They are inhibitory for eubacteria only, and Gram-positives including mycobacteria are particularly sensitive (Tables 2 and 3). Gram-negatives usually react, too, but at much higher concentrations. A remarkable exception are the myxobacteria themselves which are very sensitive to the sorangicins. This is reminiscent of

their behavior towards another inhibitor of RNA synthesis, actinomycin D, to which they also are much more sensitive than most Gram-negatives[5]. The unusual sensitivity may, however, be connected with penetration rather than the mechanism of action, which in the details differs substantially for the two inhibitors, anyway. Eukaryotic cells are completely resistant. Of the two structure variants of sorangicin, compound B appears to be slightly more efficient, while by glycosylation the potency of both is reduced drastically.

The primary site of action of sorangicin A, the only structure variant investigated in this respect, appears to be RNA synthesis in *S. aureus* (Figs. 5, 6 and 7). Experiments with isolated RNA polymerase from *E. coli* confirmed that this enzyme is indeed the molecular target. The high *in vitro* sensitivity of the enzyme further suggests that the observed resistance of Gram-negatives may be due to impeded penetration. Kinetic studies clearly demonstrated that the *E. coli* enzyme is no longer inhibited by sorangicin A after RNA polymerization has been initiated. This is the same kind of interference known for rifampicin[6,7] but it differs from that of corallopyronin and myxopyronin, two other inhibitors of bacterial RNA synthesis recently isolated by us from myxobacteria[4,8].

Acknowledgments

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