Sorangiolid A, a New Antibiotic Isolated from the Myxobacterium *Sorangium cellulosum* So ce 12†

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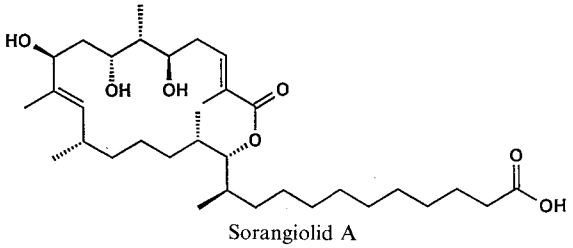
Beside sorangicin[1,2], disorazol[4,5] and chivosazol[3], we found a fourth biologically active substance in the XAD extract of the fermentation broth of *Sorangium cellulosum* strain So ce 12. It turned out to be a new structure (Fig. 1). We propose the name sorangiolid A. Here we report on the biological activity of the new substance. The isolation and structure elucidation is described elsewhere[6].

Sorangiolid A was active against Gram-positive bacteria. The MIC values were between 5 and 20 μg/ml. Yeasts and fungi were not influenced, but mammalian cells were sensitive to the same degree as Gram-positive bacteria (Table 1).

When the effect of sorangiolid A (10 μg/ml) on the syntheses of various cellular macromolecules (DNA, RNA, protein, cell wall) was determined in *Staphylococcus aureus*, it could be seen that all were inhibited simultaneously and immediately after the addition of the antibiotic. Incorporation of radioactive precursors were done as described[1]. This suggested interference with some fundamental cellular structure or biochemical reaction, like energy metabolism or membrane integrity. The effect of sorangiolid on the permeability of *Staphylococcus aureus* and of sheep erythrocytes is shown in Fig. 2. *Staphylococcus aureus* cells were centrifuged down, washed with 50 mM phosphate buffer, pH 7.2, and suspended in the same buffer to give an OD_{623} of 2. Erythrocytes from sheep blood were prepared according to STECK and KANT[7]. To one half of each suspension sorangiolid A (15 and 20 μg/ml, respectively, from a stock solution of 5 mg/ml methanol) was added, to the other half the equivalent amount of methanol as the control. The cell suspensions were incubated at 30°C or at room temperature, respectively, under shaking. At different times aliquots were taken, the cells were centrifuged down, and the extinction of the supernatant was measured at 260 nm. As can be seen, substantially more UV absorbing material was released from both types of cells, when sorangiolid A was present. The effect of sorangiolid A on *Staphylococcus aureus* was bactericidal (Fig. 3). Cells were suspended either in nutrient broth or, after washing, in 50 mM Tris-HCl buffer, pH 7.2, with or without sorangiolid (3 × MIC). At different times aliquots were diluted and plated on nutrient agar. The number of viable cells decreased rapidly within two hours after the addition of the antibiotic: In nutrient broth, the number was reduced to about 1%, in buffer to about 0.01%, perhaps because in growing cells damages can be better compensated by newly synthesized membrane material. The reason for the strong bactericidal action seems thus to be a destruction of the membranes, leading to an efflux of UV absorbing material. Correspondingly, sorangiolid A induced hemolysis in sheep erythrocytes. Though the number of surviving *Staphylococcus* cells reached a constant level after about two hours, the remaining cells were not resistant to sorangiolid A. The

Fig. 1. The chemical structure of sorangiolid A.



Sorangiolid A

A minor structural variant, sorangiolid B, with an additional OH-group in the side chain was also present[6].

Table 1. The antibiotic spectrum and MIC values of sorangiolid.

Test organism[a]	Diameter of inhibition zone[b] (mm)	MIC (μg/ml)
Staphylococcus aureus	8	5
Bacillus subtilis	8	10
Micrococcus luteus	8	20
Mycobacterium phlei	10	7.5
Nocardia corallina	8	
Streptococcus faecalis	0	
Escherichia coli	0	>120
Escherichia coli tol C[c]	0	>120
Salmonella typhimurium	0	
Candida albicans	0	
Rhodotorula glutinis	0	
Saccharomyces cerevisiae	0	
Mucor hiemalis	0	
Aspergillus niger	0	
Botrytis cinerea	0	
Mous fibroblast cells L 929		4

[a] The media for the test organisms were as described[1].
[b] The antibiotic (10 μg) was applied to paper discs of 6 mm diameter.
[c] Mutation with altered outer membrane.

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Fig. 2. Effect of sorangiolid on permeability of the cell membranes of *Staphylococcus aureus* and of sheep erythrocytes measured as efflux of UV absorbing material.

● *Staphyloccus aureus*, control; ○ *S. aureus* +15 μg sorangiolid/ml; ▼ erythrocytes, control; ▽ erythrocytes, +20 μg sorangiolid/ml.

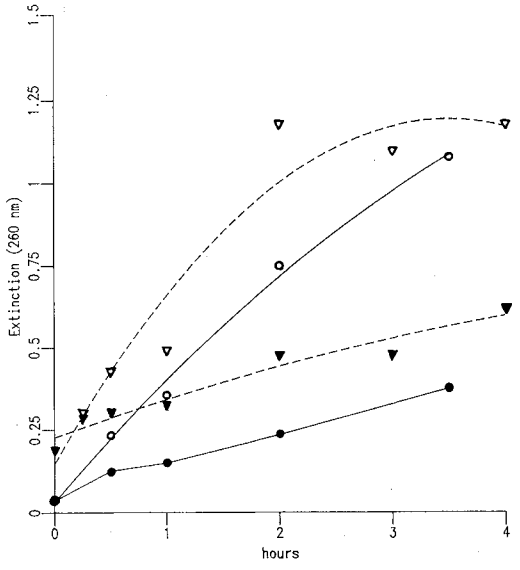
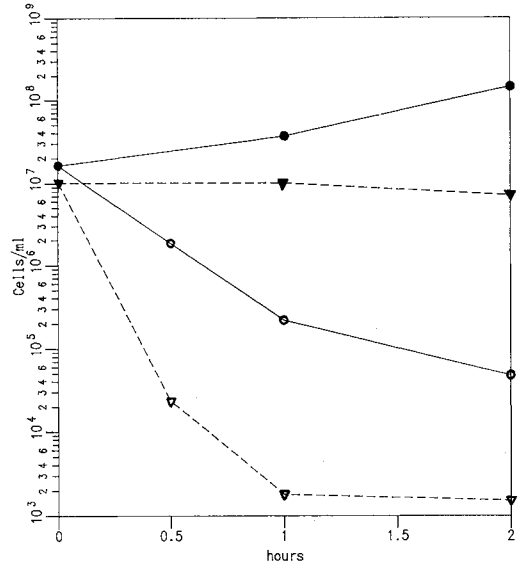


Fig. 3. Effect of sorangiolid on viability of *Staphylococcus aureus*.

● Nutrient broth, control; ○ nutrient broth + sorangiolid; ▼ Tris buffer, control; ▽ Tris buffer + sorangiolid.



reason for their survival may be removal of sorangiolid A by binding to cells or cell debris. This hypothesis is supported by a clear dependence of the MIC on the cell densitiy of the treated culture (2.5 μg/ml with 10^3, 5 μg/ml with 10^5, 10 μg/ml with 10^7 cells/ml). Also, if the broth with sorangiolid A was first incubated with 10^9 cells/ml and the cells were then removed by centrifugation, the MIC in the supernatant rose by a factor of 3. The time length of the preincubation did not make any difference as was to be expected if the antibiotic would have been destroyed.

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