The Tartrolons, New Boron-containing Antibiotics from a Myxobacterium, *Sorangium cellulosum*†

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New antibiotics were isolated from the culture broth of the myxobacterium, *Sorangium cellulosum*, strain So ce 678. The antibiotics were active against Gram-positive bacteria and mammalian cells. They were named tartrolon A and B. Tartrolon B contains a boron atom. The boron binding region of tartrolon is identical with that of boromycin and aplasmomycin.

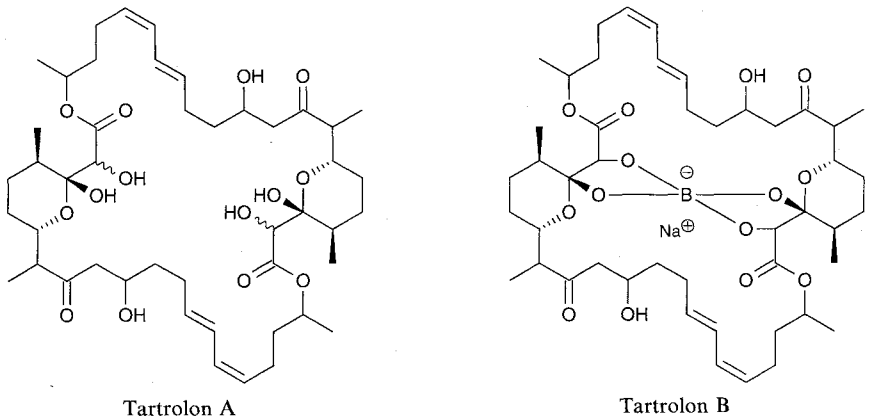
In our screening program for secondary metabolites from myxobacteria, we found an inhibitor of Gram-positive bacteria. It was produced by *Sorangium cellulosum*, strain So ce 678, and turned out to be a macrocyclic ring composed of two identical halves. There were two components; one of them contained a boron atom (Fig. 1). We propose the name tartrolon. In the following we report on the production and biological properties of the new compounds. The structure elucidation has been published elsewhere[1].

Production of the Antibiotic

The producing organism was isolated by us in 1990 from a soil sample collected near Braunschweig. It was identified as a strain of the cellulose-degrading myxobacterium, *Sorangium cellulosum*. Stock cultures were kept on vy/2-agar (bakers' yeast, 0.5%; $CaCl_2 \cdot 2H_2O$, 0.1%; cyanocobalamin, 0.5 μg/ml; agar, 1.6%; pH 7.2) at 30°C. In liquid cultures in shake flasks on a medium containing potato starch, 0.8%; yeast extract, 0.2%; defatted soja meal, 0.2%; glucose · H_2O, 0.2%; $MgSO_4 \cdot 7H_2O$, 0.1%; $CaCl_2 \cdot 2H_2O$, 0.1%; Na-Fe (III)-EDTA, 8 mg/liter; pH 7.2, the strain grew in lumps, so that an exact correlation of growth stage and antibiotic content was not possible. To get a clearer idea of the production phase during cultivation, changes in glucose content were estimated using glucose test strips (Boehringer, Mannheim). When the glucose content had decreased to about 0.1%, which happened 9 days after starting the culture with an inoculum of 3% (v/v), the total tartrolon content was 39 mg/liter. Three days later the glucose was used up completely, and the culture contained 47 mg/liter. After another two days the content was at 48 mg/liter, and the culture was harvested.

To produce greater quantities of tartrolon, the strain was cultivated in a 70 liter bioreactor (Giovanola Frères

Fig. 1. The chemical structures of tartrolons A and B.



Tartrolon A Tartrolon B

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SA, Monthey, Switzerland), equipped with an impeller stirring system and containing 65 liters of the medium described above. A neutral polystyrene adsorber resin, XAD-16 (Rohm and Haas, Darmstadt), was present from the beginning (1%, v/v). The fermentation was started with a 2.8 liters inoculum grown in Erlenmeyer flasks under shaking. The temperature was 30°C, the aeration 0.15 m^3 air per hour, the stirrer speed 150 rpm. The fermentation was stopped when the glucose was used up after 9 days. The XAD together with cell lumps was separated from the supernatant, and the antibiotic was isolated as described elsewhere[1]. The yields of tartrolons A and B are given in Table 1. As the main product was tartrolon A by far, experiments were done to find the source of the boron. The data in Table 1 indicate that boron came mainly from the glass flasks.

Physico-chemical Data

Tartrolon is chemically related to boromycin[2,3] and aplasmomycin[4].

Fig. 2 shows the electronic spectrum of tartrolon B in methanol. The IR spectrum in KBr gives absorption at $\tilde{\nu}$ = 3544 (m), 3006 (w), 2969 (m), 2932 (s), 2901 (m), 1731 (vs), 1702 (vs), 1460 (m), 1434 (w), 1415 (w), 1393

Table 1. Influence of the fermentation vessel on the formation of tartrolons A and B. Cultivation medium was as described in the text.

Fermentation vessel	Tartrolon (μg/ml)	
	A	B
Stainless steel bioreactor	42	2
Glass flask	5.6	38.7
Glass flask + 0.005% $Na_2B_4O_7$	4.7	41

Fig. 2. Electronic spectrum of tartrolon B in methanol.

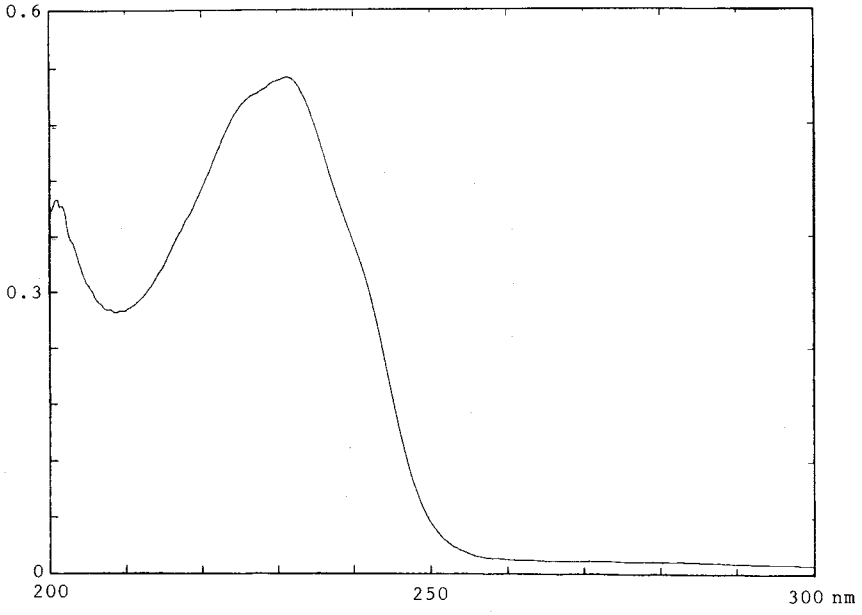
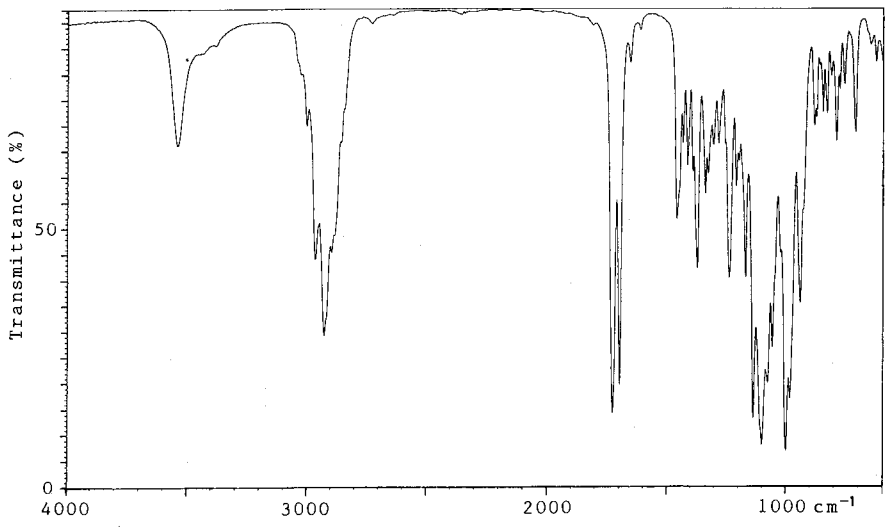


Fig. 3. IR spectrum of tartrolon B in KBr.



(w), 1373 (m), 1340 (w), 1240 (m), 1140 (vs), 1103 (vs), 1079 (s), 1060 (s), 1003 (vs), 986 (s), 944 (m), 885 (w), 842 (w), 794 (m), 714 (w) cm^{-1}.

Biological Properties

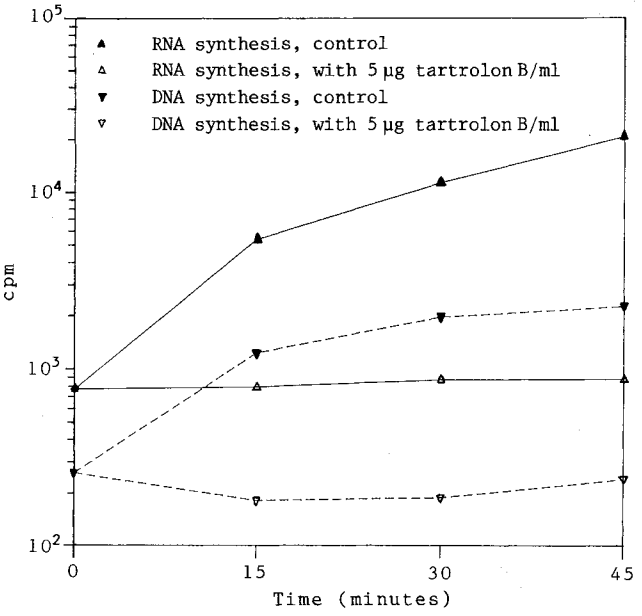
The antibiotic spectrum of the tartrolons is given in Table 2. Both tartrolons acted against Gram-positive bacteria with similar MIC values. Gram-negative bacteria, yeasts and fungi were insensitive. But mammalian cells were strongly inhibited, especially by tartrolon B. The effect of tartrolon B on macromolecular syntheses in *Staphylococcus aureus* is shown in Figs. 4~5. DNA and RNA syntheses were inhibited immediately after the addition of tartrolon (Fig. 4). Protein synthesis continued with a reduced rate for some minutes and then also stopped completely (Fig. 5). Table 3 shows the effects of the tartrolons on two key enzymes of nucleic acid synthesis. The data indicate that neither RNA polymerase nor DNA polymerase from *Escherichia coli* were influenced. Fig. 6 gives the results of conductivity measurements. *Staphylococcus aureus* was incubated overnight in EBS medium[6] containing 0.1 M KCl. After 14 hours the OD_{623} was 2.9. The following steps and measurments were done in plastic tubes. The cells were centrifuged down and washed twice with a 2% glucose solution. Then they were suspended in a 2% glucose solution to give an OD_{623} of 7.2. After the addition of the antibiotics, conductivity was measur-

Table 2. Antibiotic spectrum of tartrolons A and B.

Test strain	Inhibition zone[a] (mm)		MIC (μg/ml)	
	A	B	A	B
Arthrobacter simplex	13	9		
Bacillus megaterium	13	9		
Bacillus subtilis	15	11	0.62	0.31
Micrococcus luteus	17	11	1.25	1.25
Mycobacterium lacticola	13	10	1.56	1.56
Staphylococcus aureus	16	11	0.62	0.62
Nocardia corallina	15	12	0.62	0.62
Streptococcus faecalis	12	11	>40	10
Escherichia coli	0	0	>40	>40
Escherichia coli tol C[b]	11	0	>40	>40
Pseudomonas fluorescens	0	0		
Salmonella typhimurium	0	0		
Serratia marcescens	0	0		
Candida albicans	0	0		
Saccharomyces cerevisiae	0	0		
Rhodotorula glutinis	0	0		
Hansenula anomala	0	0		
Schizosaccharomyces pombe	0	0		
Mucor hiemalis	0	0		
Aspergillus niger	0	0		
Trichoderma harzianum	0	0		
Mouse fibroblast cells L929			0.46	0.017

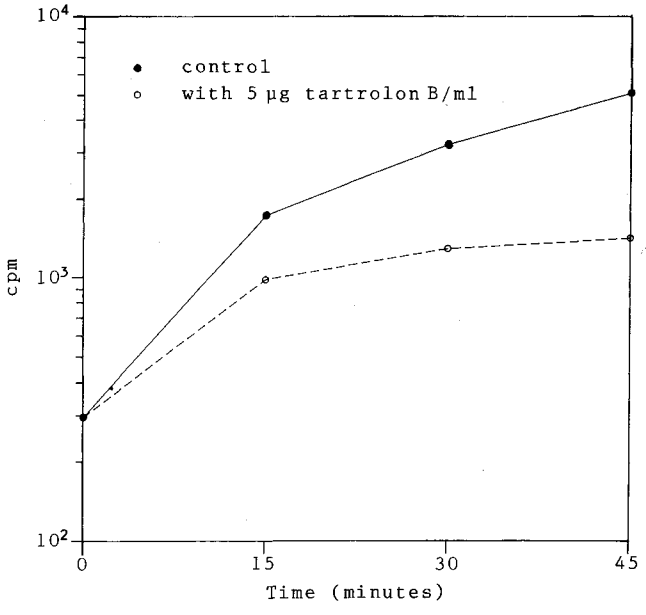
[a] With paper disks of 6 mm diameter and 10 μg tartrolon per disk. Test conditions as described in the ref.[6].
[b] Mutant with damaged outer membrane.

Fig. 4. Effect of tartrolon B on RNA and DNA synthesis in *Staphylococcus aureus*.



Measured as incorporation of [2-^{14}C]uracil (specific activity 52 Ci/mol) and [methyl-^{3}H]thymidine (specific activitiy 53.2 Ci/mmol, Amersham, Braunschweig) into perchloric acid (PCA) insoluble material. The precipitated cells were collected on glass fiber filters, Whatman GF/B. Radioactivity was determined with a Beckman liquid scintillator LS 1801. Tartrolon was added at time 0.

Fig. 5. Effect of tartrolon B on protein synthesis in *Staphylococcus aureus*.



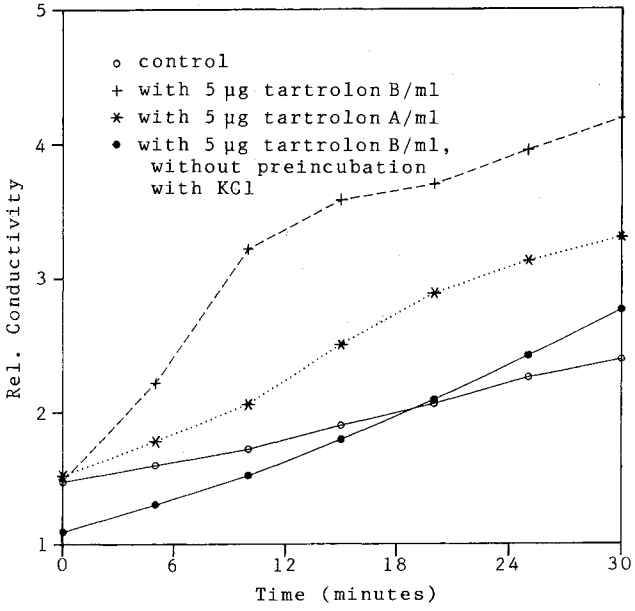
Measured as incorporation of [*U*-^{14}C]alanine (specific activity 165 Ci/mol, Amersham, Braunschweig) into PCA insoluble material.

Table 3. Influence of tartrolons A and B on RNA polymerase (E.C. 2.7.7.6) and DNA polymerase I (E.C. 2.7.7.7) of *Escherichia coli*. The enzymes were from Boehringer, Mannheim. Test conditions were as described in the application sheets.

Antibiotic	Activity (as cpm) of		
	RNA polymerase	DNA polymerase with the template poly d(A-T)	poly DNA[a]
Control	3,538	27,463	3,055
Tartrolon A, 50 μg/ml	2,790		
Tartrolon B, 50 μg/ml	3,299		
Tartrolon A, 40 μg/ml		23,824	
Tartrolon B, 40 μg/ml		25,719	
Tartrolon A, 80 μg/ml			2,484
Tartrolon B, 80 μg/ml			2,478

[a] DNA was from calf thymus (Boehringer, Mannheim). It was prepared as described in the ref.[7].

Fig. 6. Effect of tartrolons A and B on conductivity of cell suspensions of *Staphylococcus aureus* in a 2% glucose solution.

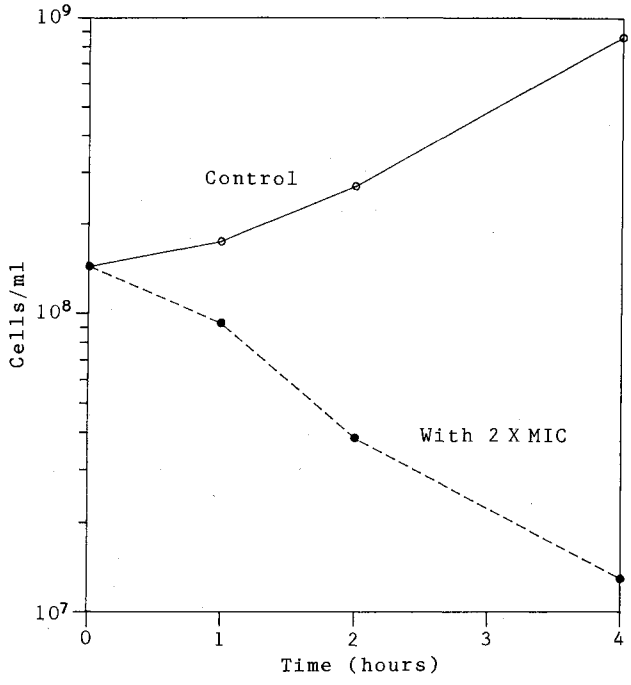


ed in a conductivity measuring cell (conductometer LF 42, WTW, Weilheim, Germany). As can be seen, the tartrolons caused a rise of conductivity of the supernatant. The effect of tartrolon B was greater than that of tartrolon A. Substantial changes of conductivity were observed only after preincubation with KCl.

With 0.1 M KCl in the medium, the MIC against *Staphylococcus aureus* increased from 0.62 to 1.24 μg/ml.

The effect of tartrolon B was bactericidal for *Staphylococcus aureus*. Cells were suspended in nutrient broth. Then the culture was divided. One half was the control, the other one contained two times the MIC of

Fig. 7. Effect of tartrolon B on viability of *Staphylococcus aureus*.



tartrolon B. Both cultures were incubated at 30°C under shaking. At different times, aliquots were diluted and plated on nutrient agar. Figure 7 shows that the number of viable cells of the tartrolon containing culture decreased in the course of the cultivation. After 4 hours about 90 % of the cells were killed.

Discussion

The tartrolons are macrolides which are structurally related to boromycin[2,3] and aplasmomycin[4]. The three antibiotics are able to bind boron by forming covalent bonds between boron and adjacent hydroxyl groups of the antibiotic. The boron binding regions of the three compounds are identical.

The formation of tartrolon A (without boron) or B (with boron) could be regulated by the culture conditions. When the culture was not in contact with glass, mainly tartrolon A was isolated. After cultivation in glass flasks, or after adding sodium tetraborate to the culture, the main product was tartrolon B (Table 1). Tartrolons A and B inhibited Gram-positive bacteria (Table 2).

The antibiotic spectrum and the MIC values of the tartrolons are comparable to those of boromycin[2] and aplasmomycin[5]. Figs. 4 to 5 show that in *Staphylococcus aureus* the syntheses of several important cellular macromolecules were inhibited immediately after the addition of tartrolon B. Isolated RNA polymerase and DNA polymerase from *Escherichia coli* were not influenced by the antibiotics (Table 3). This could mean that either tartrolon acts specifically on enzymes of Gram-positive bacteria or, more probably, that the

antibiotics interfere with energy delivery or membrane integrity. Boromycin is known to increase the potassium efflux from sensitive cells[8], so that the energy metabolism breaks down. Figure 6 indicates that tartrolon acts in a similar way. From the rise of conductivity of potassium loaded cell suspensions upon the addition of tartrolon, without a concomitant efflux of UV absorbing material (data not shown), we conclude that the tartrolons also cause a loss of potassium ions. The tartrolons are rather toxic for mammalian cell cultures (Table 2). In this case, component B is more efficient than A.

Acknowledgement

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References

1) SCHUMMER, D.; H. IRSCHIK, H. REICHENBACH & G. HÖFLE: Tartrolons: New boron-containing macrodiolides from *Sorangium cellulosum*. Liebigs Ann. Chem. 1994: 283～289, 1994
2) HÜTTER, R.; W. KELLER-SCHIERLEIN, F. KNÜSEL, V. PRELOG, G. C. RODGERS Jr., P. SUTER, G. VOGEL, W. Voser & H. ZÄHNER: Stoffwechselprodukte von Mikroorganismen. Boromycin. Helvetica Chimica Acta 50: 1533～1539, 1967
3) PACHE, W.: Boromycin. *In* Antibiotics (*Ed.* J. W. CORCORAN *et al.*) Springer New York, Vol. 3: 585～587, 1975
4) NAKAMURA, H.; Y. IITAKA, T. KITAHARA, T. OKAZAKI & Y. OKAMI: Structure of aplasmomycin. J. Antibiotics 30: 714～719, 1977
5) OKAMI, Y.; T. OKAZAKI, T. KITAHARA & H. UMEZAWA: A new antibiotic, aplasmomycin, produced by a streptomycete isolated from shallow sea mud. J. Antibiotics 29: 1019～1025, 1976
6) IRSCHIK, H.; R. JANSEN, K. GERTH, G. HÖFLE & H. REICHENBACH: The sorangicins, novel and powerful inhibitors of eubacterial RNA polymerase isolated from myxobacteria. J. Antibiotics 40: 7～13, 1987
7) APOSHIAN, H. V. & A. KORNBERG: Enzymatic synthesis of deoxyribonucleic acid. J. Biol. Chem. 237: 519～525, 1962
8) PACHE, W. & H. ZÄHNER: Metabolic products of microorganisms. Studies on the mechanism of action of boromycin. Arch. Mikrobiol. 67: 156～165, 1969