The Jerangolids: A Family of New Antifungal Compounds from *Sorangium cellulosum* (Myxobacteria)

Production, Physico-chemical and Biological Properties of Jerangolid A†

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An antifungal activity was detected in the culture broth of the myxobacterium, *Sorangium cellulosum* strain So ce 307. The activity was excreted into the supernatant during the log and early stationary phase. When the organism was fermented in the presence of the adsorber resin XAD-16, the metabolite was quantitatively bound to the resin. The main component, jerangolid A, has structural similarities to ambruticin, which is also produced by strains of *Sorangium cellulosum*.

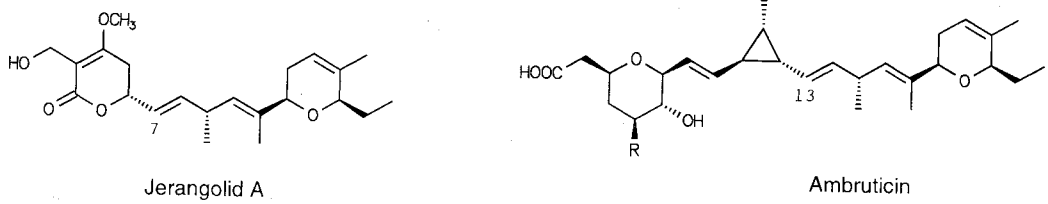
In recent years more and more interesting secondary metabolites have been discovered in culture supernatants of *Sorangium cellulosum* strains. To differentiate very early in the screening between known and new compounds, we used mutants of test organisms which are resistant to certain known antibiotics.

This method worked very well, and, as a rule, the results were confirmed by HPLC analysis. However in extracts of two strains, So ce 307 and 405, which were identified as producers of ambruticin[1,2] by their inhibition spectrum, no ambruticin could be detected by HPLC. Instead a main peak with an unknown UV spectrum, which in addition differed in both extracts, was seen in the XAD eluates. The active metabolite of strain So ce 405 was identified as pyrrolnitrin. This is the first time that a strain of the suborder Sorangineae produced a secondary metabolite known from strains of the suborder Cystobacterineae. Pyrrolnitrin, first found in pseudomonads[3], is also produced by strains of *Myxococcus*[4], *Cystobacter* and *Corallococcus*. This paper deals with the identification and biological characterization of the activities of strain So ce 307, which were due to new compounds, the jerangolids. The structure elucidation of jerangolid A (Fig. 1) is reported elsewhere[5].

Microorganism and Culture Conditions

The producing organism, *S. cellulosum* So ce 307, was isolated at the GBF in 1987 from a soil sample collected in Jerusalem, Israel. The organism was inoculated from yeast agar plates into 250 ml Erlenmeyer flasks containing 100 ml of the following basic medium (in g/liter distilled water): potato starch (Maizena) 10, glucose (Maizena) 1, soy bean meal 5, $MgSO_4 \cdot 7H_2O$ 1, $CaCl_2 \cdot 2H_2O$ 1, ethylendiaminetetraacetic acid, iron(III)-sodium salt 0.008. The pH was adjusted to 7.2 with KOH before autoclaving. For continuous adsorption of lipophilic metabolites 2% (v/v) of XAD-16 (Rohm and Haas, Frankfurt/M) was added. So ce 307 grew as a homogeneous cell suspension to a high cell density.

Fig. 1. The structures of jerangolid A[5] and ambruticin VS3 [R = $N(CH_3)_2$][2].



Jerangolid A Ambruticin

† Art. No. 72 on antibiotics from gliding bacteria. Art. No. 71: KUNZE, B.; R. JANSEN, F. SASSE, G. HÖFLE & H. REICHENBACH: J. Antibiotics 48: 1262~1266, 1995.

Optimization of the Antibiotic Production

The influence of peptone and yeast extract on growth and antibiotic production was studied by adding 0.2, 0.5 and 1% of the respective nitrogen-source to the basic medium. These experiments were performed in 250 ml Erlenmeyer flasks with 50 ml of medium. With increasing concentrations of peptone growth was stimulated while production was not influenced up to 0.5%. Addition of 1% peptone inhibited production completely. In contrast, yeast extract stimulated growth only slightly and was inhibitory at 1%. Production, however, was stimulated at a concentration of 0.2% by a factor of 2.5 and inhibited at 0.5% and 1%. For antibiotic production therefore 0.2% of yeast extract (Marcor) was added to the basic medium.

To study the effect of oxygen on the production of jerangolid, So ce 307 was grown in 250 ml Erlenmeyer flasks with increasing volumes of culture broth tested up to 150 ml. Above 75 ml a continuous decline in productivity was seen, while growth was not influenced.

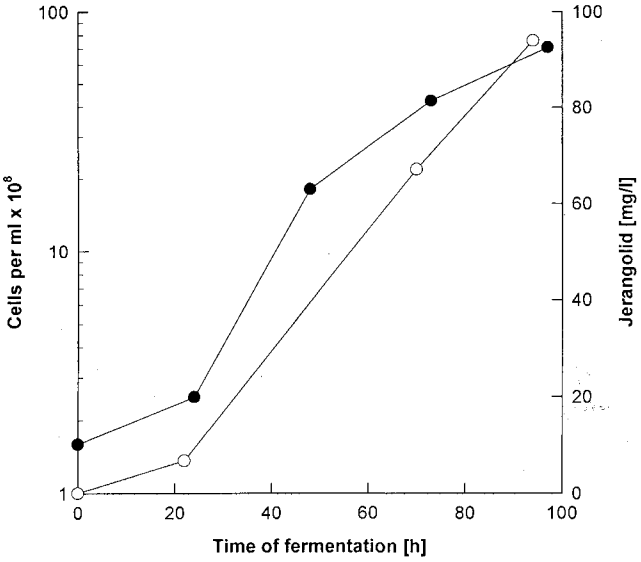
Production

A 100-liter bioreactor (Giovanola Frères, Monthey, Switzerland) equipped with a flat-blade turbine stirrer and containing 60 liter of the production medium was inoculated with 3 liter of a 4-day old preculture grown in the same medium in 1-liter Erlenmeyer flasks with 500 ml medium under shaking (160 rpm, 30°C). For continuous adsorption of the produced antibiotic, 1 liter of the adsorber resin XAD-16 (Rohm and Haas, Frankfurt/M) was added to the bioreactor before autoclaving. To prevent foam formation, 10 ml silicone antifoam (Tegosipon, Goldschmidt AG, Essen) was added. The fermentation was run for 4 days at 32°C with an aeration rate of 700 liters air per hour and a stirrer speed of 250 rpm. The pH was maintained at 7.2 with 10% KOH (Fig. 2).

At the end of the fermentation, the adsorber resin was separated from the broth by sieving. After washing the product was eluted with five bed volumes of methanol. The extract was then concentrated *in vacuo* at 40°C. The isolation of jerangolid by chromatography is described in detail elsewhere[5].

Fig. 2. Fermentation of jerangolid A.

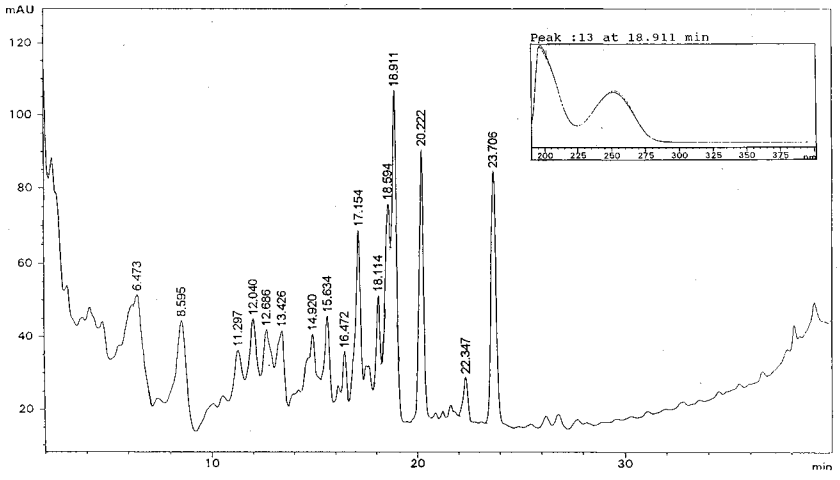
● Cell number per ml; ○ jerangolid production.



Physico-chemical Properties

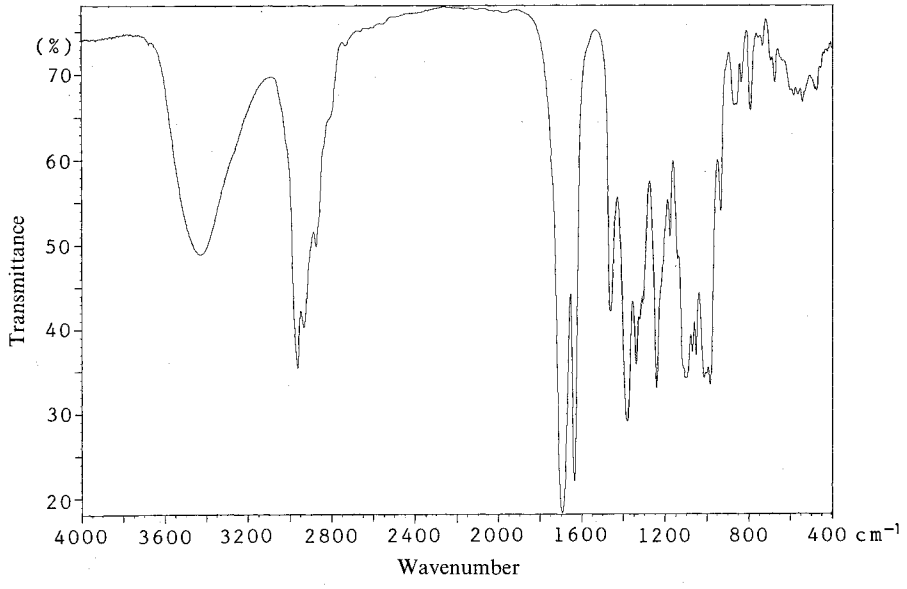
The antibiotic was analyzed by thin-layer chromatography (Silica gel Si 60 F_{254}, Merck, Darmstadt) with ethyl acetate as the solvent. Jerangolid A was detected

Fig. 3. HPLC run of a broth extract of So ce 307 grown without XAD.



UV spectra at the upslope and the downslope of the peaks are normalized and overlaid for peak purity check. The spectra of all labeled peaks are identical. Peak at 18.911 minutes corresponds to jerangolid A.

Fig. 4. IR spectrum of jerangolid A in KBr.



as a dark spot at an Rf value of 0.55 by its UV absorption.

HPLC analysis was done on Nucleosil RP-18, 5 μm (Macheey & Nagel, Düren) using a 2 × 125 mm microbore column. A methanol-water gradient from 45:55 to 70:30 within 25 minutes and a flow rate of 0.5 ml/minutes was used. Jerangolid A was detected after 20 minutes by its characteristic diode array spectrum. In the presence of XAD, jerangolid A was the main component, and only traces of four closely related compounds, the jerangolids B~E, were detected. In the absence of the adsorber resin, 16 compounds with identical diode-array spectra and different retention times could be seen (Fig. 3).

The most polar compound eluted after 6.4 minutes, and the least-polar variant was detected after 23.7 minutes. The IR spectrum of jerangolid A in KBr (Fig. 4) was measured with an FT-IR spectrometer 20 DXB (Nicolet). High-resolution FAB mass spectroscopy gave a protonated molecular ion m/z 377.2322, which is in good agreement with the elemental composition of $C_{22}H_{32}O_5$ derived from spectroscopic data and elemental analysis[5].

Antimicrobial Spectrum

The antimicrobial spectrum of jerangolid A was determined by the paper disk method. While bacteria were not inhibited, numerous yeasts and fungi were very sensitive to the antibiotic (Table 1). The minimum inhibitory concentrations (MICs), determined by the serial dilution assay in liquid growth medium, ranged from 0.07 to 7 μg/ml. The IC_{50} for the mouse cell line

Table 1. Antifungal spectrum of jerangolid A.

Test organism	Diameter of inhibition zone[a] (mm)	MIC (μg/ml)
Absidia glauca CBS[c] 100.59	45	
Mucor hiemalis DSM[b] 2655	40	0.07
Athelia rolfsii	25	
Nematospora coryli CBS 2608	—	
Schizosaccharomyces pombe Tü[d] 501	—	
Nadsonia fulvescens CBS 2596	—	
Hansenula anomala DSM 70130	35	0.07
Pichia membranaefaciens DSM 70366	30	0.4
Debaryomyces hansenii DSM 70238	18	0.4
Lipomyces lipofer DSM 70305	—	
Saccharomyces cerevisiae BT 27C-2A YGSC[e]	—	
Aspergillus clavatus CBS 121.45	—	
Penicillium capsulatum CBS 301.48	52	
Alternaria solani DSM 2954	10	
Chaetomium cochliodes DSM 1909	10	
Fusarium oxysporum DSM 2018	25	
Trichoderma hamata	40	7.0
Cladosporium resinae DSM 63423	33	
Botrytis cinerea DSM 877	40	7.0
Sclerotina sclerotiorum DSM 1946	28	
Psilocybe montana CBS 703.20	—	
Candida albicans CBS 1893	14	4.2
Rhodotorula glutinis DSM 70398	—	
Trichosporon terrestre CBS 66.97	50	0.13

[a] Paper disks (6 mm diameter) with 10 μg of jerangolid A.
[b] Deutsche Sammlung von Mikroorganismen, Braunschweig.
[c] Centraalbureau voor Schimmelcultures, Baarn.
[d] Strain collection, University Tübingen.
[e] Yeast Genetic Stock Center of Berkeley.

Table 2. Cross-resistance of yeast strains to jerangolid, ambruticin VS3, and pyrrolnitrin.

Test organism	MIC for jerangolid (μg/ml)	MIC for ambruticin (μg/ml)	MIC for pyrrolnitrin (μg/ml)
Trichosporon terrestre	0.13	2.1	4.2
Trichosporon terrestre R^{jer}	>70	>70	33
Hansenula anomala	0.07	0.03	0.13
Hansenula anomala R^{amb}	>70	>70	33

R^{jer} = mutant resistant against jerangolid.
R^{amb} = mutant resistant against ambruticin.

Fig. 5. Effect of jerangolid A on growth of *Hansenula anomala*.

● Untreated control culture; ■ 0.2 μg/ml of jerangolid added at time 0.

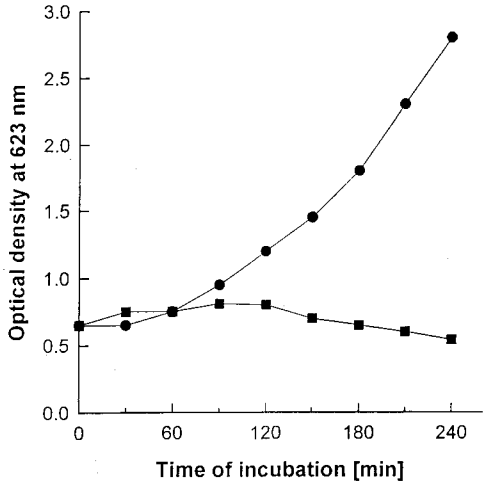


Fig. 6. Effect of jerangolid (0.2 μg/ml) on survival of growing and non-growing cells of *Hansenula anomala*.

■ Untreated control culture; ◆ culture with jerangolid incubated at 0°C; ▼ cell suspension with jerangolid in buffer at pH 7.2; ▲ culture with jerangolid at 30°C.

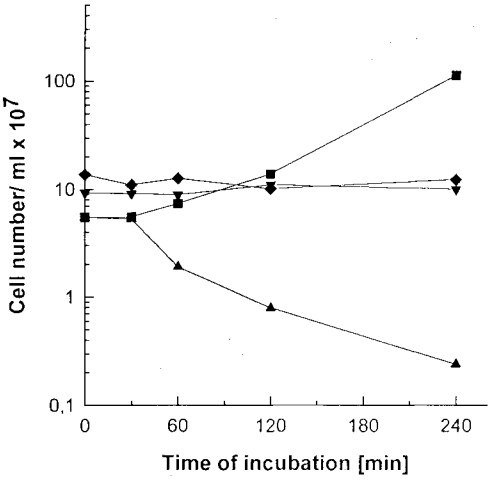
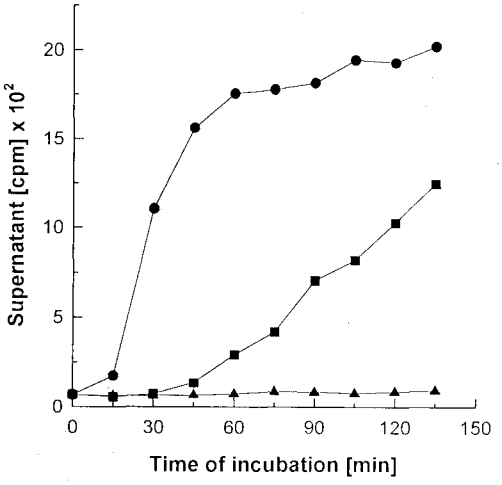


Fig. 7. Effect of jerangolid on membrane integrity.

▲ Untreated control culture; ■ culture incubated with 0.2 μg/ml of jerangolid; ● culture treated with 0.08 μg/ml of amphotericin B.



Washed cells of *H. anomala* were incubated for 30 minutes at 30°C in HEPES buffer pH 7.4 after the addition of 0.2% of glucose and [^{14}C-]2-amino-isobutyric acid. Then the cells were washed two times with buffer and resuspended in buffer with glucose. The effect of jerangolid or amphotericin B on membrane permeability was measured by determining radioactivity in the culture supernatant.

L929 was 1.4 μg/ml. An ambruticin-resistant mutant of *Hansenula anomala* showed cross resistance to jerangolid, and ambruticin was not active against a jerangolid-resistant mutant of the yeast *Trichosporon terrestre*. Both mutants proved also to be resistant to pyrrolnitrin (Table 2). In both cases the MIC values of the reference compound soraphen[6] were 0.5 μg/ml.

Biological Effects of Jerangolid A

First investigations on the mechanism of action were done with the yeast *Hansenula anomala*. Growth, measured as an increase of optical density at 623 nm, was completely stopped after the addition of 0.2 μg/ml of the antibiotic at point zero (Fig. 5). Growing cells were killed, while non-growing cells suspended in buffer or in culture broth at 0°C remained unaffected (Fig. 6).

The influence of jerangolid A on the basic metabolism of *Hansenula* was investigated by studying the incorporation of radioactive precursors into high molecular weight material. During 6 hours after the

addition of the antibiotic, the incorporation of radioactivity from glucose, alanine, acetate and uracil did not deviate from that of the control culture. The same was observed with the antifungal compound, ambruticin.

The effect of jerangolid on membrane integrity was tested with cells of *Hansenula anomala* loaded with [^{14}C]-2-aminoisobutyric acid. In the presence of jerangolid, increasing amounts of radioactive material were measured in the culture supernatant (Fig. 7). Thus the antibiotic appeared to cause changes in membrane permeability.

Indeed, efflux of material absorbing UV radiation at 262 nm was seen with cells incubated in the presence of jerangolid at 30°C, but not with a parallel culture incubated at 0°C.

Discussion

In our screening of 665 *Sorangium cellulosum* strains, 6.6% of the isolates were identified as producers of ambruticin by use of an ambruticin-resistant mutant of *Hansenula anomala*. By HPLC the new antifungal compound jerangolid A was detected in XAD eluates of three strains of this strain population. With a frequency of 0.45% of all isolates, the jerangolids were indeed rare metabolites of *S. cellulosum* strains.

The cross-resistance between ambruticin and jerangolid is also reflected in identical biochemical effects on *Hansenula anomala*. The antibiotic spectrum of both antifungal compounds is the same. They act at very low concentrations, with an MIC of 0.07 and 0.03 μg/ml, respectively, on *Hansenula anomala* (Table 2). These similarities can be explained by the structural relationship of both metabolites (Fig. 1).

The right part of jerangolid beginning with carbon 7 is identical with the right part of ambruticin VS3 from carbon 13. This interesting finding suggests that only the right parts of the two molecules are responsible for their biological activity. The cross-resistance of pyrrolnitrin, as seen with the ambruticin-resistant *Hansenula* and the jerangolid-resistant *Trichosporon* mutant, can not be explained from the chemical structure. Perhaps the mechanism of action, which is, however, not known for any of the compounds, or the transport mechanisms are closely related. The effect of jerangolid on membrane permeability of growing cells may reflect some interference with membrane synthesis.

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