# MYXOCHELIN A, A NEW IRON-CHELATING COMPOUND FROM *ANGIOCOCCUS DISCIFORMIS* (MYXOBACTERALES)†

## PRODUCTION, ISOLATION, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

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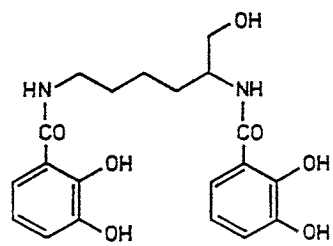
Myxochelin A, a new catechole siderophore, was isolated from the culture broth of the myxobacterium, *Angiococcus disciformis* strain An d30. As is the case with other iron-chelating compounds the production of myxochelin A could be markedly increased up to 44 mg/liter by fermentation at low iron concentrations ($10^{-7}$ M $FeCl_3$). The new substance showed weak activity against some bacteria.

The myxobacterium *Angiococcus disciformis* strain An d30 has been described to produce the antibiotics angiolam[1,2] and myxothiazol[3~5]. The latter was first isolated from the myxobacterium, *Myxococcus fulvus* strain Mx f16. In some fermentations minor amounts of an additional third, weak antibacterial activity were detected. The activity showed a positive reaction with $FeCl_3$ on TLC and was named myxochelin A. Further investigations proved that the production of the substance depended to a great extent on the iron concentration in the culture medium, and the accumulation of the compound was markedly increased by cultivation at low iron levels. Spectroscopic data revealed myxochelin A to be a new phenolic compound with the structure *N,N*-bis-(2,3-dihydroxybenzoyl)-lysinol (Fig. 1). It is related to *N,N*-bis-(2,3-dihydroxybenzoyl)-L-lysine isolated from iron-deficient cultures of *Azotobacter vinelandii* growing on nitrate[6]. In this paper we describe the production, isolation and some of the physico-chemical and biological properties of myxochelin A, while the structure elucidation will be published elsewhere.

### Microorganism and Culture Conditions

The producing organism was *A. disciformis* strain An d30 (=AfB10 Dawid). It was normally cultivated on standard peptone liquid medium (PEP l.m.: Peptone from casein, tryptically digested from Merck, Darmstadt 1%, $MgSO_4 \cdot 7H_2O$ 0.1%, pH 6.8) as described before[1]. For cultivation at low iron levels a concentrated peptone solution was adjusted to pH 7.0 and

Fig. 1. The chemical structure of myxochelin A.

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depleted of its metal ions by stirring it with 20% (w/w) of the chelating resin Chelex 100 (Bio-Rad Laboratories, Richmond, California) for 1 hour. After removal of the chelating resin, the peptone solution was diluted to 1% and supplemented with 0.1% $MgSO_4 \cdot 7H_2O$ (w/w) and 1 ml/liter of an iron-free standard trace element solution. To test the effect of iron on myxochelin A production different concentrations of $FeCl_3$ were added to the 'iron free' basal medium.

## Production

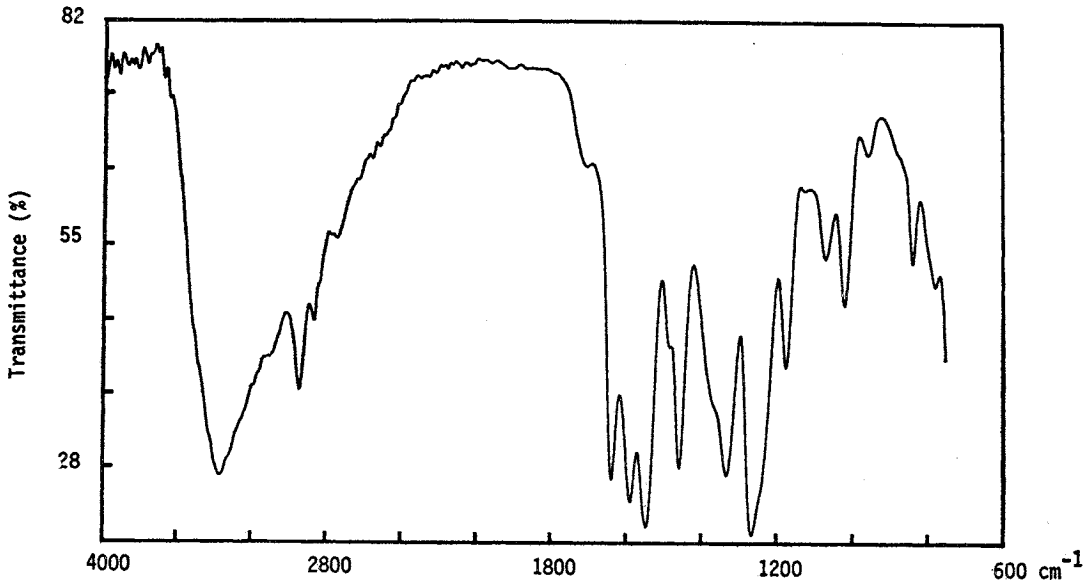
In shake cultures the best yields of myxochelin A were obtained when the 'iron free' peptone medium described above was supplemented with $FeCl_3$ to a final concentration of $10^{-7}$ M. Without the addition of $FeCl_3$, or with a final concentration of $10^{-6}$ M $FeCl_3$ the yields of myxochelin A were relatively low, and at concentrations of $10^{-5}$ M $FeCl_3$ and higher the biosynthesis of myxochelin A was strongly repressed.

Myxochelin A production on a larger scale was performed in 'iron free' medium supplemented with $FeCl_3$ to a final concentration of $10^{-7}$ M. Four liters of culture grown for 24~36 hours in this medium on a rotary shaker were inoculated into 60 liters of the same medium in a type b 50 bioreactor (Giovanola Frères, Monthey, Switzerland). The fermentor was kept at 30°C and agitated at 200~300 rpm with a turbine plate stirrer. The aeration rate was 0.052~0.078 v/v/m. The pH was not regulated and rose during fermentation from 7.0 to about 8.0. The $pO_2$ in the culture was recorded continuously with a polarographic oxygen electrode. At the beginning of the fermentation it was at about 90% saturation and fell within the first 13 hours to about 70% where it remained till the end of the fermentation at 44 hours. The concentration of myxochelin A in the culture broth was determined during the fermentation by HPLC analysis (columns: Nucleosil-$C_6H_5$ 7 $\mu$m from Macherey-Nagel, Düren, FRG; solvent: methanol - water, 65 : 35) and was 44 mg/liter at the end of the fermentation.

## Isolation

The culture broth (60 liters) was separated from the cells by centrifugation. The cells were discarded

Fig. 2. IR spectrum of myxochelin A.



and the activity in the supernatant was transformed into a water soluble Cu(II)-complex by stirring the broth with 10 g of Cu(II)-acetate for 10 minutes. Then the broth was extracted twice with ethyl acetate at pH 8.0 and 6.0, respectively. The ethyl acetate extracts containing inactive impurities were discarded, and the Cu(II)-complex in the remaining water phase was decomposed by stirring the broth with 20 g of EDTA for 15 minutes. After adjusting the water phase to pH 4.0, myxochelin A could be extracted with ethyl acetate and was purified by column chromatography on Sephadex LH-20 and HDSIL RP18.

Table 1. Antimicrobial spectrum of myxochelin A[a].

| Test organism[b] | Diameter of inhibition zone (mm) |
|---|---|
| *Bacillus brevis* DSM 30 | 13 |
| *B. cereus* DSM 621 | 15 |
| *B. megaterium* DSM 32 | 14 |
| *B. subtilis* DSM 10 | 10 |
| *B. thuringiensis* DSM 2046 | 16 |
| *Micrococcus luteus* GBF 26 | 10 |
| *Staphylococcus aureus* GBF 16 | 12 |
| *Arthrobacter simplex* DSM 20130 | 10 |
| *Brevibacterium linens* DSM 20425 | 11 |
| *Corynebacterium fascians* DSM 20131 | 14 |
| *Nocardia corallina* ATCC 13258 | 14 |
| *Escherichia coli* DSM 498 | (9) |
| *Salmonella typhimurium* DSM 50912 | 0 |
| *Serratia marcescens* GBF 61 | 0 |
| *Candida albicans* GBF 129 | 0 |
| *Saccharomyces cerevisiae* GBF 36 | 0 |
| *Mucor hiemalis* Tü 189 | 0 |
| *Paecilomyces variotii* GBF 159 | 0 |
| *Rhizoctonia solani* CBS 177.44 | 0 |

[a] Determined by the agar diffusion assay, using paper discs of 6 mm diameter with 80 $\mu$g of myxochelin A.

[b] The organisms were tested on standard complex media. The size of inoculum was $5\times10^6$ cells/ml.

### Physico-chemical Properties

Myxochelin A was soluble in methanol, acetone, ethyl acetate and chloroform, sparingly soluble in ether and almost insoluble in hexane. It had an Rf value of 0.5 on TLC (Nano-Sil $C_{18}$-50 $UV_{254}$ from Macherey-Nagel, Düren, FRG) developed with water - acetonitrile (60 : 40) and gave dark blue-violet spots after spraying with $FeCl_3$ - HCl reagent. $^1H$ NMR, $^{13}C$ NMR and mass spectroscopy established the molecular formula of $C_{20}H_{24}N_2O_7$ and the molecular weight of 404. The electronic absorption spectrum of myxochelin A dissolved in methanol was recorded with a Zeiss DMR 21 spectrophotometer and had characteristic maxima at 210 nm (log $\varepsilon$ 4.76), 245 (sh, 4.3) and 310 (3.9). The IR spectrum of myxochelin A measured with a Nicolet 20DXB FT-IR spectrometer is shown in Fig. 2.

### Antimicrobial Activity

The antibiotic activity of myxochelin A was determined by the agar diffusion test using paper discs. As can be seen from Table 1, myxochelin A showed weak activity against several Gram-positive bacteria. Gram-negative bacteria, yeasts and fungi were mostly resistant. The MIC, for *Staphylococcus aureus*, determined by the serial dilution assay, was 25 $\mu$g/ml.

## Discussion

Myxochelin A is besides angiolam[1,2] and myxothiazol[3~5] the third biologically active compound isolated from the myxobacterium *A. disciformis* strain An d30. The three compounds are structurally unrelated and show different modes of action. Physico-chemical studies revealed that myxochelin A is a new catechol siderophore. Its biosynthesis was highly stimulated by iron deficiency. It is structurally related to azotochelin, *N,N*-bis-(2,3-dihydroxybenzoyl)-L-lysine, from *A. vinelandii*[6]. While *A. vinelandii* produces several iron chelators under iron-limited conditions *viz.* besides azotochelin 2,3-dihydroxybenzoic acid[6], the peptide azotobactin[7] and the recently described aminochelin[8], we could find only myxochelin A in iron-limited cultures of strain An d30. Like certain other sidero-

phores myxochelin A showed a weak antibacterial activity which is probably caused by the chelator effect of the compound although not necessarily only by iron deficiency.

A great variety of iron-chelating compounds, generally phenolates and hydroxamates, has been described from many bacteria and fungi[9]. Myxochelin A is the first compound of this kind isolated from a myxobacterium and is possibly involved in the iron metabolism of the organism. In the meantime we detected myxochelin A also in cultures of *A. disciformis* strain Ang I1, but with the other 28 tested myxobacteria we could not observe any myxochelin A production.

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