# Ratjadon: A New Antifungal Compound from *Sorangium cellulosum* (Myxobacteria)

## Production, Physico-chemical and Biological Properties†

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An antifungal activity, ratjadon, was detected in the culture broth of *Sorangium cellulosum* (Myxococcales) strain So ce360. The metabolite was quantitatively bound to the adsorber resin XAD-16, which was added to the medium at the beginning of the fermentation. The antibiotic spectrum was narrow, but some important phytopathogenic fungi, especially species of Oomycetes, were inhibited at very low concentrations.

About 400 new isolates of *Sorangium cellulosum* were cultivated in an appropriate culture medium in the presence of 2% of the adsorber resin XAD-16. The concentrated methanol eluates were then screened both biologically by agar diffusion assays and chemically by HPLC, which at the same time gave us retention times of peaks and the diode array spectra of those metabolites that adsorbed light between 200 and 400 nm.

Numerous metabolites were typical *Sorangium* products and appeared quite often in the extracts, while others were only produced by one or a few strains. The combined biological and chemical screening allowed us to eliminate very early those strains, the biological activity of which could be explained by known active metabolites.

Strains So ce360 and So ce464, both active against some fungi, stood out because no known metabolite was detected by HPLC, while at the same time a new, prominent peak was found. This paper deals with the identification and characterization of the biological activity. The responsible compound turned out to be a new structure and was named ratjadon (Fig. 1). The structure elucidation of ratjadon is reported elsewhere[1].

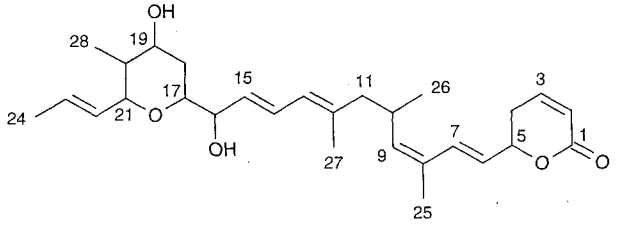
### Microorganism and Culture Conditions

The producing organism, *Sorangium cellulosum* So ce360, was isolated at the GBF in 1989 from a soil sample collected at Cala Ratjada (Mallorca, Spain). Seed cultures on yeast agar were inoculated into 250-ml Erlenmeyer flasks containing 100 ml of medium. The basic medium for growth and production had the following composition (in g/liter distilled water): potato starch (Maizena) 8; glucose (Maizena) 2; $MgSO_4 \cdot 7H_2O$ 1; $CaCl_2 \cdot 2H_2O$ 1; ethylenediaminetetraacetic acid iron(III)-sodium salt 0.008. The pH of the medium was adjusted to 7.2 with KOH before autoclaving. The addition of peptone (4 g/liter) as a nitrogen source was growth-stimulating but inhibited production completely. To obtain good production a polymeric nitrogen source had to be supplied, *e.g.*, soy bean meal 4 g/liter (production medium). In both media So ce360 grew in small lumps, so that growth could not be measured optically or by counting the cell numbers.

### Production

A 100-liter bioreactor (Giovanola Frères, Monthey, Switzerland) with 60 liters of the production medium was inoculated with 10 liters of a 4-day old preculture grown in the same medium in 1-liter Erlenmeyer flasks

Fig. 1. The structure of ratjadon[1].




† Art. No. 69 on antibiotics from gliding bacteria.
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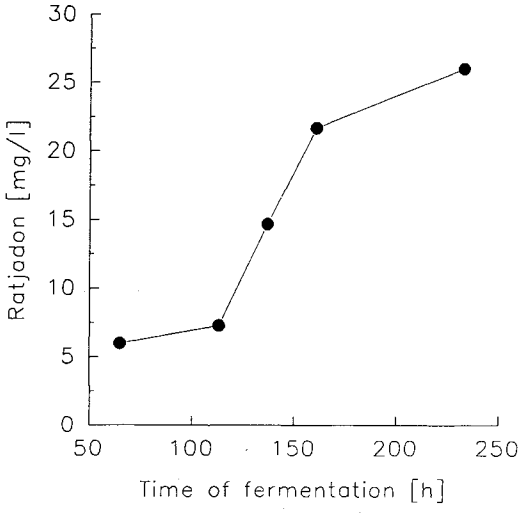
with 500 ml medium under shaking (160 rpm, 30°C). For continuous adsorption of the produced antibiotic, 1 liter of the adsorber resin XAD-16 (Rohm and Haas, Frankfurt/M) was added before autoclaving. To prevent foam formation, 10 ml silicone antifoam (Tegosipon, Goldschmidt AG Essen) was added. The fermentation was run for 10 days at 32°C with an aeration rate of 300 liter air per hour and a stirrer speed of 300 rpm. Ratjadon was excreted into the culture broth during the growth phase and became quantitatively adsorbed to the resin (Fig. 2).

At the end of the fermentation, the adsorber resin was separated from the broth by sieving. After washing it was eluted with five bed volumes of methanol. The extract was concentrated *in vacuo* at 40°C. The isolation of ratjadon by chromatography is described in detail elsewhere[1].

Fig. 2. Production of ratjadon in a 100-liter bioreactor.




Aliquots of the XAD resin were removed from the culture at the time intervals indicated and analyzed for ratjadon.

## Physico-chemical Properties

The antibiotic was analyzed by thin-layer chromatography (Silica gel Si 60 $F_{254}$, Merck, Darmstadt) with the solvent system: dichloromethane-methanol, 95:5. Ratjadon was detected at an Rf value of 0.26 by UV absorption.

HPLC analysis was done on Nucleosil RP-18, 5 $\mu$m (Macherey & Nagel, Düren) using a 2×125 mm microbore column. A methanol-water gradient from 45:65 to 70:30 within 25 minutes and a flow rate of 0.5 ml/minute was used. Ratjadon was detected after 20 minutes by its typical diode array spectrum (Fig. 3).

The IR spectrum of ratjadon in chloroform (Fig. 4) was measured with an FT-IR spectrometer 20 DXB (Nicolet), the $^1H$ NMR spectrum in $CDCl_3$ (Fig. 5) with an AM-400 spectrometer 400 MHz (Bruker, Karlsruhe). Low and high-resolution EI mass spectroscopy gave a molecular ion *m/z* (%) 456.2910 which is in good agreement with the calculated mass 456.2876 for $C_{28}H_{40}O_5$.

## Biological Activity

The antimicrobial spectrum was determined by the paper disk method. While bacteria were not inhibited, some yeasts and several fungi were very sensitive to the antibiotic (Table 1). The minimum inhibitory concentrations (MICs), determined by the serial dilution assay, ranged from 0.04 to 0.6 $\mu$g/ml. The oomycete *Phytophthora drechsleri* was inhibited at 40 ng/ml. Ratjadon was also highly active in animal cell cultures. The $IC_{50}$ for the mouse cell line L929 was 50 pg/ml, and for the HeLa cell line KB3.1 it was 40 pg/ml.

Fig. 3. HPLC run of an XAD-eluate from So ce360, and the diode array spectrum of ratjadon.

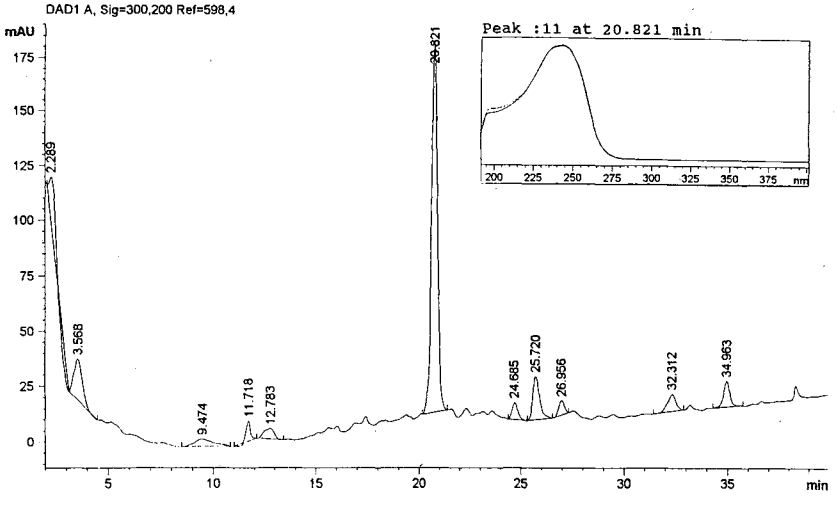


Fig. 4. IR spectrum of ratjadon in chloroform.

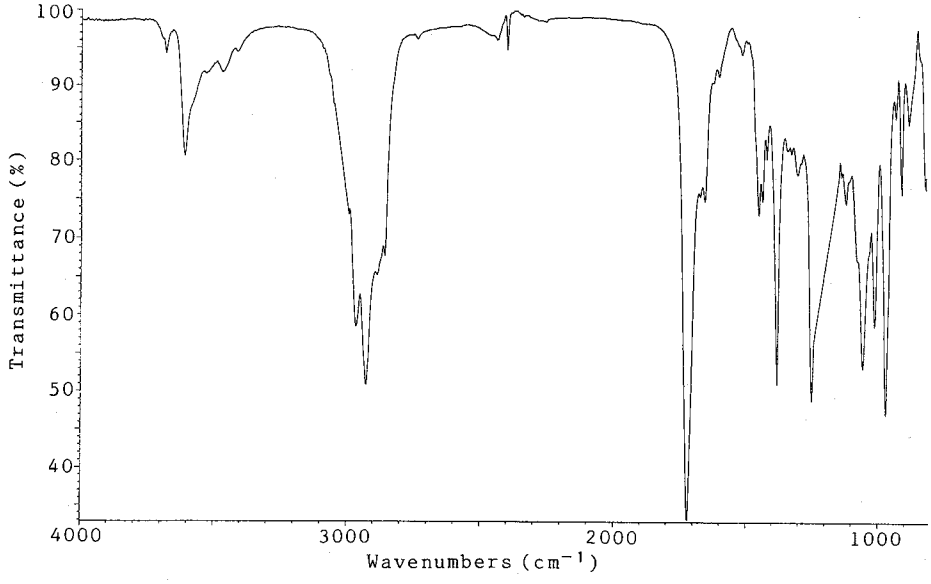
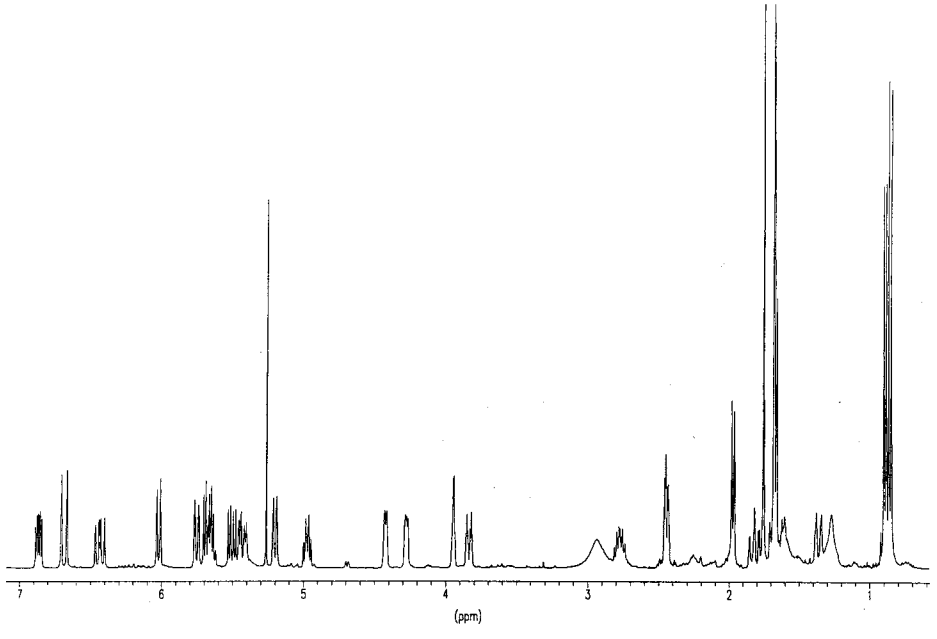



Fig. 5. $^1H$ NMR spectrum of ratjadon in $CDCl_3$.




First investigations into the mechanism of action were done with the yeast *Schizosaccharomyces pombe*. Growth, measured as an increase of optical density at 623 nm, was drastically reduced in the presence of ratjadon, but a complete inhibition was not obtained within 5 hours of incubation. An increase of the ratjadon concentration from 100 to 500 ng/ml did not improve the inhibitory effect. However, ratjadon caused striking changes in yeast morphology. The cells became elongated and sometimes began to branch like a fungal mycelium. The influence on the basic metabolism was investigated by studying the incorporation of radioactively labeled precursors into high molecular weight material. The incorporation of radioactive protein hydrolysate, of thymidine, adenine, acetate and glycerol declined in the presence of 0.5 $\mu$g ratjadon per ml, but was not completely stopped within 8 hours.

## Discussion

So ce360 with its very narrow antifungal activity is the first strain of *Sorangium cellulosum* which was selected

Table 1. Antifungal spectrum of ratjadon.

| Test organism | Diameter[a] of inhibition zone (mm) | MIC (μg/ml) |
|---|---|---|
| *Pythium debaryanum* DSM[b] 62946 | 30 | 0.3 |
| *Phytophthora drechsleri* DSM 62679 | 30 | 0.04 |
| *Absidia glauca* CBS[c] 100.59 | 35 | 0.3 |
| *Mucor hiemalis* DSM 2655 | 30 | 0.09 |
| *Cunninghamella echinulata* DSM 1905 | — | |
| *Nematospora coryli* CBS 2608 | — | |
| *Schizosaccharomyces pombe* Tü[d] 501 | 38 | 0.09 |
| *Nadsonia fulvescens* CBS 2596 | — | |
| *Hansenula anomala* DSM 70130 | — | |
| *Pichia membranaefaciens* DSM 70366 | — | |
| *Saccharomyces cerevisiae* BT 27C-2A YGSC[e] | — | |
| *Taphrina deformans* DSM 4398 | 20 | |
| *Aspergillus clavatus* CBS 121.45 | 8 | |
| *Penicillium capsulatum* CBS 301.48 | — | |
| *Ceratocystis ulmi* BBA[f] 686 | 10 | 0.6 |
| *Alternaria solani* DSM 2954 | 10 | 0.6 |
| *Chaetomium cochliodes* DSM 1909 | 10 | |
| *Fusarium oxysporum* DSM 2018 | — | |
| *Ascobolus immersus* DSM 968 | — | |
| *Cladosporium resinae* DSM 63423 | — | |
| *Botrytis cinerea* DSM 877 | — | |
| *Sclerotina sclerotiorum* DSM 1946 | — | |
| *Monilia brunnea* DSM 1362 | 40 | 0.3 |
| *Tilletia caries* DSM 4526 | — | |
| *Sporidiobolus ruineniae* DSM 3453 | — | |
| *Psilocybe montana* CBS 703.20 | 38 | 0.08 |
| *Candida albicans* CBS 1893 | — | |
| *Rhodotorula glutinis* DSM 70398 | — | |
| *Trichosporon terrestre* CBS 66.97 | — | |
| *Sporobolomyces holsaticus* DSM 70580 | — | |

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

by us for the isolation of a metabolite by a chemical screening because of an unknown diode array spectrum of a prominent peak. The compound with the unusual UV-spectrum, ratjadon, proved indeed identical with the antibiotic activity. Ratjadon, a typical polyketide with a molecular weight of 456, is synthesized by head to tail condensations of acetate and propionate units[1].

While the right part of the antibiotic (C-1 to C-11) is identical with the left part of the antitumor antibiotic anguinomycin A (C-13 to C-24)[2], the rest of the molecule is completely different. In addition to the structural similarities between ratjadon, the anguinomycins and the closely related leptomycins[3] and kazusamycin[4], all those compounds resemble each other strikingly in their biological effects. While prokaryotes are not inhibited at all and the antifungal spectrum is narrow, the yeast *S. pombe* is very sensitive and the cytotoxicity is high[5,6]. At low concentrations of ratjadon (5 times the MIC) no significiant effect on the basic metabolism of *S. pombe* could be observed. In parallel to growth inhibition, the intermediary metabolism slowly declined. A similar behavior of *S. pombe* was described for the leptomycins[7]. An effect of leptomycin on DNA synthesis was observed only at very high concentrations (100 times the MIC)[7]. Recently, the molecular action of leptomycin B was elucidated[8]. It inhibits the function of the *crm-1-809* gene which is required for maintaining higher order chromosome structure, for correct gene expression, and for cell growth in the fission yeast. The strong *in vitro* effect of ratjadon against some Oomycetes, which are of special phytopathological interest, have not been published for the structurally related compounds.

Acknowledgments

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## References

1) Schummer, D.; K. Gerth, H. Reichenbach & G. Höfle: Ratjadon: A new antifungal metabolite from *Sorangium cellulosum*. Liebigs Ann. Chem. 685~688, 1995
2) Hayakawa, Y.; K. Adachi & N. Komeshima: New antitumor antibiotics, anguinomycins A and B. J. Antibiotics 40: 1349~1352, 1987
3) Hamamoto, T.; H. Seto & T. Beppu: Leptomycins A and B, new antifungal antibiotics II. Structure elucidation. J. Antibiotics 36: 646~650, 1983
4) Komiyama, K.; K. Okada, H. Oka, S. Tomisaka, T. Miyano, S. Funayama & I. Umewzawa: Structural study of a new antitumor antibiotic, kazusamycin. J. Antibiotics 38: 220~223, 1985
5) Hamamoto, T.; S. Gunji, H. Tsuji & T. Beppu: Leptomycins A and B, new antifungal antibiotics I. Taxonomy of the producing strain and their fermentation, purification and characterization. J. Antibiotics 36: 639~645, 1983
6) Umezawa, I.; K. Komiyama, H. Oka, K. Okada, S. Tomisaka, T. Miyano & S. Takano: A new antitumor antibiotic, kazusamycin. J. Antibiotics 37: 706~711, 1984
7) Hamamoto, T.; T. Uozumi & T. Beppu: Leptomycins A and B, new antifungal antibiotics III. Mode of action of leptomycin B on *Schizosaccharomyces pombe*. J. Antibiotics 38: 1573~1580, 1985
8) Nishi, K.; M. Yoshida, D. Fujiwara, M. Nishikawa, S. Horinouchi & T. Beppu: Lepotomycin B targets a regulatory cascade of crm1, a fission yeast nuclear protein, involved in control of higher order chromosome structure and gene expression. J. Biol. Chem. 269: 6320~6324, 1994