Chivosazol A, a New Inhibitor of Eukaryotic Organisms Isolated from Myxobacteria†

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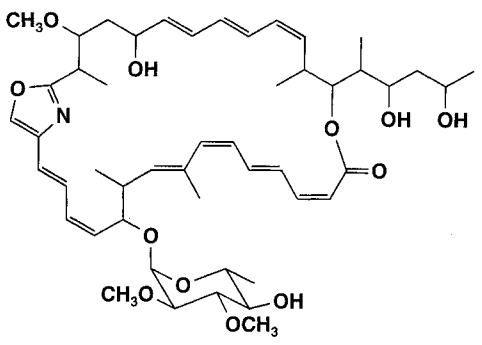
A new antibiotic, chivosazol, was isolated from the culture broth of the myxobacterium *Sorangium cellulosum* strain So ce12. It is a macrocyclic ring with one oxazol ring and a glycosidically bound 6-deoxyglucose (quinovose) at C-11. The antibiotic shows antimicrobial activity against yeasts and filamentous fungi, and is especially potent against mammalian cells. It was not active against bacteria.

In addition to sorangicin and disorazol, we found a third substance in the XAD extract of fermentation broths of *Sorangium cellulosum* strain So ce12[1,2,4,5]. It was a macrocyclic ring with one oxazole ring and a quinovose (Fig. 1). We propose the name chivosazol. In this paper we report on the discovery, production and biological properties of this new compound.

Occurrence and Production

Chivosazol was first discovered in one of the early strains of *Sorangium cellulosum* that have been screened by us. Later it was found that 9% of the strains of that species (206 strains investigated) produce this compound. When the process was optimized for sorangicin production, synthesis of chivosazol was also observed.

Fig. 1. The chemical structure of chivosazol A.



Chivosazol A

Beside this main component, 5 structural variants have been elucidated. They differ from each other in the number of methoxy-groups; one is a structural isomer differing in the position of a double bond.

The substance was produced particularly under fed-batch conditions, as described earlier[5]. Table 1 gives the yields under different fermentation conditions. Without the adsorber resin XAD-16 (Rohm and Haas, Darmstadt, Germany) in the culture broth, production was only 40% of that with XAD-16, and more than 80% of the antibiotic was found in the supernatant. In the presence of XAD-16 nearly all of the substance was bound to the resin. XAD-16 is a polystyrol-based neutral adsorber resin. Nonpolar substances are bound to it by hydrophobic interactions and are thus removed from the culture broth. This often leads to a stabilization of substances and prevention of feed back inhibitions of biosynthesis.

Isolation and Physico-chemical Data

Chivosazol (Fig. 1) was discovered and detected by HPLC with diode array detection in fractions of the XAD-16 extract which still showed biological activity after the main components, sorangicin and the di-

Table 1. Production of chivosazol by *Sorangium cellulosum* So ce12[a].

Culture conditions	Average production rate (mg/liter and day)	Maximal yield (mg/liter)
+XAD 16[b], without fed batch		2
+XAD 16, with fed batch	3	31
−XAD 16, with fed batch	n.t.	13

[a] Medium and fed-batch strategy were as described[5].
[b] Amberlite adsorber resin (Rohm and Haas).

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sorazoles had been removed[4]. It was purified by HPLC, and its structure[3] was elucidated mainly by 2D NMR spectroscopy (JANSEN *et al.*, in preparation). Figs. 2 and 3 show the UV absorption spectrum in methanol and the IR spectrum in KBr. The molecular weight is 865.

Biological Properties

Chivosazol was active against yeasts and at higher concentrations some filamentous fungi (Table 2). The antibiotic was inactive against bacteria, but it was rather toxic to animal cell cultures (Table 3). The effect of chivosazol was fungicidal as shown with *Saccharomyces cerevisiae* (Fig. 4). An overnight culture was adjusted to 7.9×10^6 cells/ml with nutrient broth. Then the culture was divided and different concentrations of the antibiotic were added to the aliquots. Incubation was at 30°C under

Fig. 2. Electronic absorption spectrum of chivosazol in methanol.

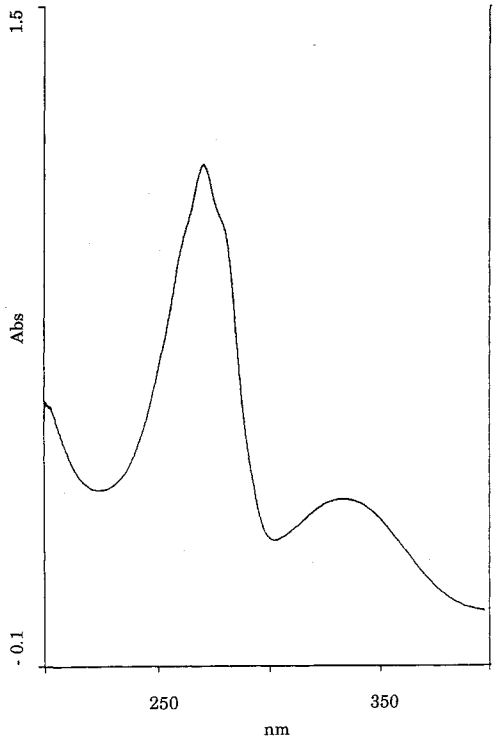


Table 2. Antimicrobial spectrum of chivosazol[a].

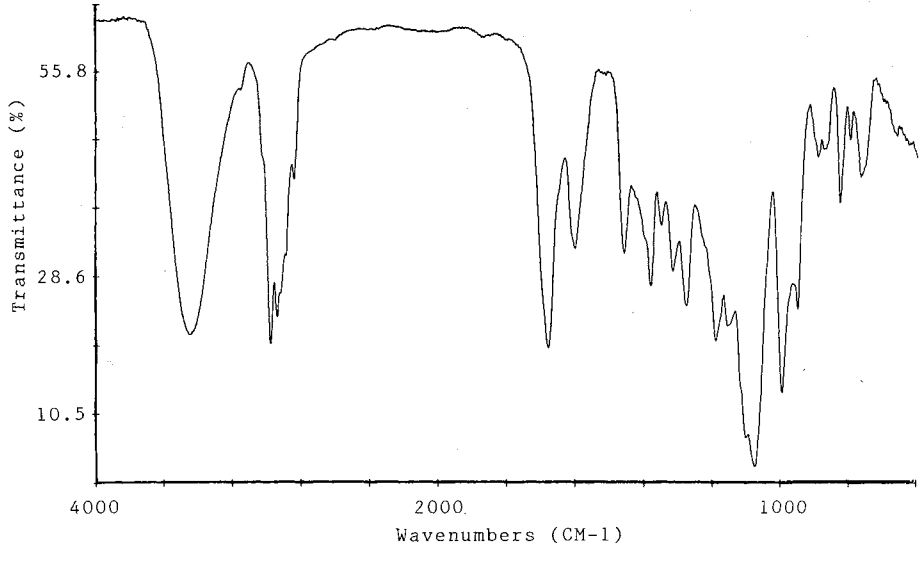
Test organism	Diameter of inhibition zone[b] (mm)	MIC (μg/ml)
Mucor hiemalis	10	10
Rhodotorula glutinis	10	10
Aspergillus niger	10	
Pythium debaryanum	0	
Botrytis cinerea	12	
Fusarium oxysporum	0	
Rhizopus arrhizus	9	
Sclerotina sclerotiorum	10	
Ustilago maydis	13	0.17
Saccharomyces cerevisiae	16	0.3
Candida albicans	17	0.6
Debaryomyces hansenii	13	0.25
Hansenula anomala	17	0.125
Bacillus subtilis	0	
Staphylococcus aureus	0	>50
Micrococcus luteus	0	
Streptococcus faecalis	0	
Escherichia coli	0	
Salmonella typhimurium	0	

[a] Test conditions as described in[1].
[b] 6 mm disk diameter, 2 μg chivosazol per paper disc.

Table 3. Cytostatic effect of chivosazol on mammalian cell lines.

Cell line	MIC (μg/ml)
Mouse fibroblast cells L 929	9×10^{-3}
HeLa cells ATCC CCL 2	9×10^{-3}

Fig. 3. IR spectrum of chivosazol in KBr.



shaking. After various time intervals the suspensions were plated. At 2 times the MIC of chivosazol, the number of viable cells was reduced to about 10% after 7 hours, while at 10 times the MIC only 0.2% of cells were viable. The colonies that survived antibiotic treatment were not resistant to the compound. The effect of chivosazol on the synthesis of some cellular macromolecules is shown in Figs. 5A to 5D. An overnight culture was adjusted to an OD_{623} of 0.4 with MYC medium and then incubated until an OD of 0.8 was reached. This culture was used for the incorporation of different radioactive precursors. The antibiotic was added at time zero. Incorporation of methionine (Fig. 5A), N-acetylglucosamine (Fig. 5B), uracil into perchloric acid insoluble material after NaOH digestion[6] (Fig. 5C) and uracil (Fig. 5D) without digestion was only slightly affected within the first hours. To see whether chivosazol had an effect on the membranes of yeasts, *Saccharomyces cerevisiae* cells were washed and resuspended in 50 mM phosphate buffer, pH 7.4. When chivosazol was added, UV absorbing material (260 and 280 nm) was slowly released into the medium (Fig. 6). On the other hand, sheep erythrocytes were not lysed by chivosazol (10 μg/ml). In nutrient broth[5] the

Fig. 4. Influence of chivosazol on the viability of *Saccharomyces cerevisiae* in nutrient broth.

● With 2 × MIC, ○ with 10 × MIC, × control.

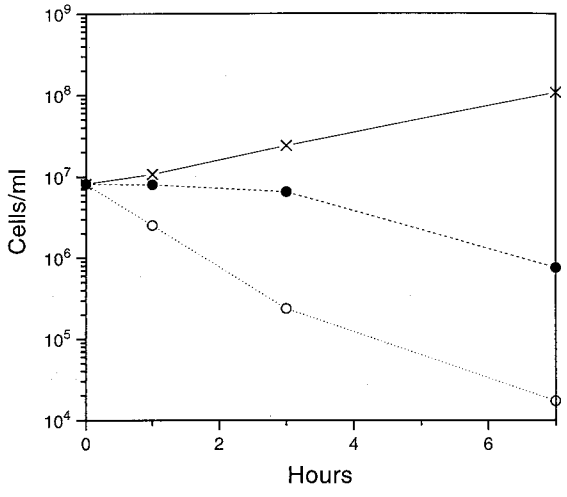
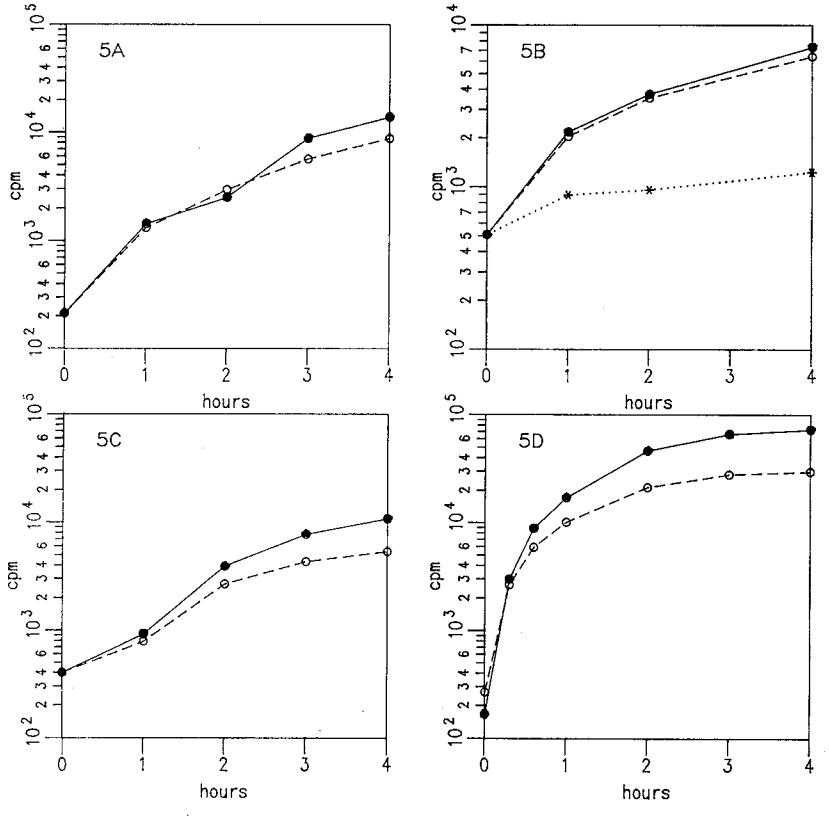


Fig. 5. Effect of chivosazol on the syntheses of biomacromolecules by *Saccharomyces cerevisiae*.

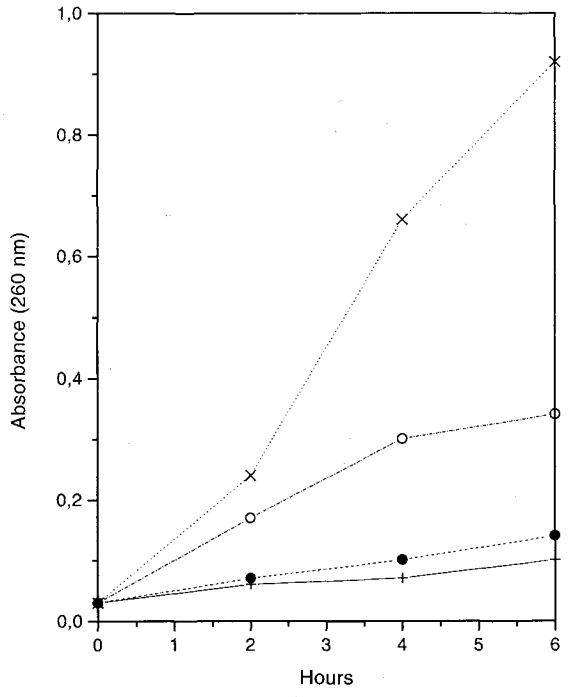


Closed circles: control; open circles: with 5 μg chivosazol/ml; stars: with 250 μg 2-deoxyglucose/ml. A: Protein synthesis, measured as incorporation of [methyl-^{14}C]-methionine (specific activity 55 Ci/mol, Amersham) into perchloric acid (PCA) insoluble material. The precipitated cells were collected on glas fiber filters, Whatman GF/A. Radioactivity was determined in a Beckman liquid scintillation counter LS 1801. B: Cell wall synthesis, measured as incorporation of N-acetyl-D-[1-^{14}C]-glucosamine (specific activity 56 Ci/mol, Amersham) in PCA insoluble material. C: DNA synthesis, measured as incorporation of [2-^{14}C]uracil (specific activity 52 Ci/mol, Amersham) into PCA insoluble material after digesting the RNA with 2N NaOH overnight[6]. D: RNA synthesis, measured as incorporation of [2-^{14}C]uracil (specific activity 52 Ci/mol, Amersham) into PCA insoluble material.

morphology of growing yeast cells changed in the presence of chivosazol. Some cells became dark under the phase contrast microscope and the cell envelope wrinkled, and many cells had burst at one of the cell poles (Fig. 7). This effect could be seen at the earliest 6 hours after the addition of chivosazol depending on the concentration of the antibiotic. On the other hand, the morphology of cells that were suspended in buffer could not be distinguished from that of the control cells. Spores of *Mucor hiemalis* suspended in MYC medium[5] also became dark and wrinkled but did not burst. The germination of the spores was inhibited.

Fig. 6. Effect of chivosazol on permeability of the cell membrane of *Saccharomyces cerevisiae*, measured as efflux of UV absorbing material into the supernatant.

● 1.2 mg chivosazol/liter, ○ 10 mg chivosazol/liter, × 10 mg amphotericin/liter, + control.

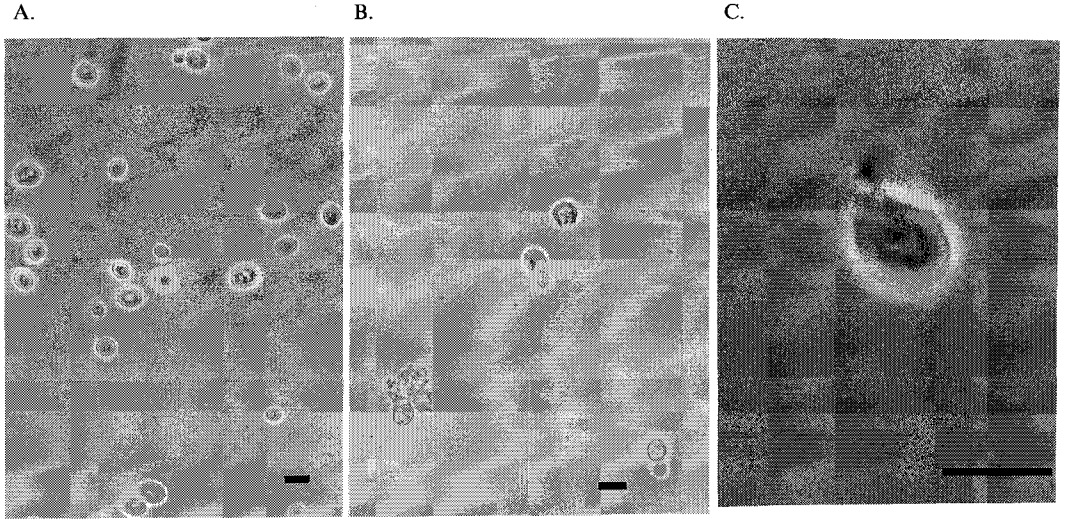


Discussion

Sorangium cellulosum strain So ce12 synthesizes at the same time four different antibiotics, sorangicin[1], disorazol[4,5], sorangiolid and chivosazol[3]. Chivosazol is a novel macrocyclic compound with an oxazol ring and a 6-deoxy-glucose (quinovose) at C-11. Since then, many more strains have been found to produce this metabolite, and in most cases together with other bioactive substances, although, usually not in the same combination as strain So ce12. The level of production of chivosazol by strain So ce12 was low compared to that of sorangicin and disorazol[5]. In contrast to disorazol, chivosazol synthesis was not inhibited by barbital[5]. Suppression of disorazol production by barbital did not lead to a substantial increase in sorangicin or chivosazol yields (data not shown). Chivosazol was active against yeasts and filamentous fungi, but much more so against animal cell cultures (Tables 2 and 3). Studies on syntheses of macromolecules in *Saccharomyces cerevisiae* showed that none of those tested was quickly or significantly inhibited. The antibiotic showed an effect on the stability and integrity of the cell membrane (Figs. 6 and 7). An effect on the morphology of yeast cells could only be seen with growing cells (Fig. 7), and not with cells suspended in buffer. As the cells were always disrupted only at one pole, chivosazol may act on the septum of dividing cells. In contrast, resting cells became simply permeable (Fig. 6). Similar effects as observed with

Fig. 7. Effect of chivosazol on the morphology of *Saccharomyces cerevisiae*.

Bar = 10 μm.



The yeasts were cultivated in MYC medium[5] for 18 hours with 3 μg chivosazol/ml. A: Control without chivosazol. B, C: Cells after chivosazol treatment.

chivosazol have been described by JOHNSON[7] for 2-deoxyglucose. But contrary to chivosazol, 2-deoxyglucose inhibits the incorporation of N-acetylglucosamine and lyses only growing cells. N-acetylglucosamine is the monomer of chitin that is located in the bud scar[8,9]. It is also a constituent of yeast mannan[10,11]. Our results indicate that chivosazol may act on the integrity of the yeast cell wall by interfering at a specific site in the envelope. This site may be situated within the budding area. If there would be an unspecific detergent effect, one would expect firstly, that the efflux of UV absorbing material would be greater than measured (Fig. 6), and secondly, that the synthesis of macromolecules would be inhibited to a greater extend than detected (Fig. 5). Furthermore, erythrocytes were not lysed at 10 μg/ml of chivosazol, even though this compound was highly toxic to animal cells. It appears that normal and transformed cells do not differ in their sensitivity to chivosazol.

Acknowledgment

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