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Isolation and identification of the principal siderophore of the plant pathogenic fungus *Botrytis cinerea*

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Summary. The plant pathogenic hyphomycete *Botrytis cinerea* has been shown to produce several trihydroxamate siderophores under conditions of low-iron stress. The total siderophores amounted to approximately 30 mg/l culture filtrate after 5 days of incubation in an asparagine/salt/glucose medium. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) on a reversed phase indicated that ferrirhodin is the predominant siderophore of this fungus. Chemical characterization of the principal siderophore by fast-atom-bombardment (FAB) mass spectrometry, nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) and comparison with a reference revealed the identity with ferrirhodin. NMR studies performed on desferri-ferrirhodin (desferrirhodin) in dimethylsulfoxide and water revealed the existence of two conformers in D_2O resulting from a *cis-trans* isomerization of the hydroxamic acid groups. Comparative iron-uptake studies showed the following order of uptake in *B. cinerea:* ferrichrysin (100%), ferrirubin (57%), ferrirhodin (45%), hexahydroferrirhodin (45%), coprogen 6%. Concentration-dependent uptake of ferrirhodin resulted in saturation kinetics only in the low concentration range of 0–30 μM ($K_m = 2.5$ μM, $V_{max} = 80$ pmol min^{-1} mg^{-1}). A non-saturable, linear uptake was observed in the high concentration range of 30–80 μM. The low concentration range appears to be the physiologically significant range, where siderophore-mediated iron transport in *B. cinerea* occurs.

Key words: Siderophore identification — Ferrirhodin — *Botrytis cinerea* — NMR studies — Transport

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Introduction

The grey mould *Botrytis cinerea* is a common plant pathogen causing infections of all kinds of plant surfaces, crops, fruits and seedlings. Field and glasshouse vegetables, apples, small berry fruits, strawberries, raspberries and grapes become fairly often affected, when the humidity is high. Rapid invasion occurs in damaged and undamaged leaves and fruits.

The present stage of knowledge of the biology of *Botrytis* is summarized in a comprehensive treatise (Coley-Smith et al. 1980). According to the latest taxonomic treatment, the conidiospore-producing mould of the form genus *Botrytis* is regarded as the imperfect state (anamorph) of the perfect or sexual state (teleomorph) of *Botryotinia*. Many isolates of *Botrytis cinerea* strains are probably the imperfect states of *Botryotinia fuckeliana*.

Although the conidia of *B. cinerea* can survive fairly well in the field, the sclerotia are considered to be the principal survival structures. Sclerotia usually overwinter and produce reproductive structures such as conidia during the following spring and summer which then give rise to a further spread of infections. Mycelial development in infected tissues seems to proceed after direct penetration of germ tubes from conidia as well as from swollen tips or branched hyphal structures which are then called appressoria. The final death of the host cell is assumed to be caused by cell-wall-degrading enzymes in connection with the production of toxic compounds.

Although it is well known that siderophore-mediated iron transport occurs in a variety of saprophytic and parasitic fungi (Winkelmann and Huschka 1987), no information is so far available on the iron-uptake routes in *B. cinerea*. In the

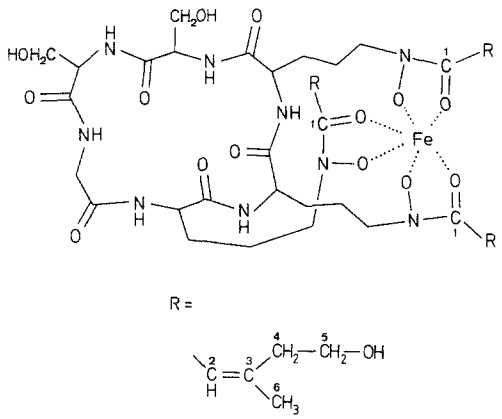

Fig. 1. Structural formula of ferrirhodin with *cis*-anhydromevalonyl residues numbered for NMR assignment

present paper we describe the isolation and chemical characterization of ferrirhodin (see Fig. 1) as the principle siderophore of *B. cinerea* and investigate its role in iron transport in the producing cells.

Materials and methods

Strains and cultivation conditions. The siderophore-producing strain of *Botrytis cinerea* B-1 was isolated from roses and was found to have a more intense coloured aerial mycel than most other laboratory strains. However, a later screening for siderophores in strains from the culture collection of our institute revealed that all strains studied produced nearly the same siderophore pattern with ferrirhodin as the principle siderophore. Strains were grown on agar slants containing yeast extract (0.4%), malt extract (1%) and glucose (0.4%). The same agar medium was used for the production of conidiospores which were harvested after at least 4 weeks of growth at room temperature.

Siderophores. Ferrichrysin was isolated from *Aspergillus viridinutans* CBS 127.56 (Diekmann and Krezdorn 1975). Ferrirubin was kindly provided by W. Keller-Schierlein, ETH Zürich. Hexahydroferrirhodin was prepared by hydrogenation of ferrirhodin with10% Pd on charcoal according to Keller-Schierlein (1963) and the purity was checked by TLC (Konetschny-Rapp et al. 1988). $^{55}FeCl_3$ in 0.1 M HCl (carrier-free) was purchased from Amersham International, England.

Siderophore production. The siderophore production medium contained per liter: L-asparagine (2.5 g), $K_2HPO_4 \times 3H_2O$ (1 g), $MgSO_4 \times 7H_2O$ (1 g), $CaCl_2$ (0.5 g), $ZnSO_4$ (20 mg) with glucose (20 g), autoclaved separately. The pH was adjusted to 6.0 prior to autoclaving at 121° C for 15 min. Conidiospores harvested from two 500-ml conical flasks each containing 100 ml YMG-agar were suspended in 10 ml of a 0.9% NaCl solution containing 1 drop of Tween 80 and used to inoculate a 100-ml preculture. After 2 days of growth, the preculture was transferred to 1.5-l production medium and incubated on a rotary shaker at 22° C for 7 days.

Isolation of siderophores. After filtration of the culture, $FeSO_4 \times 7H_2O$ was added to the culture filtrate and the solution was stirred until the typical brown colour of the hydroxamate siderophores had developed. The siderophores were adsorbed on an XAD-2 column (Serva, Heidelberg, FRG), washed with two volumes of distilled water and desorbed with one volume of methanol. The methanol eluate was evaporated to dryness, redissolved in methanol and separated by column chromatography on silica gel 60, 0.063–0.200 mm (Merck, Darmstadt, FRG) using chloroform/methanol/water (70:24:4) and further purified by gel filtration on Sephadex LH20 (Pharmacia, Freiburg, FRG). Final purification was achieved by semipreparative HPLC on a Nucleosil RP-18 column (250 × 8 mm internal diameter) with precolumn (Grom, Ammerbuch, FRG) using an isocratic elution with acetonitrile/water (20:80, *v/v*). Iron removal was achieved by the 8-hydroxyquinoline method as described earlier (Wiebe and Winkelmann 1975; Wong et al. 1983).

Amino acid analysis. Samples (1 mg) of the desferri-siderophore were hydrolysed with 6 M HCl (0.5 ml) and with 57% HI (0.1 ml) in sealed vials at 110 °C for about 12 h. The amino acid analysis of the total hydrolysates were performed by ion-exchange chromatography (LKB Biochrom 4150 Amino acid analyser). The absolute configuration of the amino acids was determined by gas chromatography (Frank et al. 1977) on Duran glass capillary column, coated with Chirasil-Val (Siemens Sichromat 1, Siemens Autosampler 200, C.A.T. Autoderivat 100, Spectra Physics SP 4270 Data System). Trifluoroacetylated amino acid *n*-propyl esters were obtained by esterification of the total hydrolysates (100 µmol) with 2 M HCl- propanol (0.5 ml) at 110° C for 30 min followed by acylation with trifluoroacetic anhydride (0.2 ml) at 150° C for 10 min.

High-performance liquid chromatography. Analytical HPLC studies were carried out on an RP-18 column according to the procedures described earlier (Konetschny-Rapp et al. 1988).

Thin-layer chromatography. TLC was performed on precoated plates of silica gel 60 (Merck, Darmstadt, FRG) using chloroform/methanol/water (70:24:4) as a solvent system.

Spectroscopic measurements. NMR spectra were recorded on a Bruker WM 400 instrument at 400.13 MHz (^{1}H-NMR) and 100.6 MHz (^{13}C-NMR). As solvents D_2O, $(^{12}CD_3)_2SO$ and $^{12}CD_3OD$ were used. Assignments were made by comparison with known ferrichromes (Llinas et al. 1977; Jalal et al. 1983; Jalal et al. 1985) and confirmed by *J*-modulated spin-echo experiments.

Absorption spectra. A Lambda 5 UV/VIS spectrophotometer (Perkin Elmer) was used ($c = 0.05$ mmol/l, methanol) for recording the ultraviolet absorption spectra. Circular dichroic spectra were measured over 200–600 nm on a Jasco-J-20A dichrograph ($c = 0.1$ mmol/l, water, pH 7.0).

Positive FAB spectra were obtained on a Varian-MAT 711 instrument coupled with an SS 200 data system. The FAB mass spectra were measured in a glycerol matrix. Temperature of the ion source was 30° C.

Transport studies. Transport of [^{55}Fe]ferrirhodin and several other ^{55}Fe-labelled siderophores was carried out using young mycelia of *B. cinerea* grown for 48 h from conidia in an asparagine/salt/glucose medium. The mycelia were washed with two volumes of fresh medium by repeated sedimentation. ^{55}Fe-labelled siderophores were prepared by mixing unlabelled siderophores (1 µmol/ml) with desferri-siderophores (10 nmol) and [^{55}Fe]Cl_3 (10 nmol; 2.5 MBq). Time-dependent

assays were performed with washed mycelial suspensions in 20 ml asparagine medium plus glucose (1%) and the addition of 200 µl [^{55}Fe]ferrirhodin or [^{55}Fe]ferrirubin (2.6 MBq each). Samples of 1 ml were taken at intervals and filtered through cellulose nitrate filters (8 µm, SM 113, Sartorius, Göttingen, FRG). The radioactivity of the filters was measured in a liquid scintillation counter (Kontron MR 300). The assays for measuring concentration-dependent uptake assays contained 800 µl cell suspension and 200 µl ^{55}Fe-labelled siderophores (10–80 nmol) covering a concentration range of 1–80 µM. After an incubation period of 5 min the mycelia were filtered, washed with fresh medium and the radioactivity counted in a liquid scintillation counter. The amount taken up was expressed as nmol/mg dry mass.

Results and discussion

In the present investigation we have shown that ferrirhodin is the principal siderophore of *Botrytis cinerea*. The producing strain was isolated from plant material (roses). However, we also confirmed that several of our laboratory strains which we routinely use for the detection of antifungal antibiotics are likewise able to synthesize ferrirhodin in a glucose/asparagine/salt medium. The production of siderophores by *B. cinerea* is accompanied by a rapid acidification of the medium with pH < 4.

The pattern of siderophores produced by *B. cinerea* is shown in Fig. 2. Besides the main siderophore ferrirhodin (retention time 39.64 min, $R_F = 0.38$) several minor peaks were detected which could not be assigned to any known fungal siderophores. The total amount of siderophores produced in a glucose/asparagine/salt medium was approximately 30 mg/l. Purification of ferrirhodin was achieved by column chromatography on silica gel, Sephadex LH20 and on a semipreparative RP-18 column by HPLC. From the absorption spectrum of the purified siderophore (Fig. 3) it was deduced that the isolated siderophore contained three anhydromevalonic acid residues ($A_{252\,nm}/A_{436\,nm} = 0.16$; Konetschny-Rapp et al. 1988).

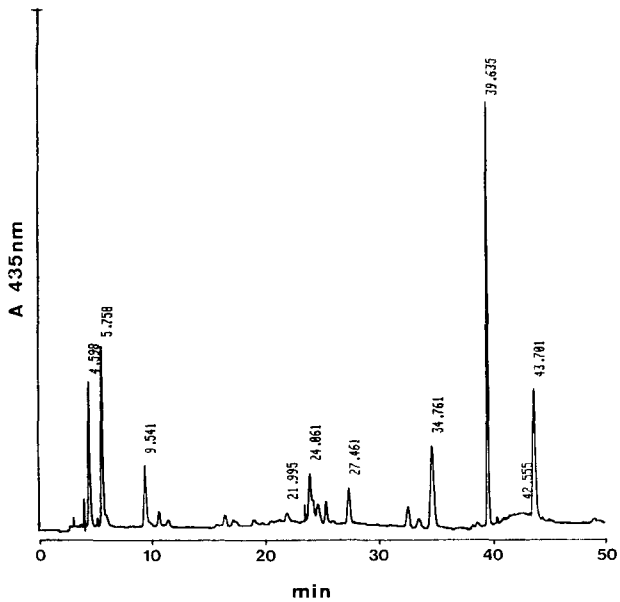

Fig. 2. HPLC separation of the total siderophores of *B. cinerea* on a Nucleosil C_{18} column (250 × 4.6 mm internal diameter) using a gradient of 10 mM phosphate buffer, pH 3 and acetonitrile using an elution program as described earlier (Konetschny-Rapp et al. 1988). *Flow rate:* 1 ml/min, *detector wavelength:* 435 nm. The main peak with a retention time of 39.635 min represents ferrirhodin. No other peaks could be identified

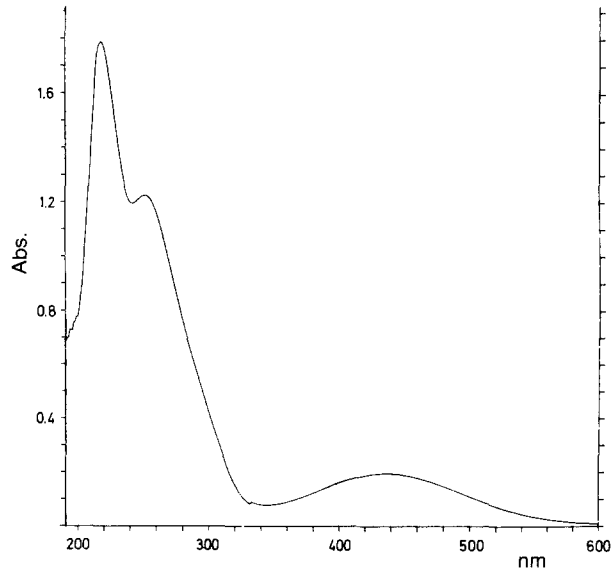

Fig. 3. Absorption spectrum of ferrirhodin recorded in methanol ($c = 0.05$ mol/l)

Hydrolysis of the iron-free main siderophore with 6 M HCl yielded a Gly/Ser/Ala/Orn ratio of 1.0:1.82:0.30:0.36, whereas hydrolysis with 57% HI revealed a ratio of 1.0:1.09:0.73:3.34 indicating the presence of ornithine N^{δ}-hydroxamate groups. The configuration of Ser and Orn was determined as L, Ala appeared racemically and seemed to arise from serine during hydrolysis (Keller-Schierlein 1963). Therefore, the amino acid composition was assumed to be Gly/Ser/Orn in a ratio of 1:2:3 which corresponded to the ferrichrysin-type of siderophores including ferrirhodin and ferrirubin.

Positive FAB spectroscopy of the iron-free siderophore (Fig. 4) revealed a molecular ion peak at m/z 958 corresponding to $[M+H]^+$ and an additional peak at m/z 942 which was assigned to $[M+H-O]^+$, a characteristic fragment of hydroxamate compounds. The FAB spectrum of the

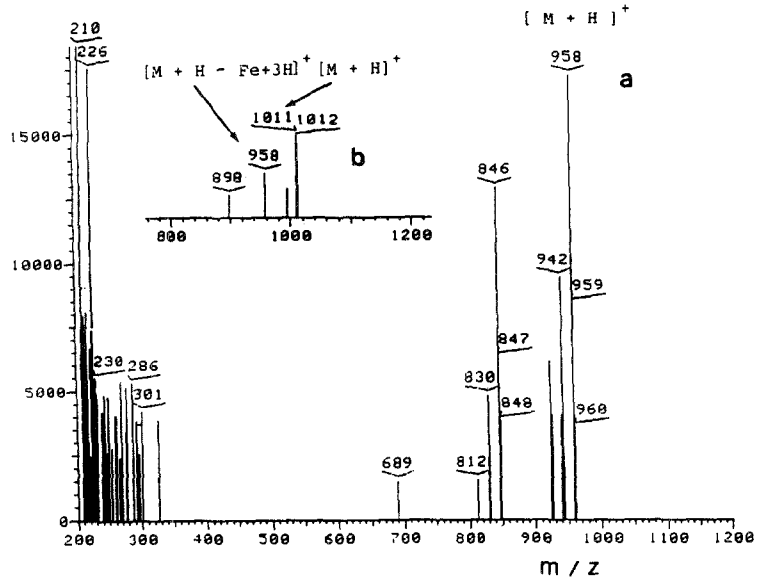

Fig. 4. Positive fast-atom-bombardment mass spectra of **(a)** desferrirhodin (iron-free) with a molecular mass peak $[M+H]^+$ at 958 Da and **(b)** ferrirhodin *(insert)* with a molecular mass peak $[M+H]^+$ at 1011 Da and a characteristic peak at 958 Da assigned to the iron-free ligand $[M+H-Fe+3H]^+$

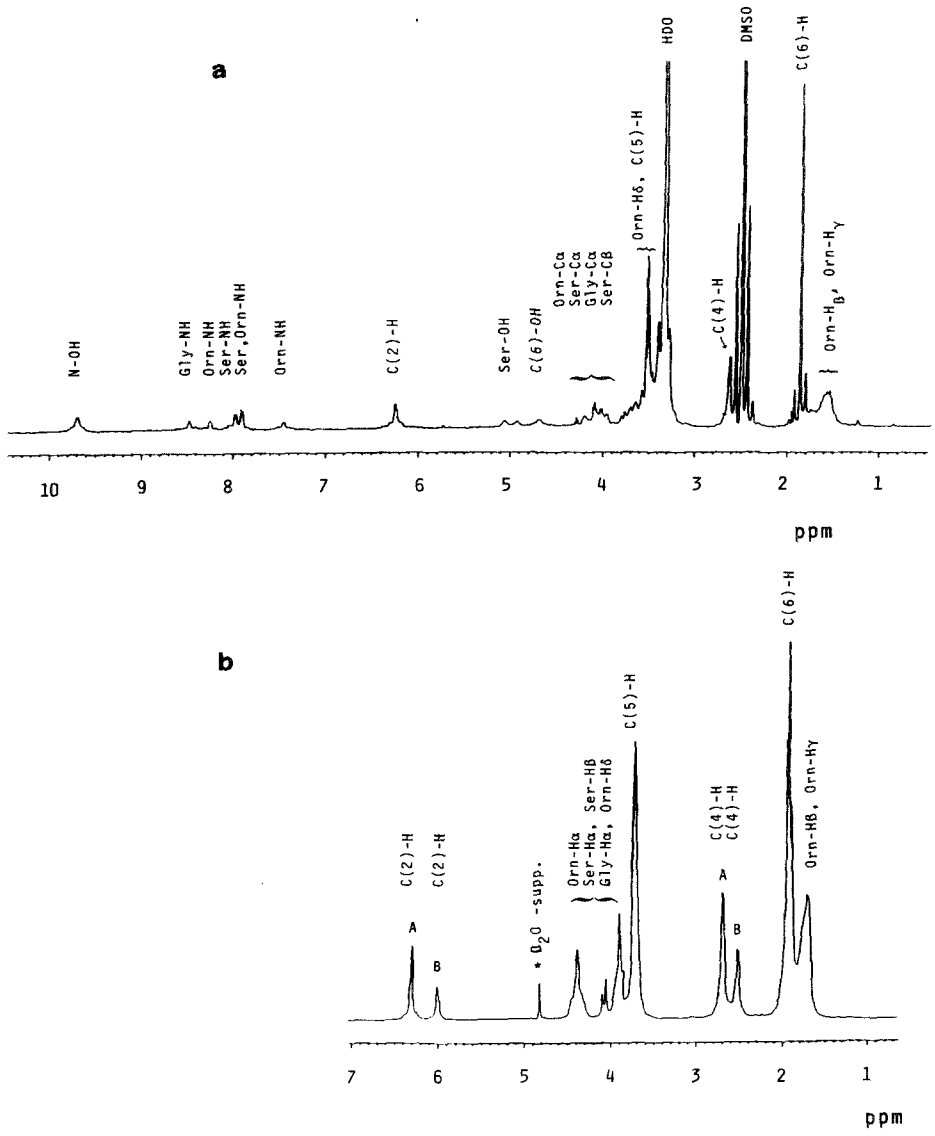

Fig. 5a, b. ^{1}H-NMR spectra of desferrirhodin **(a)** in $(CD_3)_2SO$ and **(b)** in D_2O. For assignment see Table 1

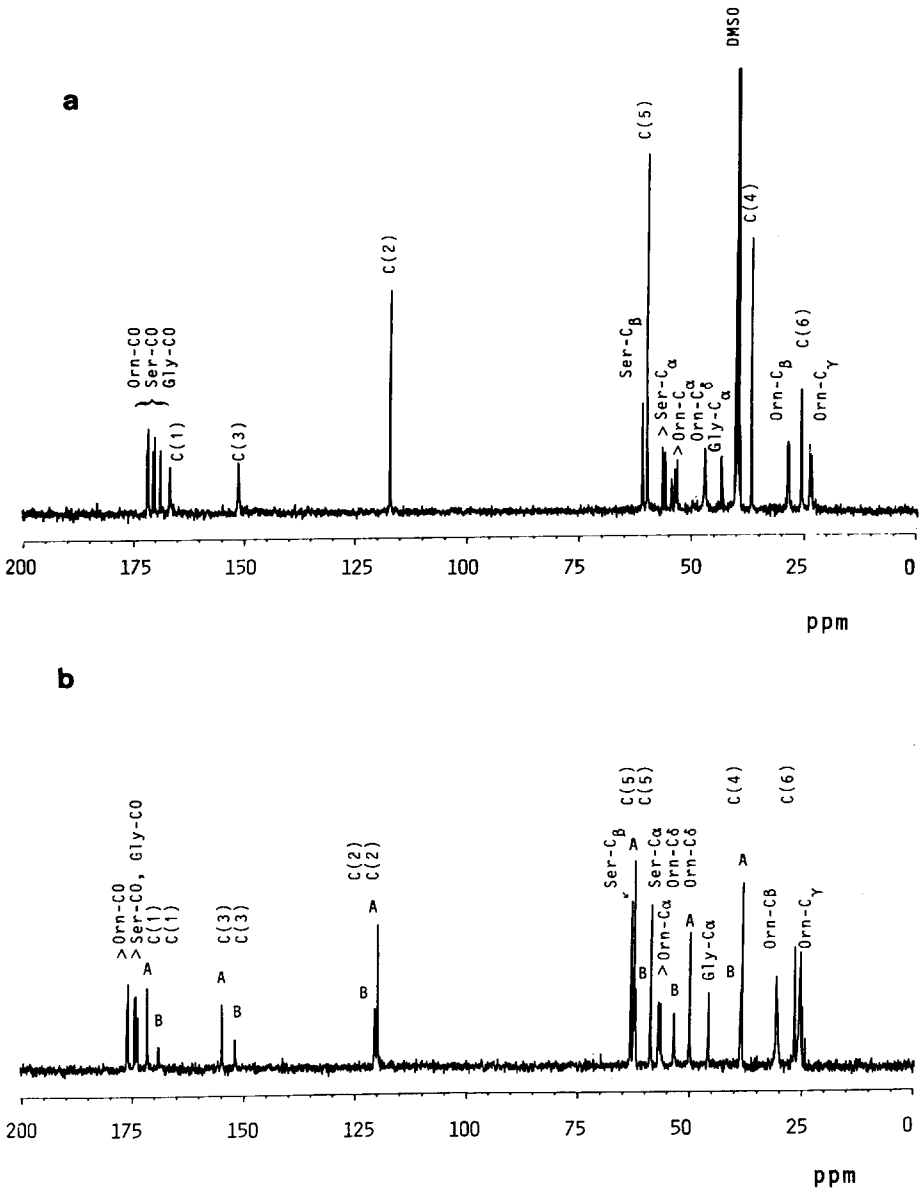

Fig. 6a, b. ^{13}C-NMR spectra of desferrirhodin **a** in $(^{12}CD_3)_2SO$ and **b** in D_2O. For assignment see Table 2. DMSO marks the solvent peak in **a**

iron-containing siderophore (Fig. 4 insert) showed, besides the molecular ion peak *m/z* 1011 $[M+H]^+$, the expected release of iron $[M+H-Fe+3H]^+$, *m/z* 958 (Dell et al. 1982) and an additional fragment *m/z* 898 produced by the detaching of an anhydromevalonic acid (AMA) residue $[M+H-AMA]^+$.

Further evidence for the structure of the principal siderophore of *B. cinerea* was obtained from ^{1}H-NMR and ^{13}C-NMR spectra of the desferri compound measured in $(CD_3)_2SO$ (Figs. 5a, 6a; Tables 1, 2). Comparison of the chemical shifts of the non-peptide signals with those reported for 5-hydroxy-3-methylpent-2-enoyl residues in various fungal siderophores (Jalal et al. 1985) indicated unequivocally the presence of three *cis*-configurated anhydromevalonic acid residues in the molecule. Thus ferrirhodin was confirmed as the principle siderophore of *B. cinerea*.

^{1}H-NMR and ^{13}C-NMR spectra of desferrirhodin were also measured in D_2O (Figs. 5b and 6b; Tables 1 and 2). They showed the expected loss of resolution compared to those recorded in $(CD_3)_2SO$ due to a less defined structure of desferrirhodin in aqueous solution (Llinas et al. 1977). Furthermore a duplication of the signals originating from the protons and carbon atoms located near the hydroxamic acid functions was observed. In Figs. 5 and 6 the signals of the doublets are identified by A and B. Following the li-

Table 1. ^{1}H-NMR chemical shifts of ferrirhodin

Chemical shift δ in $(CD_3)_2SO$ (ppm)	Assignment	Chemical shift δ in D_2O (ppm)
9.69	N-OH	
8.46	Gly-NH	
8.25–8.23	Orn-NH	
7.98–7.96	Ser-NH	
7.90	Ser-NH, Orn-NH	
7.46	Orn-NH	
6.23	C(2)-H	6.30 (1.92 H) (A), 5.99 (1.08 H) (B)
5.05–4.92	Ser-OH	
4.67	C(5)-OH	
4.18	Orn-H(α)	(4 H) suppressed by D_2O
4.11–4.05	Orn-H(α)	
4.03–3.96	Ser-H(α)	
3.78–3.75	Gly-H(α)	4.38 (4 H)
3.69–3.63	Ser-H(β)	4.08–3.85 (1 H)
3.59–3.56	Gly-H(α)	3.94–3.85 (4 H)
3.52–3.50	C(5)-H, Orn-H(δ)	3.73 (10 H)
2.61	C(4)-H	2.69 (3.84 H) (A), 2.51 (2.16 H) (B)
1.85	C(6)-H, Orn-H(β)	1.93–1.89 (12 H)
1.75–1.52	Orn-H(β), Orn-H(γ)	1.71 (9 H)

Table 2. ^{13}C-NMR chemical shifts of ferrirhodin

Chemical shift δ in $(CD_3)_2SO$ (ppm)	Assignment	Chemical shift δ in D_2O (ppm)
171.7	Orn C=O	176.2
171.5	Orn C=O	
171.4	Orn C=O	175.9
170.3	Ser C=O	174.5
169.9	Ser C=O	174.1
168.7	Gly C=O	173.7
166.5	C(1)=O	171.5 (A), 169.0 (B)
151.2	C(3)	154.8 (A), 151.8 (B)
117.0	C(2)	120.4 (B), 120.0 (A)
60.7	Ser-C(β)	63.1, 63.0
59.7	C(5)	62.4 (A), 61.9 (B)
56.2	Ser-C(α)	58.6
55.6	Ser-C(α)	
54.1	Orn-C(α)	56.7
53.3	Orn-C(α)	56.2
52.3	Orn-C(α)	
46.6	Orn-C(δ)	53.2 (B), 49.7 (A)
43.1	Gly-C(α)	45.6
36.4	C(4)	38.5 (B), 38.2 (A)
28.5	Orn-C(β)	
28.3	Orn-C(β)	30.6
28.0	Orn-C(β)	
25.3	C(6)	26.5
23.5	Orn-C(γ)	25.4
23.3	Orn-C(γ)	25.2
23.0	Orn-C(γ)	

gand chain, the additional peaks B were alternately shifted to lower and higher chemical shift values compared to those of the A signals. The maximum chemical shift differences were shown by the H and C atoms in the vicinity of the hydroxamic acid groups, but they were also significant for the H and C atoms in the rigid anhydromevalonic acid residues. In pure D_2O the ratio of the double peaks was 1:0.56 (A/B). When $(CD_3)_2SO$ was added to the D_2O solution the minor peaks decreased approximately linearly with the portion of $(CD_3)_2SO$ in the solvent mixture. They disappeared completely at about 80% $(CD_3)_2SO$. Thus, the major peaks (A) corresponded to the equivalent signals observed in $(CD_3)_2SO$. There was only one set of signals as in $(CD_3)_2SO$ when CD_3OD was used as solvent.

The appearance of these double signals may be explained by the presence of two conformers of desferrirhodin in aqueous solution originating from a *cis-trans* isomerization of the hydroxamic acid groups as described for the siderophore dimerum acid by the van der Helm group (Jalal et al. 1986). The partial double bond character of the C-N bond of hydroxamic acids provokes either a planar *trans* or *cis* conformation. The rotational energy barrier for the isomerization of the two conformers is relatively low, less, for example, than those found in amide bonds because of the reduced electron density in the C-N bond. Temperature-dependent proton resonance experiments performed with desferrirhodin in aqueous solution gave further evidence for such an isomerization. The energy barrier of the C-N bond rotation as free enthalpy of activation ΔG^* was calculated from the 'slow exchange peak separation' $\Delta\nu$ and the coalescence temperature T_c of the peak couples at 6.30/5.99 ppm ($\Delta\nu = 120.70$ Hz, $T_c = 327$ K) and at 2.69/2.51 ppm ($\Delta\nu = 72.58$ Hz, $T_c = 320$ K) in the D_2O spectrum. With respect to the unequal doublet A/B, the calculation method of Shanan-Atidi and Bar-Eli (1970) was applied, which yielded $\Delta G^*_A = 66.9 \pm 0.8$ kJ/mol (16.0 ± 0.2 kcal/mol) and $\Delta G^*_B = 65.2 \pm 0.8$ kJ/mol (15.6 ± 0.2 kcal/mol). These values agree largely with those reported for the isomerization of formhydroxamic acids (Kolasa 1983). They are somewhat reduced since the substituents at the hydroxamic acid

groups of desferrirhodin are more bulky compared to the formhydroxamic acids so that their planar conformation is of higher energy content which facilitates rotation about the C-N bond.

However, assignment of the two signal groups (A) and (B) to a distinct conformer is rather difficult. It was suggested that the *trans* conformer is the predominant conformer of dimerum acid in D_2O, since the O atom in the N—OH group comes closer to the $-CH=$ proton of the anhydromevalonic acid residue explaining the deshielding of this proton compared to the equivalent proton in the minor conformer. The *trans* conformation will also be favoured from a spatial point of view, since the steric interaction of the *N*- and *C*-alkyl residues of the hydroxamic acid groups should be smaller. It was found that the introduction of an acetyl group instead of a formyl group leads to an increased amount of *trans*-conformers (Kolasa 1983). A preferred *cis* conformation of hydroxamic acids in solution was reported, based on investigations on the carbonyl stretching frequencies in their infrared spectra and on dipole measurements of solutions in benzene and dioxane (Smith and Raymond 1980).

The ability to synthesize hydroxamate siderophores is common among various fungal genera, such as *Fusarium* (Diekmann and Zähner 1967; Sayer and Emery 1968), *Ustilago* (Neilands 1952), *Neurospora* (Keller-Schierlein and Diekmann 1970), *Aspergillus* and *Penicillium* (Zähner et al. 1963; Wiebe and Winkelmann 1975; Diekmann and Krezdorn 1975; Jalal et al. 1983; Adjimani and Emery 1987, 1988), *Gliocladium* (Jalal et al. 1986) the majority of which represent saprophytic strains. The yeast *Saccharomyces* seems to be unable to synthesize siderophores (Neilands et al. 1987). However, several genera of the heterobasidiomycetes yeasts have been shown to produce siderophores (Atkin et al. 1970; Deml et al. 1984). Recently the structures of siderophores from *Stemphylium botyrosum* (Manulis et al. 1987) and of *Alternaria alternata* (Jalal et al. 1988) have been reported.

Botrytis cinerea is a plant pathogen of eminent importance especially for the storage of fruits and berries. Therefore, it is interesting to know in detail the mechanisms of siderophore biosynthesis and its corresponding uptake mechanisms. It is clear from the present investigation that ferrirhodin is the main siderophore of all *B. cinerea* strains which we have studied so far. However, approximately 30% of the siderophores produced still represent unknown siderophore components.

Transport measurements with ^{55}Fe-labelled siderophores have revealed that ferrichrysin, possessing an identical peptide backbone to ferrirhodin, is taken up most rapidly (100%), followed by ferrirubin (57.5%) and ferrirhodin (45%). It is interesting to note that ferrirhodin and ferrirubin showed similar uptake rates although the configuration of the anhydromevalonic acid residues is *cis* in ferrirhodin and *trans* in ferrirubin (Figs. 7 and 8). *B. cinerea* obviously does not recognize different configurations of the iron-surrounding *N*-acyl residues. This is in contrast to the findings with *Neurospora crassa,* where ferrirubin is not taken up while ferrirhodin is (Huschka et al. 1986). This is further supported by our observation that, after reduction of the three double bonds in ferrirubin which yielded hexahydroferri-

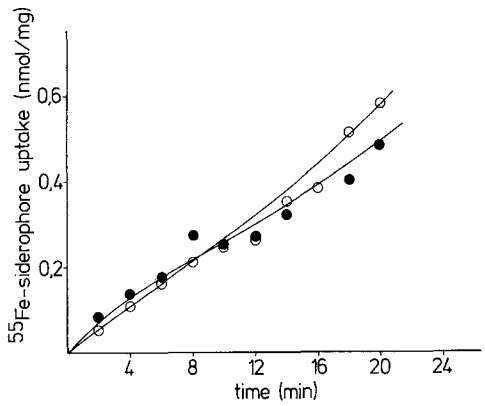

Fig. 7. Time-dependent uptake of [^{55}Fe]ferrirhodin (●) and [^{55}Fe]ferrirubin (○) in *B. cinerea*. Washed cells were incubated with ^{55}Fe-labelled siderophores (10 µM) and samples were taken at intervals, filtered, washed and radioactivity measured as described in Materials and methods

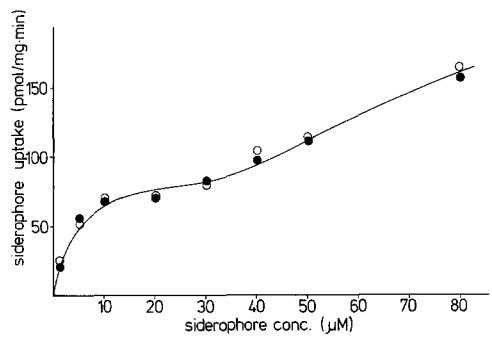

Fig. 8. Concentration-dependent uptake of [^{55}Fe]ferrirhodin (●) and [^{55}Fe]ferrirubin (○) in *B. cinerea*. Initial uptake rates were determined by incubating cells for 5 min with increasing concentrations of labelled siderophores as described in Materials and methods and calculated as rate of uptake by the dry mass (pmol mg^{-1} min^{-1})

Table 3. Iron uptake from ^{55}Fe-labelled siderophores by young mycelia of *B. cinerea*

Siderophore	Uptake after 20-min incubation (pmol mg^{-1})	Comparative uptake (%)
Ferrichrysin	1044	100
Ferrirubin	600	57.5
Ferrirhodin	470	45.0
Hexahydroferrirhodin	468	44.8
Coprogen	65	6.2

Conidiospores of *B. cinerea* were incubated in an asparagine/salt/glucose medium for 2 days. The young mycelia obtained were washed with fresh medium and incubated further in 20 ml medium plus ^{55}Fe-labelled siderophores (10 μM). Samples of the cell suspension were filtered, washed and radioactivity measured in a liquid scintillation counter

rubin (=hexahydroferrirhodin), the transport rates remained unaltered (Table 3). Concentration-dependent uptake of ferrirhodin was, however, biphasic showing saturation kinetics only at lower concentrations (1–30 μM) and a linear relationship at higher concentration, suggesting a diffusion-like uptake. It seems difficult to discuss these observations in a mechanistic way but it is obvious that the low concentration range is the physiologically relevant one since the production rate is also comparatively low.

Siderophore production is accompanied by a rapid acidification of the incubation medium which may be an important factor for improving iron assimilation in vitro. However, in vivo the buffer capacity of the host cells has to be taken into consideration. An intracellular function of fungal siderophores within the host cells has never been demonstrated. Alternatively *B. cinerea* may need ferrirhodin for its saprophytic life cycle during survival in the field. Another interesting aspect of siderophore production has been emphasized recently (Matzanke et al. 1987, 1988). Siderophores seem also to be required for the formation of conidiospores and are stored in appreciable amounts in these propagules. Thus in a variety of fungi, siderophore-mediated iron transport and storage seems to be metabolically connected with spore formation which may be regarded as crucial for the propagation of the pathogenic fungi.

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