Iron Uptake System of some Members of the Genus *Tolypocladium*: Crystal Structure of the Ligand and its Iron(III) Complex†

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A new type of fungal hydroxamic acid (1'*S*,2'*R*,4'*S*,6'*R*)-3-(2',4'-dimethyl-6'-vinylcyclohexyl)-1,4-dihydroxy-2(1*H*)-pyridone (tolypocin, HL) has been isolated from the mycelium of the fungus *Tolypocladium geodes*. The acid ($C_{15}H_{21}NO_3$) crystallized from methanol [monoclinic, space group $P2_1$, $Z = 4$, $a = 12.28(1)$, $b = 9.958(5)$, $c = 12.737(4)$ Å, $\beta = 94.91(5)°$]. The structure was solved by direct methods and refined to a final R value of 0.055 for 2393 unique observed reflections. The iron(III) tris-chelate complex of this ligand [FeL_3] has been isolated from the mycelium of the fungus *Tolypocladium terricola*. Its methanol solvate ($C_{45}H_{60}FeN_3O_9 \cdot 2MeOH$) was crystallized from methanol [orthorhombic, space group $P2_12_12$, $Z = 4$, $a = 20.27(3)$, $b = 19.35(5)$, $c = 13.43(4)$ Å]. The structure was solved by direct methods and refined to a final R value of 0.062 for 5562 observed reflections. The absolute configuration of the complex as determined from CD spectra and anomalous X-ray dispersion has been shown to be Λ-*cis* both in solution and in the solid state.

The iron-limiting conditions facilitated by the pronounced insolubility of iron(III) hydroxide at physiological pH ($K_{sp} = 10^{-39}$ mol^2 dm^{-6}) engendered the evolution of iron(III)-specific sequestering agents, termed siderophores, which can dissolve, transport and make available this essential element for aerobic organisms.[1] Iron(III) complexes of siderophores possess an octahedral geometry and the co-ordinated metal ion is d^5, high spin, and rapidly exchangeable due to kinetic lability.[2] Siderophores exhibit remarkable affinity for Fe^{3+}, little affinity for other ions differing in charge and/or size, and, in particular, little or no affinity for Fe^{2+}.[3] Since the stability constants of siderophores for Fe^{3+} are usually 10^{30} dm^3 mol^{-1} or greater, it was proposed that the release of iron from such complexes is accompanied with its reduction. Other mechanisms, such as metal substitution or enzymatic hydrolysis may, however, participate in this process as well.[4] The conformation of the metal complexes seems to be a primary factor in the selective transport which was found to be highly stereospecific.[5] Two geometric isomers, *cis* and *trans*, are possible for an octahedral complex formed by co-ordination of Fe^{3+} by three equivalent optically active hydroxamate groups, and each such isomer can exist as Λ and Δ optical isomers. X-Ray crystal structure determinations of several complexes and their circular dichroism spectra in solutions revealed that tris(hydroxamate) complexes form preferably Λ- and Δ-*cis* complexes depending on the ligand.[6] Hence, owing to the kinetic lability of iron(III) complexes, the question regarding the structure–function relationship of individual isomers remains to be solved.

In mammals free iron is strongly bonded to transport and functional proteins such as transferrin.[7] To overcome this part of the non-specific defence mechanism of their host, pathogens must be able to compete with the complexation ability of the host iron-binding proteins in order to obtain sufficient iron for their own growth and multiplication. Consequently, the pathogenicity and virulence of certain microbial infections has been found to be associated with the ability of these organisms to take up iron.[8]

A number of different siderophores has been identified so far in bacteria and fungi, most containing catecholate or hydroxamate functional groups.[1] Representative catecholate siderophores have been described, *e.g.* from the bacteria *Salmonella typhimurium* (enterobactin) or *Vibrio cholerae* (vibriobactin).[9] Representative examples of the hydroxamate siderophores are the cyclic peptide ferrichromes from the fungus *Ustilago sphaerogena*, coprogens, linear trihydroxamate siderophores containing N^5-acyl-N^5-hydroxy-L-ornithine from the fungi *Pilobolus*, *Penicillium* and *Neurospora* or cyclic N^5-hydroxy-L-ornithine-derived rhodotorulic acid and fusarinines from fungi of the genera *Rhodotorula*, *Fusarium*, *Aspergillus* and *Penicillium*.[10]

To date, most studies of siderophores have been carried out on vertebrate pathogens or non-pathogenic fungi and bacteria.[1] Since there is some indication of possible correlations between the production of siderophores by pathogens and their ability to facilitate systemic disease,[8] such a relationship would be useful for the evaluation of the efficacy of pathogens used for biocontrol. In this paper we report the isolation and structure determination of a new type of siderophore isolated from entomopathogenic fungi of the genus *Tolypocladium*.

Experimental

Physical Measurements.—Melting points were determined between cover plates in air and are uncorrected. Infrared spectra were recorded with a Perkin Elmer 584 spectrometer with a PE-7000 data station, UV/VIS spectra in methanol with a Varian DMS 300 spectrometer, CD spectra in methanol with a Jobin-

† *Supplementary data available:* see Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1993, Issue 1, pp. xxiii–xxviii.

Yvon 5 automatic recording spectropolarimeter at ambient temperature, and NMR spectra of the ligand tolypocin [3-(2',4'-dimethyl-6'-vinylcyclohexyl)-1,4-dihydroxy-2(1*H*)-pyridone (HL)] on a VXR-400 Varian spectrometer with a 400 MHz observing frequency for the ^1H and 100 MHz for the ^{13}C resonances. The chemical shifts are reported in ppm (δ) downfield from tetramethylsilane used as an internal standard. The NMR spectra were recorded in deuteriated chloroform, dimethyl sulfoxide (dmso), dimethylformamide (dmf) and methanol at ambient temperature. The *J* resolved (HOM2DJ), homonuclear correlation (COSY) and heteronuclear correlation (HETCOR) two-dimensional NMR spectra were recorded with the standard pulse sequences and programming as supplied by Varian Associates.

Positive-ion electron-impact (EI) mass spectra of ligand HL were recorded on a Finnigan MAT 90 double sector instrument of *BE* geometry (magnetic sector preceding electrostatic sector) [ionizing energy 70 eV (*ca.* 1.12×10^{-17} J), source temperature 250 °C, emission current 1 mA, accelerating voltage 5 kV, direct inlet]. High-resolution measurements were carried out by the peak-matching method using perfluorokerosene as an internal standard. The resolution was approximately 9000, 10% valley. The products of metastable collisionally activated decompositions in the first field-free region of the instrument (helium as a collision gas, applied amount of helium suppressed the ion beam by 50%) were analysed by the following linked scans: daughter ions, B/E constant; parent ions, B^2/E constant; neutral losses, (B^2/E^2) $(E_0 - E)$ constant where B is the magnetic field, E is the electrostatic voltage and $E_0 - E$ is the electrostatic voltage offset, using the manufacturer's software. For positive and negative ion FAB mass spectral measurements, high-purity xenon 4.0 was used as a FAB gas [1×10^{-5} mbar (10^{-3} Pa)]. The standard saddle-field atom gun FAB11NF (Ion Tech, Teddington) was operated at 2 mA current and 6 kV energy. A rotatable FAB target was used with approximately 2 μg of the complex [FeL_3] (dissolved in 1 μl of matrix) on one side and caesium iodide on the other side as lockmass for accurate mass assignment. For low-resolution measurements *m*-nitrobenzyl alcohol (Fluka) was found to be an excellent matrix. High-resolution data were obtained using monothioglycerol (acidified with heptafluorobutyric acid) and enabled rotation of the FAB target without mixing of the matrix with the reference standard. For the peak-matching method the resolution was adjusted to 7000 (10% valley).

Crystallographic Studies.—*Crystal data for tolypocin* (HL). $C_{15}H_{21}NO_3$, $M = 263.34$, monoclinic, space group $P2_1$ (no. 4), $a = 12.28(1)$, $b = 9.958(5)$, $c = 12.737(4)$ Å, $\beta = 94.91(5)°$, $U = 1551(1)$ Å^3 (by least-squares refinement on diffractometer angles for 15 automatically centred reflections, $\lambda = 0.710\ 73$ Å), $D_c = 1.127$ g cm^{-3}, $Z = 4$, $F(000) = 568$, colourless needles, crystal dimensions $0.9 \times 0.2 \times 0.2$ mm, μ(Mo-Kα) = 0.07 mm^{-1}.

Enraf–Nonius CAD-4 diffractometer, ω–2θ mode, graphite-monochromated Mo-Kα radiation, 293 K. 6104 Reflections measured (h 0–15, k −12 to 12, l −15 to 15, $2\theta_{max} = 53°$), 2393 of which were observed [$I \geqslant 2\sigma(I)$]; no corrections for absorption, extinction or anomalous dispersion. Direct methods followed by standard Fourier series and full-matrix least squares, hydrogen atoms in calculated positions, and OH hydrogens constrained in positions found from a ΔF map. Function minimized $\Sigma w(|F_o| - |F_c|)^2$, $w = 1/\sigma^2(F_o)$, 343 parameters refined, ratio of maximum least-squares shift to e.s.d. $(\Delta/\sigma) = 0.06$. The refinement converged to $R = 0.055$, $R' = 0.050$, $S = 0.965$, with the largest residual peaks of −0.22 and 0.22 e Å^{-3}. Programs used were SHELXS 86, CRYSTALS and PARST.[11] Coordinates of non-H atoms are presented in Table 1 and selected bond lengths and angles in Table 2.

Crystal data for terricolin methanol solvate, [FeL_3]·2MeOH. $C_{47}H_{68}FeN_3O_{11}$, $M = 904.9$, orthorhombic, space group $P2_12_12$ (no. 18), $a = 20.27(3)$, $b = 19.35(5)$, $c = 13.43(4)$ Å, $U = 5268(2)$ Å^3 (by least-squares refinement on diffractometer angles for 20 automatically centred reflections, $\lambda = 0.710\ 73$ Å), $D_c = 1.141$ g cm^{-3}, $Z = 4$, $F(000) = 1932$. Deep red prismatic crystal of dimensions *ca.* $0.9 \times 0.5 \times 0.5$ mm, in a capillary with 20 μl of mother-liquor, adjustment with silicon grease, μ(Mo-Kα) = 0.34 mm^{-1}.

Details as above except where listed. 7470 Reflections measured (h −24 to 24, k 0 to 22, l 0 to 10, $2\theta_{max} = 50°$), 5562 observed [$I \geqslant 3\sigma(I)$]; no absorption and extinction corrections. Direct methods followed by standard Fourier series and block-diagonal least squares, hydrogen atoms in calculated positions. Positions of OH hydrogens were not found.

559 Parameters refined, ratio of maximum least-squares shift to e.s.d. $(\Delta/\sigma) = 0.2$. Taking in account the anomalous dispersion the last refinement converged to $R = 0.062$, $R' = 0.078$, $S = 1.9$, for the Λ and $R = 0.067$, $R' = 0.081$ for the Δ enantiomer with the largest residual peaks of −0.5 and 1.7 e Å^{-3}. The Flack[12] enantiomorph parameter $|F(h,x)|^2 = (1 - x)|F(h)|^2 + x|F(-h)|^2$ refined with other parameters converged at $x = 0.03(4)$. Coordinates of non-H atoms are presented in Table 3 and selected bond lengths and angles in Table 4.

Additional material available for both structures from the Cambridge Crystallographic Data Centre comprises H-atom coordinates, thermal parameters and remaining bond lengths and angles.

Culture of Fungi.—Strains[13] of *Tolypocladium geodes* CCEF 2198 and *terricola* CCEF 2201 were grown for 21 d at 25 °C in polyethylene bags on the surface of sterilized Sabouraud medium supplemented with 2% casein hydrolysate. Mycelium was filtered off and extracted twice with methanol (1:10, w/v). The pooled extracts were evaporated to dryness.

Isolation of Tolypocin.—The crude mycelium extract from *T. geodes* (50 g) was subjected to column liquid chromatography on silica gel L 100/400 (Lachema, Czech Republic) with a stepwise gradient of methanol in chloroform (to 20% v/v MeOH in $CHCl_3$). The bactericidal activity of the collected fractions was monitored by a standard technique on agar plates against *Bacillus subtilis*, *Proteus mirabilis*, *Serratia marcenscens*, *Erwinia herbicola*, *Neiseria sp.* and *Micrococcus sp.* Active fractions were pooled and evaporated. Pure tolypocin was obtained by repeated crystallization from its hot solution in methanol–water (9:1, v/v), as colourless prismatic crystals (200 mg), sublimes at *ca.* 220 °C (Found: C, 68.2; H, 8.2. $C_{15}H_{21}NO_3$ requires C, 68.40; H, 8.05%). Positive-ion EI mass spectrum: *m/z* 263.1519 ($M^{\cdot+}$, $C_{15}H_{21}NO_3$, calc. 263.1521, 28), 246.1493 ($C_{15}H_{20}NO_2$, calc. 246.1494, 100), 168 (11), 167 (11), 164 (16), 162 (13), 152.0716 ($C_8H_{10}NO_2$, calc. 152.0711, 17), 150.0557 ($C_8H_8NO_2$, calc. 150.0555, 34), 148 (11), 141.0430 ($C_6H_7NO_3$, calc. 141.0426, 36), 140.0352 ($C_6H_6NO_3$, calc. 140.0348, 45), 138 (18), 124.0396 ($C_6H_6NO_2$, calc. 124.0399, 77), 123.1164 (C_9H_{15}, calc. 123.1174, 42), 122.1100 (C_9H_{14}, calc. 122.1096, 5), 81 (13), 79 (11), 77 (11), 53 (12), 41 (18) and 39 (16%). IR (Nujol): ν_{max} 1638vs and 1631vs (CO), 1566s and 1536vs cm^{-1}. UV (methanol): λ_{max} 215 (ε 35 000) and 290 nm (4600 cm^3 mol^{-1} cm^{-1}). Proton and ^{13}C NMR data are listed in Table 5. The overall fragmentation scheme is given in Fig. 3. All transitions were verified by linked scanning techniques.

Isolation of Terricolin, [FeL_3].—The crude mycelium extract of *T. terricola* (50 g) was suspended in chloroform. Crude complex **1** was removed as an insoluble part and dissolved in methanol–water (4:1, v/v). Addition of water to the filtered solution (final ratio of methanol:water = 1:3), afforded the complex as a fine precipitate which was finally purified by crystallization from methanol–ethyl acetate (1:7, v/v). Red hexagonal plates (400 mg), decomposes at 220 °C without melting (Found: C, 63.9; H, 7.4; $C_{45}H_{60}FeN_3O_9$ requires C, 64.10; H, 7.20%). FAB mass spectrum (acidified monothioglycerol as matrix): 843.377, $[M + H]^+$ (requires 843.376). IR

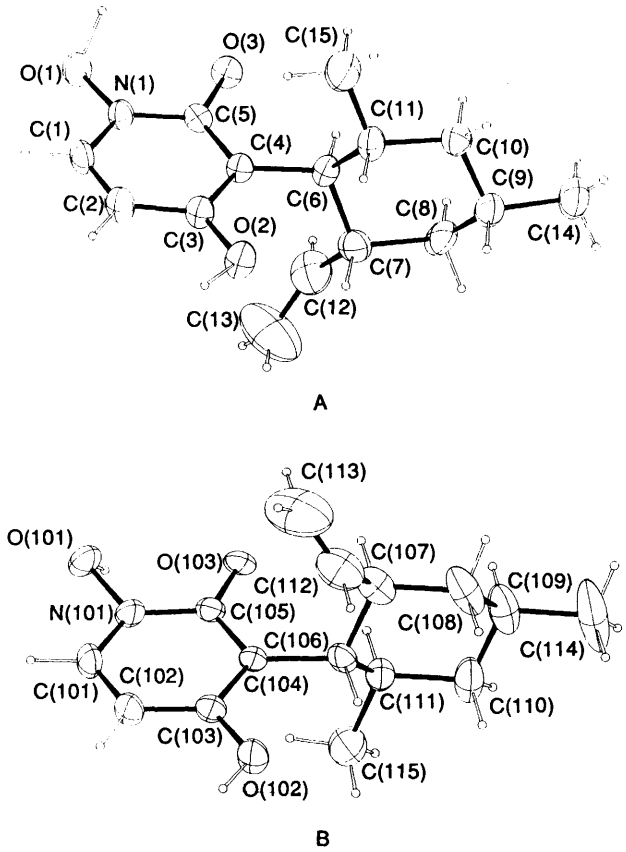

Fig. 1 The two symmetrically independent molecules of tolypocin HL denoted as A and B. Thermal ellipsoids are drawn at 50% probability

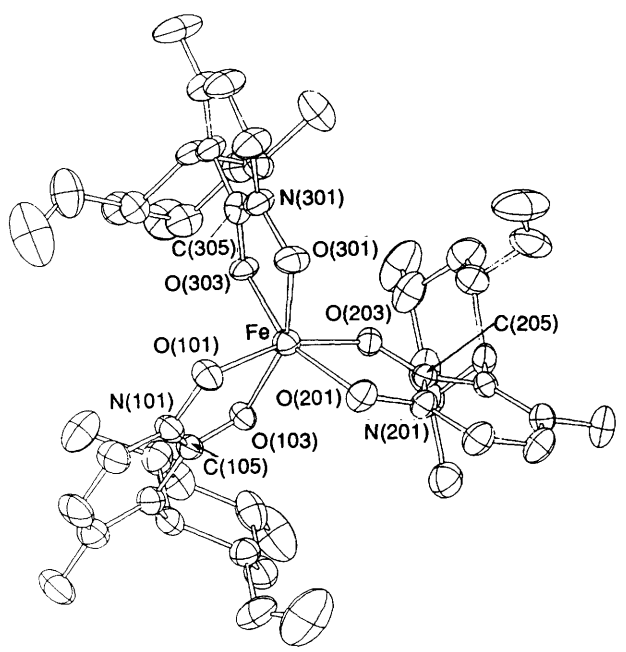

Fig. 2 The molecule of terricolin $[FeL_3]$·2MeOH with thermal ellipsoids at 50% probability. Atom numbering is not complete for clarity (see Fig. 1); ligands 1, 2 and 3 differ by +100, +200 and +300

(Nujol): ν_{max} 1629vs (CO), 1571s and 1509vs cm^{-1}. UV/VIS: λ_{max}(methanol) 221 (ε 94 500), 250(sh) (20 000), 288 (11 100) and 390 (5100); (MeOH–1 mol dm^{-3} aqueous HCl) 245 (18 000), 270 (sh) (13 200), 315 (1800) and 450 (850 dm^3 mol^{-1} cm^{-1}). Mass and CD spectra are given in Figs. 4 and 5.

Table 1 Atomic coordinates for non-H atoms of tolypocin HL with estimated standard deviations (e.s.d.s) in parentheses. The atoms of the second molecule have the same index + 100

Atom	*X/a*	*Y/b*	*Z/c*
O(1)	−0.6244(3)	0.1100(5)	−0.7198(3)
O(2)	−0.3081(3)	−0.0591(5)	−0.4260(2)
O(3)	−0.4185(3)	0.1413(5)	−0.7547(2)
O(101)	−0.3991(3)	−0.6023(5)	0.2348(3)
O(102)	−0.6863(3)	−0.4380(6)	−0.0865(3)
O(103)	−0.6114(3)	−0.6317(4)	0.2497(2)
N(1)	−0.5415(3)	0.0741(5)	−0.6442(3)
N(101)	−0.4760(3)	−0.5676(6)	0.1516(3)
C(1)	−0.5726(4)	0.0220(7)	−0.5529(4)
C(2)	−0.4965(5)	−0.0214(8)	−0.4791(4)
C(3)	−0.3862(4)	−0.0156(7)	−0.4982(4)
C(4)	−0.3538(4)	0.0385(6)	−0.5914(3)
C(5)	−0.4359(4)	0.0847(6)	−0.6668(3)
C(6)	−0.2363(4)	0.0516(6)	−0.6165(3)
C(7)	−0.1786(5)	−0.0830(7)	−0.6246(5)
C(8)	−0.0659(5)	−0.0577(9)	−0.6661(5)
C(9)	0.0036(5)	0.0411(8)	−0.5993(5)
C(10)	−0.0582(4)	0.1692(8)	−0.5846(5)
C(11)	−0.1697(4)	0.1508(7)	−0.5427(4)
C(12)	−0.2428(7)	−0.1779(9)	−0.6931(7)
C(13)	−0.281(1)	−0.290(1)	−0.668(1)
C(14)	0.1119(5)	0.070(1)	−0.6485(6)
C(15)	−0.2260(5)	0.2826(7)	−0.5323(6)
C(101)	−0.4346(4)	−0.5176(8)	0.0648(4)
C(102)	−0.5027(5)	−0.4741(8)	−0.0163(4)
C(103)	−0.6146(4)	−0.4816(7)	−0.0078(4)
C(104)	−0.6582(4)	−0.5327(7)	0.0820(3)
C(105)	−0.5847(4)	−0.5783(6)	0.1651(3)
C(106)	−0.7805(4)	−0.5364(7)	0.0903(4)
C(107)	−0.8180(5)	−0.4348(7)	0.1687(5)
C(108)	−0.9418(5)	−0.4330(9)	0.1668(6)
C(109)	−0.9892(5)	−0.5716(8)	0.1818(6)
C(110)	−0.9495(5)	−0.6704(9)	0.1047(5)
C(111)	−0.8259(4)	−0.6755(7)	0.1080(4)
C(112)	−0.7749(8)	−0.2987(9)	0.1513(7)
C(113)	−0.711(1)	−0.231(1)	0.2112(9)
C(114)	−1.1141(5)	−0.566(1)	0.1767(8)
C(115)	−0.7886(6)	−0.7818(9)	0.0331(6)

Table 2 Selected distances (Å) and angles (°) for tolypocin

O(1)–N(1)	1.387(5)	O(101)–N(101)	1.400(5)
O(2)–C(3)	1.342(6)	O(102)–C(103)	1.348(6)
O(3)–C(5)	1.288(6)	O(103)–C(105)	1.269(6)
N(1)–C(5)	1.356(6)	N(101)–C(105)	1.365(6)
C(1)–N(1)–O(1)	116.7(4)	C(101)–N(101)–O(101)	115.7(4)
C(5)–N(1)–O(1)	119.4(4)	C(105)–N(101)–O(101)	119.2(4)
C(5)–N(1)–C(1)	123.8(4)	C(105)–N(101)–C(101)	125.0(4)

Results and Discussion

Screening of the genus *Tolypocladium* indicated significant bactericidal activity of the methanolic extract of the mycelium from the 21 day-old fungus *Tolypocladium geodes*. The active principle was named tolypocin (HL). It was separated by silica gel column chromatography with a stepwise gradient of methanol in chloroform. Final purification was achieved by repeated crystallization from methanol.

The fungi *Tolypocladium geodes* and *T. inflatum* exhibit considerably higher ability to produce a broad diversity of pigments[14] than does *T. terricola*. Hence, attempts were made to characterize the principal pigment named terricolin $[FeL_3]$, which accounts for the orange colour of a methanolic mycelium extract of *T. terricola*. The solid complex was isolated by taking advantage of the fact that it is fairly soluble in solvent mixtures containing methanol but almost insoluble in all other solvents. Thus, washing with different solvents and finally crystallization

Table 3 Atomic coordinates for non-H atoms of terricolin $[FeL_3]\cdot 2MeOH$ with e.s.d.s. in parentheses. The atoms of the ligands, 1, 2 and 3 have the same index + 100, 200 and 300

Atom	*X/a*	*Y/b*	*Z/c*	Atom	*X/a*	*Y/b*	*Z/c*
Fe	0.254 35(6)	0.173 28(5)	0.910 36(9)				
Methanol							
O(1)	0.099 7(3)	0.605 5(4)	0.885 2(7)	O(2)	0.138 7(3)	0.155 6(5)	0.158 9(6)
C(1)	0.129 2(7)	0.544 2(7)	0.859(1)	C(2)	0.182 7(6)	0.190 0(9)	0.222(1)
Ligands							
O(101)	0.209 5(3)	0.254 7(3)	0.974 5(5)	C(207)	0.347 7(4)	−0.023 8(5)	0.731 3(7)
N(101)	0.152 8(3)	0.272 9(3)	0.925 5(6)	C(208)	0.367 5(5)	−0.031 0(6)	0.618 4(8)
C(101)	0.116 9(4)	0.326 8(5)	0.959 2(8)	C(209)	0.321 5(6)	0.006 2(6)	0.549 3(8)
O(102)	−0.012 7(3)	0.325 0(4)	0.771 3(6)	C(210)	0.252 2(6)	−0.023 2(4)	0.565 1(6)
C(102)	0.061 2(4)	0.344 0(5)	0.909 0(9)	C(211)	0.227 6(4)	−0.016 7(4)	0.673 9(7)
O(103)	0.177 3(2)	0.186 4(3)	0.821 5(4)	C(212)	0.394 2(5)	−0.062 5(7)	0.798 2(9)
C(103)	0.042 7(4)	0.308 6(5)	0.819 4(8)	C(213)	0.427 6(5)	−0.034 5(9)	0.862(1)
C(104)	0.082 6(4)	0.253 9(4)	0.786 1(7)	C(214)	0.343 4(7)	0.002 4(6)	0.439 9(8)
C(105)	0.139 0(4)	0.236 1(4)	0.842 2(7)	C(215)	0.157 9(5)	−0.047 5(5)	0.684 2(8)
C(106)	0.066 9(4)	0.215 9(4)	0.692 0(7)	O(301)	0.332 8(3)	0.199 7(3)	0.997 3(4)
C(107)	0.053 3(4)	0.138 3(5)	0.709 5(7)	N(301)	0.379 5(3)	0.234 3(4)	0.941 3(6)
C(108)	0.037 1(4)	0.102 6(5)	0.610 6(8)	C(301)	0.432 9(5)	0.261 0(5)	0.985 2(8)
C(109)	0.089 7(5)	0.111 8(5)	0.532 3(8)	O(302)	0.521 3(3)	0.323 1(5)	0.771 3(6)
C(110)	0.103 9(5)	0.189 4(6)	0.515 8(7)	C(302)	0.481 4(5)	0.290 5(6)	0.925 8(8)
C(111)	0.121 1(5)	0.227 9(5)	0.610 0(7)	O(303)	0.313 5(2)	0.214 1(3)	0.809 2(4)
C(112)	0.001 5(6)	0.126 5(6)	0.783 6(9)	C(303)	0.474 3(4)	0.293 7(5)	0.826 3(7)
C(113)	0.008(1)	0.089 3(8)	0.859(1)	C(304)	0.418 1(4)	0.267 4(5)	0.781 0(7)
C(114)	0.072 8(7)	0.070 1(7)	0.436 3(9)	C(305)	0.369 2(4)	0.237 4(5)	0.838 4(7)
C(115)	0.130 8(6)	0.304 0(5)	0.587 0(9)	C(306)	0.409 7(4)	0.266 6(5)	0.667 4(7)
O(201)	0.221 3(3)	0.106 5(3)	1.014 6(5)	C(307)	0.348 3(4)	0.305 6(5)	0.632 1(6)
N(201)	0.225 1(3)	0.038 7(3)	0.983 4(5)	C(308)	0.343 5(5)	0.302 6(5)	0.517 6(7)
C(201)	0.205 4(5)	−0.013 1(5)	1.039 8(8)	C(309)	0.343 7(5)	0.229 9(5)	0.478 4(8)
O(202)	0.239 9(4)	−0.158 5(3)	0.880 4(5)	C(310)	0.404 5(5)	0.191 5(6)	0.514 2(8)
C(202)	0.210 8(5)	−0.078 9(5)	1.011 8(8)	C(311)	0.410 6(4)	0.192 1(5)	0.628 2(7)
O(203)	0.270 7(2)	−0.082 9(2)	0.846 8(4)	C(312)	0.349 9(7)	0.379 0(6)	0.664 8(9)
C(203)	0.235 4(4)	0.092 2(4)	0.913 8(8)	C(313)	0.305 5(8)	0.412 1(8)	0.710(1)
C(204)	0.254 7(4)	−0.038 9(4)	0.853 5(6)	C(314)	0.340 3(6)	0.229 0(7)	0.362 8(8)
C(205)	0.250 3(4)	0.028 4(3)	0.890 7(5)	C(315)	0.472 6(5)	0.152 3(6)	0.661 3(9)
C(206)	0.276 5(4)	−0.049 5(4)	0.748 7(7)				

from a methanol–ethyl acetate mixture afforded pure complex without the necessity for a chromatographic purification.

Since the structures of HL and its complex could not be determined unambiguously from physical measurements due to the complexity of spectra, single-crystal X-ray diffraction analyses were performed. Crystals of HL were obtained by slow cooling of a saturated methanol solution. Attempts to prepare good-quality crystals of the complex were made over 3 years and mixtures of more than 40 various solvents were tested. From mixtures of methanol with acetonitrile or ethyl acetate the complex crystallizes in the form of stable thin hexagonal plates, which are too small for crystallographic study. Large crystals of $[FeL_3]\cdot 2MeOH$ were obtained from methanol. This solvate is, however, very unstable, decomposing within seconds even in an inert solvent, and can only be manipulated in the viscous deep red mother-liquor. For X-ray crystal structure determination the crystal was transferred in the mother-liquor to a Lindemann capillary, the capillary was capped with a silicon grease, the excess of solvent removed, and the crystal measured while surrounded by residual 20 μl of mother-liquor.

The structure determination finally revealed that the compound HL forms the tris-chelate complex $[FeL_3]$. The crystallographic atom numbering schemes are given in Figs. 1 and 2. The molecule HL is composed of 2(1*H*)-pyridone and cyclohexane rings connected by a single bond. The cyclohexane ring possesses a regular chair conformation with substituents in 1, 2, 4 and 6-equatorial positions. The unit cell contains two types of molecules ($Z = 4$), which differ in the rotation around the C(4)–C(6) or C(104)–C(106) bond and in the chirality of the N atom (Fig. 1). The molecular packing is stabilized by two mutual NOH · · · O=C hydrogen bonds between molecules A and B and by additional COH · · · O=C hydrogen bonds between neighbouring molecules of A,A′ and B,B′, thus creating a three-dimensional structure. Hydrogen bonding stabilizes the *S* and *R* absolute configurations of the N atoms in molecules A and B, respectively [N(1) displaced by 0.028 Å and N(101) by −0.028 Å from the C(1)O(1)C(5) and C(101)O(101)C(105) planes]. In the complex all the ligands adopt approximately the same orientation and the *R* absolute configuration of the N atom as in molecule B of the free ligand.

The 2(1*H*)-pyridone moiety is nearly planar in the ligand as well as in the complex with a maximum displacement of 0.02 Å; the bond lengths and angles indicate the aromatic character of the ring. The co-ordination of iron(III) does not influence bond lengths and angles significantly (Tables 2 and 4) except for the angles O(1)–N(1)–C(5) and N(1)–C(5)–O(3) which are smaller in the complex than in the free ligand due to formation of the five-membered ring. In the complex the pseudo-octahedral FeO_6 arrangement is markedly trigonally distorted due to the short distance between the oxygen donors (average 2.63 in HL, 2.54 Å in **1**). The deviation from octahedral symmetry is also reflected in the O–Fe–O angles (Table 4). The three five-membered chelate rings are nearly equidimensional but their conformation slightly differs for ligands 1–3, obviously due to the presence of two solvate molecules, and can be described as λ-skew, λ-envelope and δ-envelope respectively.[15] The corresponding puckering parameters[16] q and φ are 0.028(9), −90(17); 0.059(7), −26(7); and 0.062(9), −122(8)° for the FeO(1)N(1)C(5)O(3) chelate rings 1, 2, and 3, respectively. In the present structure the average N–O and N–C distances within the chelate rings are nearly equal (Table 4), while in most other iron(III) hydroxamates the N–O are systematically longer

Table 4 Selected distances (Å) and angles (°) for terricolin

Fe–O(101)	2.012(6)	O(103)–C(105)	1.268(9)
Fe–O(103)	1.982(5)	O(201)–N(201)	1.380(8)
Fe–O(201)	2.019(6)	N(201)–C(205)	1.361(9)
Fe–O(203)	1.974(5)	O(202)–C(203)	1.361(9)
Fe–O(301)	2.038(6)	O(203)–C(205)	1.276(8)
Fe–O(303)	1.977(5)	O(301)–N(301)	1.382(8)
O(101)–N(101)	1.371(8)	N(301)–C(305)	1.40(1)
N(101)–C(105)	1.36(1)	O(302)–C(303)	1.33(1)
O(102)–C(103)	1.33(1)	O(303)–C(305)	1.277(9)
O(103)–Fe–O(101)	78.6(2)	O(303)–Fe–O(203)	87.4(2)
O(201)–Fe–O(101)	93.1(2)	O(303)–Fe–O(301)	79.7(2)
O(201)–Fe–O(103)	103.7(2)	N(101)–O(101)–Fe	112.0(4)
O(203)–Fe–O(101)	162.6(2)	C(105)–N(101)–O(101)	115.7(6)
O(203)–Fe–O(103)	89.2(2)	C(105)–O(103)–Fe	116.6(5)
O(203)–Fe–O(201)	77.8(2)	O(103)–C(105)–N(101)	116.9(7)
O(301)–Fe–O(101)	84.9(2)	N(201)–O(201)–Fe	112.3(5)
O(301)–Fe–O(103)	158.1(2)	C(205)–N(201)–O(201)	115.9(6)
O(301)–Fe–O(201)	91.3(2)	C(205)–O(203)–Fe	118.5(5)
O(301)–Fe–O(203)	109.8(2)	O(203)–C(205)–N(201)	115.1(6)
O(303)–Fe–O(101)	104.8(2)	N(301)–O(301)–Fe	110.1(4)
O(303)–Fe–O(103)	90.7(2)	C(305)–N(301)–O(301)	117.1(7)
O(303)–Fe–O(201)	159.0(2)	C(305)–O(303)–Fe	117.8(6)
		O(303)–C(305)–N(301)	114.8(8)

Table 5 Proton and ^{13}C NMR parameters of tolypocin[a]

Atom	Molecule	δ_C	δ_H	^2J, ^3J/Hz
6	A′	132.91* (d)	7.541* (d)	7.7
	B′	132.97* (d)	7.554* (d)	7.6
5	A′	99.25* (d)	5.961* (d)	7.7
	B′	100.23* (d)	5.961* (d)	7.6
4	A′	160.67* (s)	—	—
	B′	162.93* (s)	—	—
3	A′	115.66* (s)	—	—
	B′	115.32* (s)	—	—
2	A′	163.51 (s)	—	—
	B′	164.19 (s)	—	—
1′	A′	48.46 (d)	2.648 (dd)	11.1, 11.1
	B′	47.47 (d)	2.485 (dd)	10.7, 10.8
6′	A′	45.52 (d)	2.873 (m)	
	B′	44.36 (d)	3.030 (m)	
5′	A′	43.94 (t)	0.946 (ddd)	12.8, 11.9, 11.9
			1.713 (m)	
	B′	44.08 (t)	0.901 (ddd)	12.8, 11.8, 11.7
			1.743 (m)	
4′	A′	33.44* (d)	1.612* (m)	
	B′	33.50* (d)	1.659* (m)	
3′	A′	46.27 (t)	0.780 (ddd)	12.9, 12.0, 11.8
			1.748 (m)	
	B′	46.17 (t)	0.734 (ddd[b])	
			1.726 (m)	
2′	A′	34.44 (d)	2.399 (m)	
	B′	33.29 (d)	2.276 (m)	
6′-α	A′	144.98* (d)	5.570 (ddd)	17.1, 10.2, 8.7
	B′	145.01* (d)	5.531 (ddd)	17.1, 10.3, 8.6
6′-β	A′	113.23* (t)	4.772 (ddd)	17.1, 2.4, 0.9
			4.627 (dd)	10.2, 2.4
	B′	113.30* (t)	4.792 (ddd)	17.1, 2.4, 0.9
			4.633 (ddd)	10.3, 2.4, 0.2
4′-Me	A′	23.42* (q)	0.946* (d)	6.6
	B′	23.46* (q)	0.946* (d)	6.7
2′-Me	A′	21.16 (q)	0.738 (d)	6.7
	B′	21.31 (q)	0.711 (d)	6.5

[a] In CD_3OD, at 25 °C. [b] Overlap with methyl groups. Asterisked signals for the same atom might be interchanged.

than the N–C distances. The C=O bond (1.274 Å) is significantly longer than the average C=O bond, *e.g.*, in peptides (1.228 Å).[17]

Compared to the known hydroxamato complexes,[6] there is a significant difference in the mode of co-ordination in $[FeL_3]$ which make its structure unique. Whereas the higher electron

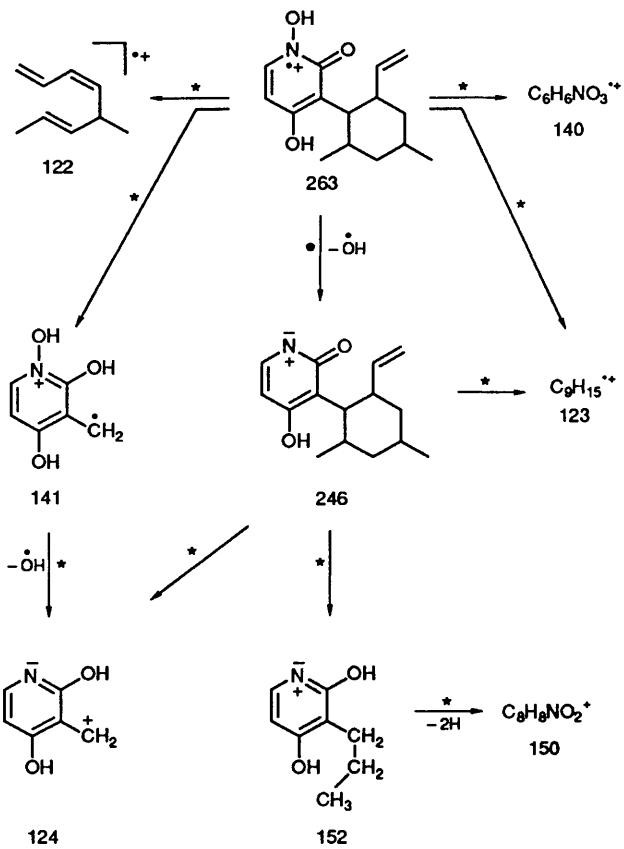

Fig. 3 Fragmentation pathways for Tolypocin under positive-ion EI conditions. Asterisks denote metastable transitions established by linked scans

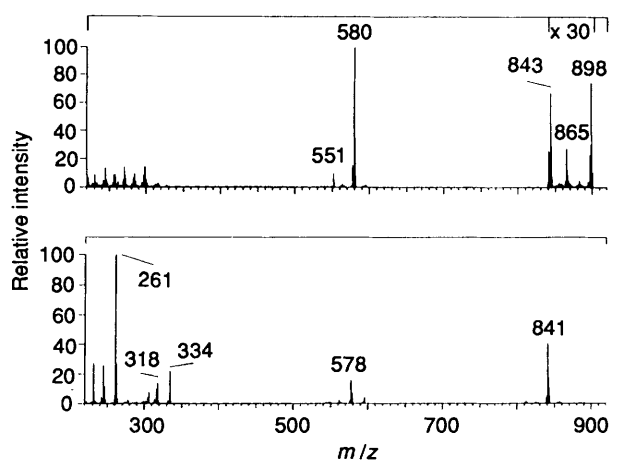

Fig. 4 The positive- (upper) and negative-ion FAB mass spectra of terricolin $[FeL_3]$

density is localized at the N–*O* donor atom and consequently Fe–O(N) bonds are shorter than Fe–O(C) ones in hydroxamato complexes, here the opposite is true. Formally $[FeL_3]$ should, therefore, be formulated as a tris(2-oxidopyridine *N*-oxide) iron(III) complex rather than a salt of hydroxamic acid. This difference can be rationalized by considering the extensive electron delocalization within the pyridine and chelate rings which is not possible in hydroxamates derived from amino acids.

The anomalous X-ray dispersion shows that the absolute configuration of the molecule is Λ-*cis*. This appears to be the preferred isomer among cyclic iron(III) hydroxamates[6] probably for steric reasons, though both *cis* and *trans* isomers of kinetically inert Cr^{III} have been prepared and resolved chromatographically.[18] The presence of a negative charge localized in one face of the octahedron apparently does not influence the

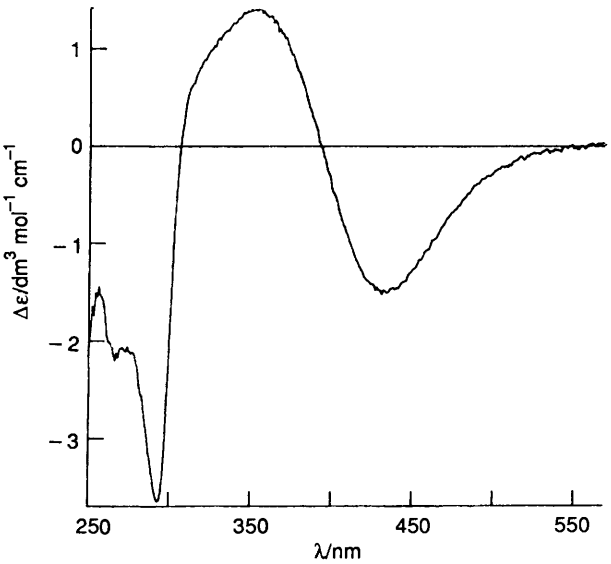

Fig. 5 The CD spectrum of terricolin

preferred geometric or optical isomerism, despite the larger charge contribution originating from an oxime or phenolate group as discussed above. The determination of the absolute configuration of $[FeL_3]$ enables also the assignment of the absolute chirality of the ligand, *i.e.* as (1′*S*,2′*R*,4′*S*,6′*R*)-3-(2′,4′-dimcthyl-6′-vinylcyclohexyl)-1,4-dihydroxy-2(1*H*)-pyridone.

The red iron(III) complex $[FeL_3]$ dissolves in methanol, ethanol, dmf and dmso to form orange solutions. The positions of the absorption maxima increase with decreasing pH. The complex is strongly retained on cation exchangers (Dowex 50W, methanol) which indicates that it exists in a protonated form in the presence of a strong acid. Iron(III) ions added to a solution of $[FeL_3]$ co-ordinate to the free phenol group, the charge-transfer band at 390 nm being broadened and colour changing from orange to deep red. In the same manner the complex is able to co-ordinate a number of other ions and thus to form polymetallic complexes. A comparison of the absolute configuration of the molecule in solution and in the solid state was made by measuring the circular dichroism of the chelate in the vicinity of the characteristic charge-transfer absorption. Comparison with the CD spectra of hydroxamates of known structures[6,18] (Fig. 5) indicates that $[FeL_3]$ possesses the same Λ-*cis* structure in solution as in the solid state.

From the bond distances as well as from NMR spectra (Table 5) it follows that the ligand HL is fully aromatic as indicated by the proton doublets in the aromatic region and the δ_C shifts. On the other hand, δ_H, δ_C and the coupling constants found for the cyclohexane ring indicate a fairly aliphatic skeleton with all the substituents in equatorial positions. In solution two molecules are present (A′ and B′) which differ in the δ_H and δ_C shifts belonging to groups adjacent to the ring junction (3–1′). The A′:B′ ratio is slightly solvent dependent [about 50:50 in CD_3OD and 60:40 in $CDCl_3$, $(CD_3)_2SO$ and $DCON(CD_3)_2$]. The two distinct conformations in solution seem likely to originate from sterically hindered rotation around the 3–1′ bond. Individual conformers cannot, however, be assigned unambiguously to the two symmetrically independent molecules A and B in the crystal structure (Fig. 1).

The positive-ion EI mass spectrum of the ligand showed $M^{\cdot+}$ at *m*/*z* 263 (28% of the base peak). Selected ions were accurately measured (see Experimental section) and the overall fragmentation scheme is given in Fig. 3. All the transitions were verified by linked scanning techniques. The EI mass spectrum of the complex afforded ions assigned to ligand fragmentation only. The positive-ion FAB mass spectrum (*m*-nitrobenzyl alcohol as matrix) exhibited $[M + H]^+$ at *m*/*z* 843 (relative intensity 2.3%), adduct ions $[M + Na]^+$ (0.9), and $[M + Fe]^+$ (2.5%) at *m*/*z* 865 and 898, respectively, and strong fragment ions at *m*/*z* 580 (100) and 551 (10%). The negative-ion mass spectrum included ions $[M - H]^-$ at *m*/*z* (40), 578 (16), and 334 (22), 318 (14), 316 (10) and 261 (100%). Those at *m*/*z* 578, 316 and 261 probably indicate the consecutive release of ligands, Fig. 4.

Though the structure of the ligand is very unusual, its biosynthesis can be inferred from some analogies. The 2(1*H*)-pyridone moeity has already been found in funiculosin, ilicolin H, tenellin and bassianin, metabolites of the fungi *Penicillium funiculosum*, *Cylindrocladium ilicola*, *Beauveria tenella* and *B. bassiana*.[19] The complexation abilities of these metabolites and their biological activities are, however, unknown. Based on the known biosynthesis of tenellin,[20] the biogenetic origin of tolypocin might be rationalized as a condensation of an acetate-derived polyketide chain with β-alanine, followed by cyclization of the C=O group with an activated double bond in the side chain. Since there is the on-going debate about the taxonomic assignment of the genera *Tolypocladium* and *Beauveria*,[21] the present work may also contribute to their chemotaxonomy. The present ligand and complex are not directly toxic to insects; their possible role in the pathogenic process is a subject of further study.

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