An EXAFS, and preliminary X-ray crystallographic, investigation of an iron-containing product from the lichen *Cladonia deformis*

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An iron-containing product in the acetone extract from the lichen *Cladonia deformis* has been investigated using chemical, spectroscopic and X-ray crystallographic methods. Visible-near UV, EPR and IR spectra indicate that the iron is present as high-spin Fe(III) and coordinates in an oxygen-containing environment arising from graciliformin (or graciliformin-like) ligands. This has been confirmed by an XAS (X-ray absorption) study using synchrotron radiation. Comparison of the EXAFS and XANES results with those obtained from a model, tris(pent-2,4-dionato)Fe(III), and detailed fitting using the single-scattering, curved-wave formalism for the EXAFS strongly supports the presence of a Fe(III) coordinated to five oxygen atoms from the graciliformin.

Introduction

Lichens contain several low-molecular-weight organic compounds which are capable of absorbing a wide variety of elements from the environment. So much so that their avid efficacy in the uptake and retention of metals and non-metals, including sulphur, has been utilized to monitor air and substrate pollution [1,2].

Several compounds have been separated and identified [3,4], yet little information is available on their complex chemistry. We report the purification, and the chemical and preliminary crystallographic characterisation, together with spectroscopic identification – including a detailed EXAFS study – of the chelate centre of a natural iron-containing product extracted from the lichen *Cladonia deformis*.

Experimental procedures

The lichen *Cladonia deformis* was collected in N. Trøndelag, Norway, and a dark red crystalline material was isolated by Dr. T. Bruun (NTH; deceased 1977), from a sample of about 4.4 kg, using extraction with chloroform. This extract was then fractionally crystal-lised several times from acetone and the acetone extracts finally separated by chromatography on an acidic (0.25 M oxalic acid) Kieselgel G column, using 95 : 5 benzene/methanol as eluant.

The fraction containing a dark red pigment was retained and further purified by crystallisation from acetone (or chloroform) to yield thin, dark red rhombic plates with boundary faces (110) and (001), the latter defining the base plane of the rhomboid. The crystals closely resemble some of the crystal forms described by Zopf [5] many years ago for bellidiflorin, which was first isolated from *Cladonia bellidiflora* (Ach.) var. *coccocephala*(Ach.). Bellidiflorin has also been found in other *Cladonia* species [4].

Elemental analyses of this iron-containing product showed that carbon, hydrogen, oxygen and iron were present. The average composition from four different analyses was: C, 62.43; H, 4.04; O, 30.05; and Fe, 2.75%. No methoxy groups are present, as deduced from standard chemical methods and IR spectra.

The crystals showed a strong tendency to aggregate on the (001) faces. They did not diffract well, and film exposures at ambient and low temperatures suggested that disorder was present.

A small, single crystal of dimensions approx. $0.2 \times 0.2 \times 0.02$ mm was used for measuring cell parameters and intensity data. All intensities were recorded photographically with an oscillating camera and Fe-filtered Co-K$_\alpha$ radiation ($\lambda = 1.7903$ Å). Exposures for time periods of 16 h were required for oscillation ranges of

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6°. Total angular ranges of 116° about [100] and 21° about [010] were explored by successive 6° exposures with 1° overlap.

EPR spectra were registered on a Varian E9 instrument equipped with standard liquid nitrogen attachment. Visible-near UV absorption spectra were measured on a Perkin-Elmer 303 instrument using matched quartz cells and IR spectra (KBr discs) on a Bruker 113 FT-IR instrument.

XAS spectra were obtained at room temperature from finely powdered samples held in kapton-covered holders at the PULS facility, INFN, Frascati. Data points for the XANES were collected at 0.035 eV intervals over the Fe-K-edge (to 125 eV beyond the onset of absorbance) and the EXAFS at 2 eV intervals over a range of 1200 eV.

Results

Crystals of the iron-containing extract from *Cladonia deformis* are orthorhombic, space group $P2_12_12_1$ from systematic extinctions: $a = 37.0$ (3), $b = 25.1$ (3), $c = 21.2$ (3) Å; $V = 19\,688(400)$ Å; $D_m = 1.415(2) Mg \cdot m^{-3}$. These values lead to a molecular weight of 2100(45) with $Z = 8$, or 4200 (90) with $Z = 4$. The former case corresponds to two crystallographically distinct molecular units in the asymmetric unit. An independent estimate of the molecular weight obtained from the elemental analysis is approx. 2030 – or 4060 – assuming one – or two – Fe atoms per molecule, respectively. The two sets of experimental values are in agreement within the 2σ limit of the data. Diffraction goes out to approx. 40° in 2θ with $Co-K_\alpha$ radiation, corresponding to a resolution (d_{min}) of approx. 2.6 Å. This fact, and the poor crystal quality constitute a major obstacle to a full crystallographic structure analysis.

The powder EPR spectrum of this Fe-containing product gives a three-line signal with apparent g-values of 8.68, 4.1 and 1.69, which are diagnostic for the presence of a high-spin Fe(III) in a rhombic environ-
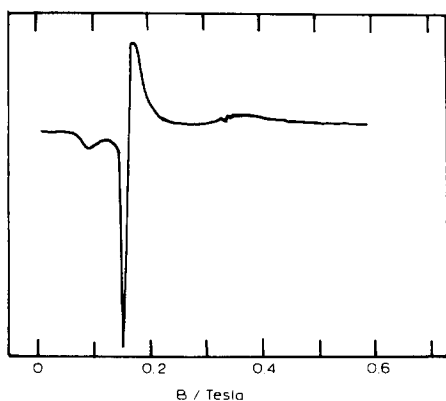
ment [6,7]. There are no signals ascribable to Fe(II) (Fig. 1). The weak lines observed at 0.32 mT are probably due to trace amounts of Cu^{II} [8,9].

The visible-near-UV spectrum (acetone solution) shows no absorbance between 1000 and 500 nm, again in agreement with the presence of a high-spin $[FeO_6]$- or $[FeO_5]$-containing complex. The spectrum may be compared with that of $Fe(pd)_3$(pd = pent-2,4-dionato ion), which gives only very weak ($\epsilon < 2$) spin-forbidden d-d transitions, at 650–750 nm [10], whereas Fe(II) (pd)₃ shows a relatively intense broad d-d band at 819.5 nm [11]. Turning to the non-d-d bands, there is a major absorbance peak at 388 nm, together with shoulders at 402, 435 and 460 nm (Fig. 2). Both the main band and major shoulder lie at energies in close agreement with those found for the bellidiflorin (387 and 465 nm) reported by Ejiri et al. [12]. The IR spectrum (in KBr disc) is very rich in detail in the region below 1200 cm^{-1}. Carbonyl frequencies at 1749 and 1704 cm^{-1} and further maxima at 1618, 1585, 1229 and 1045 cm^{-1} are in fair agreement with the data reported for bellidiflorin (in $HCCl_3$) [12]. The bellidiflorin of Ref. 12 was extracted from *Cladonia graciliformis* and it was demonstrated that removal of the iron present gave rise to graciliformin:
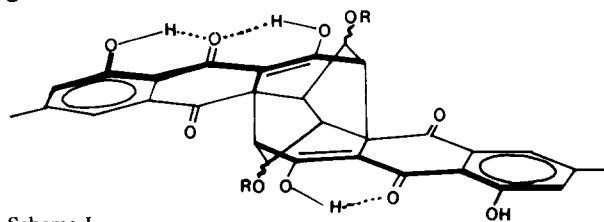
Scheme I

In sum, the presence of α,γ-ketoenolate rings suggests that coordination of the iron occurs via $O \ominus O$ moieties to give six-membered rings analogous not only as regards coordinating atoms but also in second-shell atoms ($C_\alpha$atoms) to those present in tris(pent-2,4-di-

Fig. 1. EPR spectrum (X-band, 77 K) of a powdered sample of Fe product extracted from the lichen *Cladonia deformis*.
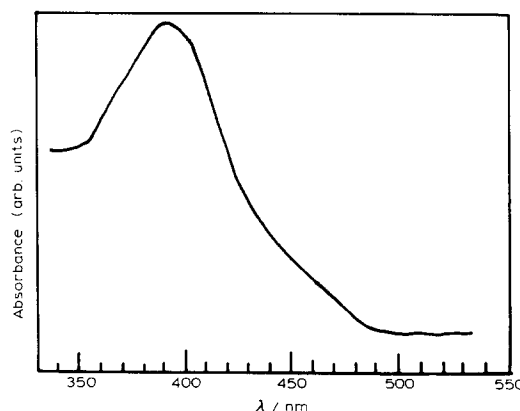
Fig. 2. Visible-near ultraviolet absorbance spectrum (25°C, acetone solution) of the Fe-product extracted from *Cladonia deformis*.

onato)Fe(III) mentioned above, the structure of which is known to contain a high-spin [FeO_6] unit [13]. The latter (prepared as in the Ref. 12 and the references therein) was used as the EXAFS 'model' for extracting the parameters necessary for analysing the data obtained for the Fe-containing product, using both standard, plane-wave formalism [14,15] and the spherical-wave formulation (see below).

Discussion

Fig. 3 shows the experimental Fe-K-edge spectra of the two compounds. The energy positions of the main peak (measured from derivative spectra) are very similar, confirming the presence of Fe(III) in the Fe-lichen product. Further, the relative energies and intensities of the other features in the spectra beyond the edge (up to approx. 60 eV) are also very similar, the minor differences – in peaks b and c – being ascribable to the influence of differences in the backbone structure of the complex ligand present in the Fe-lichen product [16]. Conversely, the spectra of both compounds differ from those of Fe(III) complexes with non-axial structures [17]. Roe et al. [18] have described the pre-edge features of some 28 synthetic iron compounds and found an inverse correlation between the intensity of this band and the coordination number of the Fe. This feature (a in Fig. 3) which may be assigned to the Fe $1s \rightarrow 3d$ transition [18], is of slightly higher intensity in the Fe-lichen product than in Fe(pd)$_3$, indicating that the former may contain the Fe(III) in a five-coordinate, rather than six-coordinate, environment.

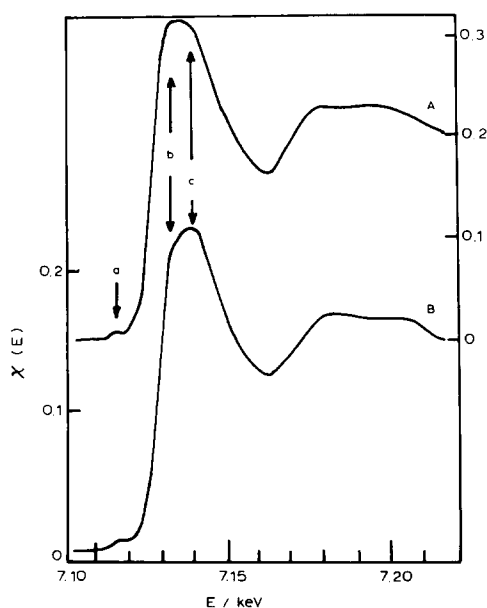The experimental extracted EXAFS of both compounds are shown in Fig. 4. They are almost superim-

Fig. 3. Experimental Fe-K-edge spectrum of Fe(pd)$_3$ (A) and the Fe-product extracted from *Cladonia deformis* (B).

posable, except for details in the regions 8–9 and 10–12 Å^{-1}. The first step in the analysis consisted of performing a series of firstshell fits to the Fourier-filtered $R = 1.5$–2.5 Å region of the EXAFS of Fe(pd)$_3$. This standard procedure [14] gave the results of Table I. A good fit was obtained for a Fe–O distance of 1.99(1) Å, in good agreement with the crystallographic mean distance of 1.99 Å (range 2.004–1.986 Å, from visually estimated intensities [13]). Spherical-wave fits were then performed over an R-range for Fourier-filtering which covered the entire molecule, i.e., such as to include the C_α, C_β and C_γ atoms, and maintaining the Fe–O bond length fixed at 1.99 Å. The ab initio phase shifts and single-scattering spherical wave formalism of Lee and Pendry [19] was used, together with the fast, curved-wave algorithm, the parameterisation of which is described in detail by Gurman et al. [20]. The refined fit obtained is shown in Fig. 5C. As shown by the intermediate stages, it was not possible to obtain good-quality simulation between 4.2–5.0 and 8.0–9.0 Å^{-1} by including the molecular distances alone. However, when a further shell, of intermolecular Fe...C contacts longer than 4.5 Å, was included there was a significant improvement in the fit index. Of particular interest is the fact that the Fe...C_β and Fe...C_γ distances are significantly shorter in the EXAFS, by 0.042 and 0.368 Å, respectively. (Inserting the crystallographic Fe...$C_{\alpha,\beta,\gamma}$ distances in the simulations gave very unsatisfactory fits.) Such EXAFS distance contractions are characteristic of the presence of multiple scattering effects, as found in imidazole-containing complexes and proteins [21]. According to Teo [22,23], multiple scattering effects become more important whenever the central interatomic angle (β) in a three-atom system is larger than approx. 140° and reaches a maximum (highest enhanced forward scattering through the central atom, oxygen in this case) when the three atoms involved are in a line (i.e., $\beta = 180$°). This agrees with the crystallographic results: Fe-O-C_α angles lie in the range 128–130°, too low for multiple scattering effects to come into play, whereas the Fe-O-C_β angles lie in the range 163–166°. This is the first observation of multiple scattering effects in such six-membered rings and further work on 'model' compounds is under way to clarify the procedures for applying corrections.

The EXAFS spectrum of the Fe-lichen product was then simulated using exactly the same procedure, i.e., progressive inclusion of outer shells in the Fourier-filtering windows, keeping the parameters (E_0, etc.) extracted from the analysis of the Fe(pd)$_3$ fixed. Again, the major Fe–O peak in the FT was fitted, varying N (number of neighbours), $\Delta\sigma^2$ (differences in Debye-Waller factors) and R (distance). The results are summarised in Table II. Regular 5, 6 or 7 coordinations gave unsatisfactory fits. Fits improved when two Fe–O distances were inserted. The best fits were obtained for
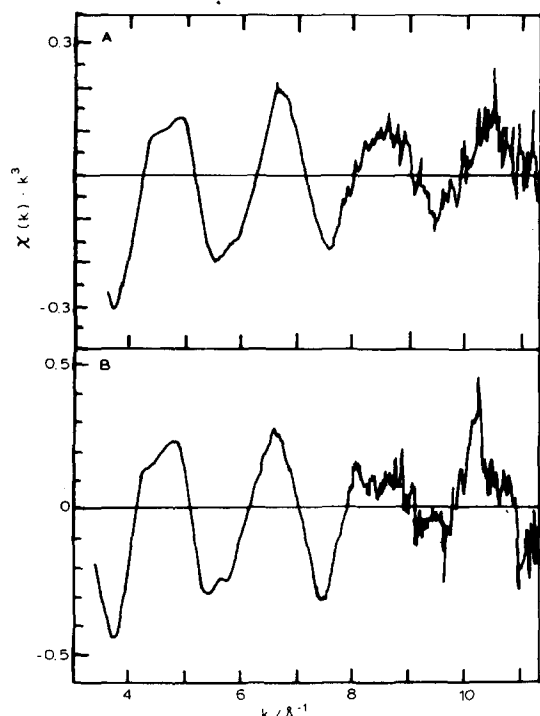
Fig. 4. Experimental extracted EXAFS spectra of the Fe product extracted from *Cladonia deformis* (A) and Fe(pd)₃ (B).

both a rhombic six-coordinate structure and five-coordination with four equal in-plane Fe–O at 1.97 Å and one longer Fe–O at 2.28 Å.

The Fe-lichen EXAFS was then simulated over the

TABLE I

Results of simulations for Fe(pd)₃

	N	$R(\text{Å})$	$2\sigma^2(\text{Å}^2)$
A FeO₆ ,	6	1.99	0.015
B Fe(pd)₃ Fe–O	6	1.98	0.014
Fe...Cα	6	2.99	0.022
Fe...Cβ	3	3.28	0.005
Fe...Cγ	6	3.93	0.035
C Fe(pd)₃+ intermolecular carbon atoms			
Fe–O	6	1.98	0.014
Fe...Cα	6	2.99	0.022
Fe...Cβ	3	3.28	0.005
Fe...Cγ	6	3.93	0.035
(int.) Fe...C	3	4.53	0.001

X-ray crystallography

	N	$R(\text{Å})$	$\sigma^2(\text{Å}^2)$
Fe–O	6	1.989	0.014
Fe...Cα	6	2.992	0.012
Fe...O	3	3.000	0.072
Fe...Cβ	3	3.330	0.012
Fe...Cγ	3	3.900	0.024
Fe...C	3	4.010	0.039
Fe...C	3	4.531	0.002

Table II

Single-shell simulations for C. deformis Fe product

F.I., fit index, defined as:

$$\text{F.I.} = \sum \frac{(Rk^{kT})}{\text{No. of points}} \times 100.$$

R, residual points at point i, $(X_{exp}(k) - X_{theor}(k))$
k, wavenumber at point i.
kT, weighting (3).

	N	R (Å)	$2\sigma^2$ (Å²)	F.I.
Four-coordinate	4	1.96₉	0.014	0.024
Tetragonal	4	1.96₈	0.013	0.011
Five-coordinate	1	2.27₉	0.038	
Five-coordinate	5	1.97₂	0.019	0.062
Seven-coordinate	5	1.97₂	0.018	0.032
	2	2.25	0.035	
Six-coordinate	6	1.97₃	0.025	0.121
Tetragonal	4	1.96₄	0.013	0.014
Six-coordinate	2	2.242	0.062	
Rhombic	2	1.96₁	0.006	
Six-coordinate	2	2.05₄	0.021	0.013
	2	2.11₃	0.048	

R-range up to 5 Å. As for Fe(III)(pd)₃, simulations were not satisfactory when only second shell Cα atoms were included; it was necessary to introduce C atoms as far as approx. 4.5 Å, especially to obtain satisfactory agreement in the range 4–5 Å⁻¹ in the back-transform. However, the 'goodness of fit' was little influenced by the number of C atoms introduced at these long distances.

Thus, the first four oxygen atoms about the Fe in the

TABLE III

Full-shell simulations for C. deformis Fe product

		N	$R(\text{Å})$	$2\sigma^2(\text{Å}^2)$	F.I.
FeO₄ + O	Fe–O	4	1.97₂	0.014	
	Fe–O	1	2.28₂	0.042	
	Fe...Cα	3	2.99₃	0.020	
	Fe...Cβ	3	3.29₅	0.025	0.011
	Fe...Cγ	3	3.88₇	0.036	
	Fe...C	3	4.47₇	0.011	
FeO₄ + 2O	Fe–O	4	1.97₇	0.044	
	Fe–O	2	2.29₁	0.071	
	Fe...Cα	3	2.98₄	0.020	
	Fe...Cβ	3	3.28₇	0.027	0.25
	Fe...Cγ	3	3.88₈	0.034	
	Fe...C	3	4.47₇	0.012	
FeO₆	Fe–O	6	1.97₇	0.025	
	Fe...Cα	2	3.00₅	0.013	
	Fe...Cβ	4	3.30₅	0.033	0.3
	Fe...Cγ	3	3.83₈	0.041	
	Fe...C	3	4.48₄	0.012	

Fe-containing product appear to be in a square-based array of a square-based pyramidal structure. Although a further (i.e., sixth) O atom along the z-axis of the molecule cannot be completely excluded, the measura-

ble shortening of the four Fe–O bonds with respect to those in $Fe(pd)_3$ are in agreement with this conclusion. (A preliminary crystal structure analysis of $(Fe(III)$ $(pd)_2Cl)$ showed it to be square-based pyramidal with
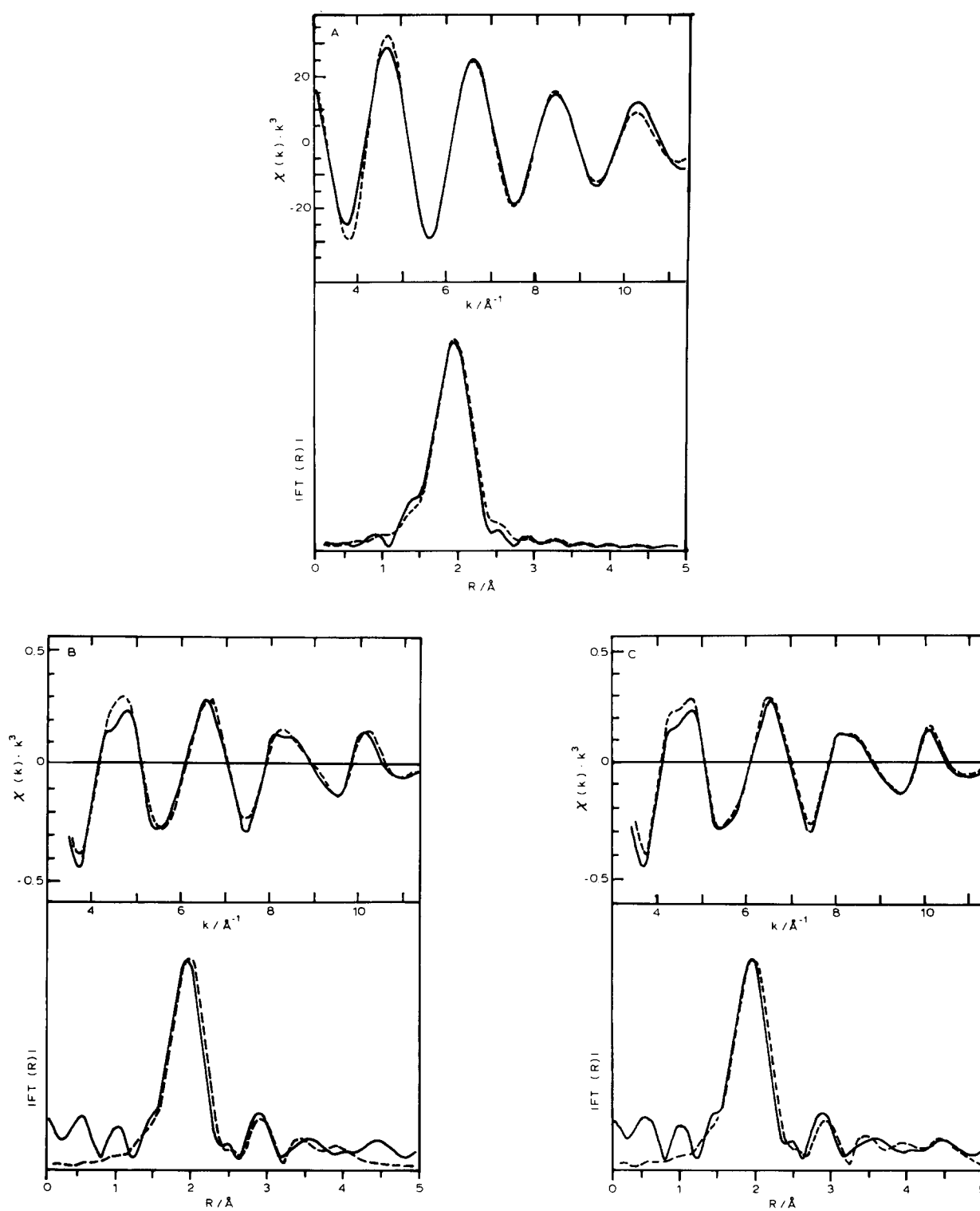

Fig. 5. Fits of the EXAFS spectrum of $Fe(pd)_3$. Fit of FeO_6 shell alone (A); the entire molecule of $Fe(pd)_3$ (B); and the entire molecule of $Fe(pd)_3$ plus the intermolecular C atoms of Table I (C).
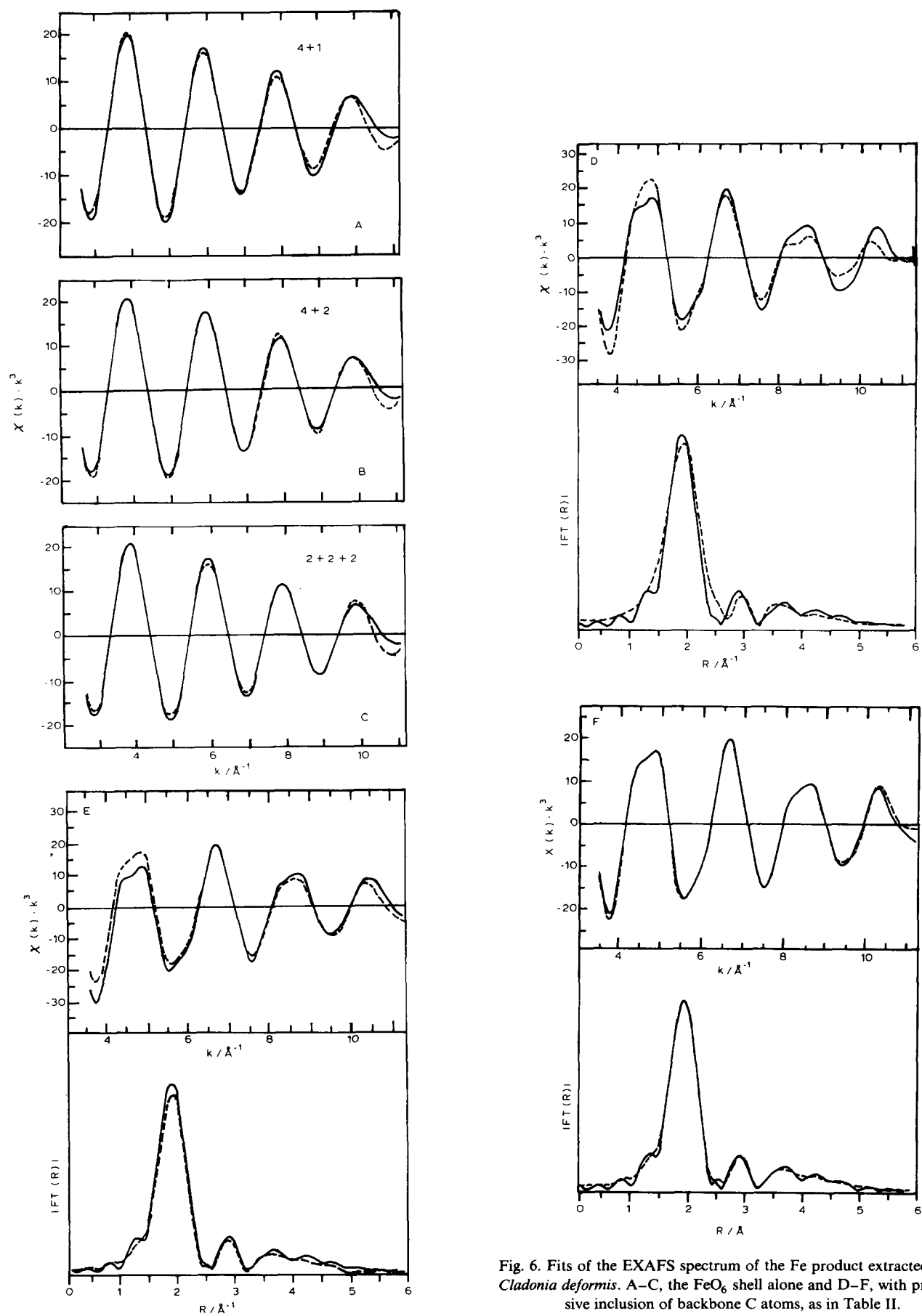
Fig. 6. Fits of the EXAFS spectrum of the Fe product extracted from *Cladonia deformis*. A–C, the FeO_6 shell alone and D–F, with progressive inclusion of backbone C atoms, as in Table II.
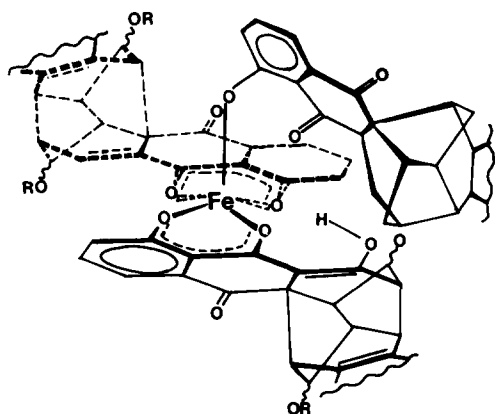
Fig. 7. The coordination (schematic) of graciliformin (or gracilifor-min-like) ligand to Fe in *Cladonia deformis* consistent with the EX-AFS analysis (and supporting evidence).

Fe–O = 1.95(1) Å – the iron being out of the basal plane of the four oxygen atoms by 0.51 Å [24]. A choice between 5- and 6-coordination in metalloproteins or materials entirely surrounded by low-Z scatterers (O, C in this case) based upon very small changes in M–O distances may be vitiated by distortion (as we determined previously [25]). In this case, it seems well-founded because of the close correspondence of fits between 'model' and 'unknown' permitted by using the curved wave formalism. It also nicely agrees with models, which show that three graciliformin (or gracilifor-min-like) molecules, which are quite rigid and bulky, cannot all coordinate as bidentate ligands around Fe(III) at accepted Fe(III)–O distances. Finally, there is no evidence from either EXAFS or XANES (or other spectroscopies) for the presence of two close Fe atoms.

Steric models suggest that the 'outer' complexing sites are involved, as shown schematically in Fig. 7 (which, in addition, leads to blocking of the sixth position). Alternative sites lead to such strong steric crowding that three ligands would not coordinate.

Acknowledgements

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