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The Solution Conformation of the Ferrichromes, II: Proton Magnetic Resonance of Metal-free Ferricrocin and Ferrichrysin, Conformational Implications(*)

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(*)A preliminary report was presented at the Joint Western Regional Meeting of the American Chemical Society and the Society for Applied Spectroscopy, San Francisco, California, October 6-9, 1970. Taken in part from the Ph.D. dissertation of M ..l. at the University of California, Berkeley, 1971. Paper I in this series is reference 13.

Abstract: Ferrichrome, ferricrocin and ferrichrysin are ferric cyclohexapeptides whose general primary composition is represented by $\overleftarrow{[Res^3\text{-}Res^2\text{-}Gly^1\text{-}Orn^3\text{-}Orn^2\text{-}Orn^1]}$, where the $Res^{2,3}$ sites are occupied by glycyl or L-seryl residues and $Orn^{1,2,3}$ stands for δ-N-acetyl-δ-N-hydroxy-L-ornithyl. The 220 MHz proton magnetic resonance spectra of deferriferricrocin and deferriferrichrysin in aqueous and in deuterodimethyl sulfoxide solutions are reported and discussed in terms of the molecular conformations. In $(CD_3)_2SO$ the chemical shift temperature dependences of the amide proton resonances are consistent with structures containing two transannular hydrogen bonds. Such hydrogen-bonding results in an antiparallel β-pleated sheet structure, as in the Schwyzer model for cyclohexapeptides. In terms of sites paired by the hydrogen bonds, however, the β-fold differs. In water a random conformation is suggested for these peptides.

INTRODUCTION

The existence of a number of "doubling reactions" in which linear tripeptides would condense, in a head-to-tail manner, to yield cyclic hexapeptides led Schwyzer, in 1958, to propose a conformational model for this type of compound which features transannular hydrogen bond pairing between two opposite residues to give an antiparallel β-pleated sheet structure (1). Since then, a number of investigators have taken up the problem. Optical rotation (2), proton magnetic resonance (PMR)* spectroscopy (3-7), hydrogen exchange (8) and theoretical calculations (9) are among the approaches employed. Indeed, X-ray studies have identified the presence of Schwyzer-type conformers in synthetic crystalline compounds (10,11). When dealing with such simple compounds, PMR appears to be the method of choice for solution studies. The recent achievement of higher, homogeneous, magnetic fields allows this type of spectroscopy to achieve greater resolution. In particular, the amide NH resonances are highly informative for conformational studies by this technique. Their chemical shift may vary over ranges of up to a few parts per million (ppm) depending on the solvent, temperature, anisotropic shielding effects arising from neighbor groups, and the extent of hydrogen bonding. Ohnishi and Urry first demonstrated the value of chemical shift temperature dependence studies: intramolecularly hydrogen-bonded or sterically buried amide hydrogens exhibit smaller dependences than those that are external and hence exposed to hydrogen-bonding to the solvent (12).

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*Abbreviations: PMR, proton magnetic resonance; p.p.m., parts per million; TMS, tetramethylsilane; TBA, tert. butyl alcohol; d_6-DMSO, deutero-dimethyl sulfoxide.

Previously we have reported a 220 MHz PMR solution conformational study of deferriferrichrome, the iron-free peptide derived from ferrichrome (13). Ferrichrome is the cyclic ferric hexapeptide of structure $\overleftarrow{[Gly^3\text{-}Gly^2\text{-}Gly^1\text{-}Orn^3\text{-}Orn^2\text{-}Orn^1]}$, where Gly stands for glycyl and Orn for the δ-N-acetyl-δ-N-hydroxy-L-ornithyl, the latter residues providing the hydroxamate ligands which chelate the iron (14). We found that while in water the six amide NH resonances of the metal-free peptide are rather equivalent in terms of their chemical shifts and temperature dependences, such is not the case in $(CD_3)SO$, a solvent of relatively lower dielectric constant. In this solvent the data were consistent with a Schwyzer-type structure where the Gly^3 and Orn^3 residues are paired by two transannular, carbonyl-amide, hydrogen bonds (see Fig. 1). It thus seemed of interest to extend that study to the analogous iron-free peptides derived from ferricrocin (→ deferriferricrocin) and ferrichrysin (→ deferriferrichrysin).

The amino acid composition of ferricrocin is known to be two moles of glycine, one of L-serine and three of δ-N-acetyl-δ-N-hydroxy-L-ornithine (15). Evidence that its primary structure corresponds to that of ferrichrome, a seryl residue substituting for a glycyl at site 2 was presented elsewhere (16,17). Ferrichrysin, in turn, differs from ferrichrome in that it has L,L-serylserine ($Ser^3\text{-}Ser^2$) substituting for $Gly^3\text{-}Gly^2$ in ferrichrome (18). Ferrichrysin differs hence from ferricrocin in having a seryl instead of a glycyl residue at site 3. Henceforth, sites occupied by Gly^1, Gly^2 and Gly^3 in ferrichrome will be referred to as sites 1, 2 and 3, respectively (Fig. 1).

The interest in studying two other analogues of deferriferrichrome is obvious: stepwise substitutions of seryl-for-glycyl residues

introduce local perturbations arising from the different steric requirements and solvation properties of the seryl sidechains which are of interest in terms of their effects on the peptide backbone conformation. The ferrichrome compounds act as iron transport agents in microbial species, and the particular conformation of these molecules is intimately related to their ability to penetrate the cytoplasmic membrane.

The spectra of deferriferricrocin and deferriferrichrysin are presented, both in water and in $(CD_3)_2SO$. By homonuclear proton spin-spin decoupling experiments most resonances are assigned by connections to the amide NH peaks. Then the temperature dependence of the chemical shift of the amide region of the spectra will be plotted for both solvents. Finally, data for deferriferrichrome (13) and the two seryl analogues will be compared for the purpose of establishing how these substitutions perturb the deferriferrichrome spectral parameters, and hence its solution conformation.

EXPERIMENTAL

The ferricrocin sample was part of a batch, the production, purification and crystallization of which was described elsewhere (16,17). Ferrichrysin was obtained from low iron cultures of Aspergillus melleus (M2853) in a medium supplemented to 25 mM acetate and 12.5 mM ornithine (19).. The culture was processed in the same way as described for ferricrocin (16,17,19) and the final powder crystallized twice from cold, anhydrous ethanol with a net yield of the ferric peptide of at least (first crop) 100 mg/l. Identity of the samples was ascertained by ascending paper chromatography in 4:1:1 n-butanol:acetic acid:water (20).

The PMR spectra of the deferripeptides confirms their purity (vide infra). The iron-free compounds were prepared by extraction of the metal with a 40-fold molar excess of 8-hydroxyquinoline, followed by chromatography of the peptide on Bio-gel P2, as in the case of deferriferrichrome (13).

The PMR instrumentation and spectroscopic methods are the same as described in Paper I (13) for the studies on deferriferrichrome. A Varian HR220 spectrometer, which operates at 220 MHz, was used. Proton spin-spin decoupling was achieved by double irradiation experiments by sideband modulation. The probe temperature was determined with ethylene glycol. Spectra are referred to internal tetramethylsilane (TMS) or to internal tert. butyl alcohol (TBA) when comparisons between amide NH resonances in H_2O and in d_6-dimethylsulfoxide were desired. The aqueous solutions were 5 mM d_4-acetic acid, adjusted to pH 5.14 with KOH. Water was quartz distilled. $(CD_3)_2SO$ (Merck, Sharp & Dohme of Canada, Ltd.) was certified to be 99.5 atom % D.

RESULTS AND DISCUSSION

The PMR spectra for deferriferricrocin and deferriferrichrysin in deutero dimethylsulfoxide, at 45°C, are shown in Fig. 2, a and b. The regions coupled by proton spin-spin interactions are shown connected by arrows. The overall spectra can be comprehended in a manner similar to that for the spectrum of deferriferrichrome taken under similar conditions (13). The latter spectrum should serve as guide for an approximate identification of the resonances in the analogues. However, the substitution of glycyl residues in ferrichrome by one and two seryl residues in ferricrocin and ferrichrysin, respectively, results in a few new resonances.

The seryl $C_\beta\underline{O}H$ resonances occur at about 4.97 to 5.12 ppm from TMS; due to coupling to the pair of β-hydrogens these resonances are triplets which appear relatively broadened. This can be attributed to hydrogen exchange between the seryl $C_\beta\underline{O}H$ and the free hydroxamic $N\underline{O}H$ an exchange which, in turn, broadens this last resonance relative to that in deferriferrichrome. The exchange, and hence the extent of broadening, are temperature dependent.

Identification of amide $\underline{N}H$ resonances as belonging to glycyl or either ornithyl or seryl residues is readily accomplished from their multiplet structure since the first appear as triplets and either of the latter two as doublets. In principle, it would be possible to distinguish between the seryl and ornithyl resonances on the basis of the sequential spin-spin coupling connections: $\underline{N}H$ (doublet) $\leftrightarrow C_\alpha\underline{H} \leftrightarrow C_\beta\underline{H} \leftrightarrow C_\beta\underline{O}H$ for the seryl and $\underline{N}H$(doublet)$\leftrightarrow C_\alpha\underline{H} \leftrightarrow C_\beta\underline{H} \leftrightarrow C_\delta\underline{H}$ for the ornithyl residues. Unfortunately, this could not be accomplished completely. The rather close proximity of the $C_\alpha\underline{H}$ resonances established by spin-spin decoupling to amide $\underline{N}H$ doublets made it difficult to ascertain which of these is itself coupled to a seryl-assigned $C_\beta\underline{H}$ (established as seryl because of coupling to a $C_\beta\underline{O}H$). The relative proximity of the ornithyl and seryl $C_\alpha\underline{H}$ region to the seryl $C_\beta\underline{H}$ resonances made it cumbersome to directly decouple these last two in the field scanning mode in which the spectrometer operates. In the case of deferriferrichrome this problem did not arise, at least for the assignment of the ornithyl $C_\alpha\underline{H}$'s, since these are all well resolved and the $C_\alpha\underline{H} \leftrightarrow C_\beta\underline{H}$ connections appear less equivocal (13). This resulted in a failure to distinguish coupled seryl $\underline{N}H$-$C_\alpha\underline{H}$ resonances from their ornithyl counterparts.

The broadening of the (seryl) $C_\beta O\underline{H}$ resonances was not a serious problem in the identification of the corresponding $C_\beta\underline{H}$ resonances by spin-spin decoupling. Upon lowering the sample temperature to about 20°C the $C_\beta O\underline{H}$ resonance sharpened into a neater triplet whose collapse by double irradiation could be detected readily. On raising the temperature back to 45°C we assume that, unlike the coupled $C_\beta O\underline{H}$, the position of the seryl $C_\beta\underline{H}$ did not shift appreciably.

Chemical shifts and assignments are given in Table I. Resonances assigned to amide $N\underline{H}$, hydroxamate $NO\underline{H}$ and seryl $C_\beta O\underline{H}$ were confirmed by the disappearance of these peaks upon dissolution in D_2O because of isotope exchange. This exchange resulted, in turn, in a collapse of the multiplets of the spin-spin coupled resonances. The complete 60 MHz spectra of these two peptides in D_2O has been reported by others (15,20).

As in the previous communication (13), we adhere to the convention of using subindices to denote the ordering of the amino acid residues in accord with their order of appearance in the PMR spectrum and superindices for designation of the absolute peptide sequence.

In $(CD_3)_2SO$ the Gly_1 $C_\alpha\underline{H}$ resonates at 3.74 ppm in deferriferrichrome (13), Gly_2 at 3.75 ppm in deferriferricrocin, and Gly at 3.77 ppm in deferriferrichrysin (Table I). Since the glycyl residue in deferriferrichrysin is necessarily at site 1, it is reasonable that these resonances be assigned to Gly^1. This lends support to the assignment of that resonance in deferriferrichrome, given in (13). Furthermore,

the Gly_3 $C_{\alpha}\underline{H}$ is at 3.90 ppm in deferriferrichrome while Gly_1 $C_{\alpha}\underline{H}$ is at 3.88 ppm in deferriferricrocin, affirming the interpretation that Gly_3 corresponds to $Gly^3(12)$. Because of the uncertainties in the assignment of the doublet $N\underline{H}$ resonances in the deferripeptides, no correlation of this type is possible between Gly_2 in deferriferrichrome and the corresponding seryl residue in deferriferricrocin or deferriferrichrysin. Furthermore, while the three ornithyl $C_{\alpha}\underline{H}$ resonances were neatly resolved as individual peaks in the deferriferrichrome spectrum (13), this is not the case for either seryl deferripeptide where the $C_{\alpha}\underline{H}$ resonances assigned to amide $N\underline{H}$ doublets by proton spin-spin decoupling experiments are more clustered together (Fig. 2, a and b, and Table I). The bulky and hydrophilic character of the ornithyl hydroxamate sidechain apparently causes these residues to be sterially more sensitive to the seryl-for-glycyl substitutions. Small rotations along the peptide backbone bonds at the ornithyl sites could result in changes in the extent of anisotropic shielding of the $C_{\alpha}\underline{H}$ from neighbor peptidyl π bonds.

The proton spin-spin coupling constants for amides $N\underline{H}$-$C_{\alpha}\underline{H}$ interaction (J_{NC}) are conformationally dependent; for completeness, these values are given in Table II for the deferripeptides in water and in $(CD_3)_2SO$. The values are averages of determinations at different temperatures within the range of the chemical shift temperature dependence studies, this treatment being again justified by an apparent independence of the J_{NC} on temperature within the experimental errors. Exchange broadening of the amide $N\underline{H}$ resonances resulted in diminished resolution of their splittings. In such cases and/or when the number of averaged data points was low, the uncertainties were found to be rather large

(e.g., deferriferricrocin in H_2O, Fig. 3a and Table II). Only those amide NH resonances, the multiplet structure of which could be resolved satisfactorily or estimated from line shapes, are reported.

In Figs. 3 and 4 the amide NH resonances of deferriferricrocin and deferriferrichrysin are shown in water (pH 5.14) and in $(CD_3)_2SO$ at three different temperatures. A feature shown by these figures and already found in the case of deferriferrichrome (13) is a more uniform temperature dependence of the amide NH chemical shifts in water than in dimethylsulfoxide. Furthermore, in water the widths of these resonances were affected by temperature (hydrogen exchange broadening), while this was not the case in $(CD_3)_2SO$, where higher temperatures resulted in net (motional?) narrowing. As a result, it was found possible to resolve satisfactorily overlapping amide NH resonances in $(CD_3)_2SO$, but not in water, simply by varying the sample temperature.

Plots of the temperature dependence of the amide NH chemical shifts, in both water and in dimethyl sulfoxide, for deferriferricrocin and deferriferrichrysin are shown in Fig. 5. The lines are least square fits of the experimental points and the slopes are indicated in parentheses. It was stated above that the slopes of these linear plots serve as useful indicators of the extent of protection (whether by steric shielding, intramolecular hydrogen-bonding or both) of the particular amides within the peptide structure. As Fig. 3a shows for deferriferricrocin in water, the complex band, which at 23.3°C occurs at about 7.3 ppm, did not allow complete resolution of the three overlapping resonances within any measurable temperature interval. At most, a single triplet appeared to shift out towards relatively lower fields

as the temperature was increased, so that a remaining triplet plus a doublet could not be resolved. Hence, only the temperature shift of the center of these last two resonances, indicated by $G_2 + D_1$, is shown in Fig. 5a. In the case of deferriferrichrysin in $(CD_3)_2SO$ (Fig. 5d), the set of points for doublet 5 (D_5) obviously does not satisfy a linear plot over the entire temperature range and a linear trend is manifest only at lower temperatures. The positive sign of the initial slope for the deferriferrichrysin D_5 NH chemical shift should be noticed, as it might imply that the predominant thermally activated process is different for these amides than for the others.

Comparison of Figs. 5a, b, c and d with the corresponding plots for deferriferrichrome under identical conditions (16) reveals that these substitutions result in the amide NH resonances of each demetallopeptide showing wider ranges both in their chemical shifts and in the slopes of the linear temperature dependence plots. These data are summarized below:

	Slope Range$_3$ (ppm/°K)x10^3		Chem. Shift Range (ppm at 23°C)	
	H_2O	$(CD_3)_2SO$	H_2O	$(CD_3)_2SO$
deferriferrichrome	1.21	2.81	0.37	0.57
deferriferricrocin	1.94	4.10	0.37	0.64
deferriferrichrysin	5.14	7.93	0.73	1.02

Although the rather serious steric restriction imposed by the cyclic nature of the peptide is a major conformational determinant, the comparative evidence between the different analogues indicates clearly that minor differences in the composition, arising from single residue

substitutions, also generate significant pressures in establishing the backbone conformation. As was observed for deferriferrichrome (13), the chemical shift and linear slope ranges for deferriferricrocin and deferriferrichrysin are wider in $(CD_3)_2SO$ than in water, suggesting again more constant environments for the amide hydrogens, or a more rigid conformation for the backbone of the peptides, in the less polar solvent. Water and dimethyl sulfoxide are solvents which amplify different effects; the first, solvation of the hydroxyl sidechain, and the second, protection of the intramolecular hydrogen bonds. It is hence relevant to mention here that addition of $DCCl_3$ to deferriferrichrysin in dimethyl sulfoxide results in an enhanced resolution of the amide NH resonances with further increase in the total spread of this region. Thus, at room temperature, a doublet shifts to lower fields out of the complex band composed of three resonances at about 6.8 ppm (see Fig. 4, spectrum at 23.3°C in $(CD_3)_2SO$).

The trend in the ranges tabulated above indicates the seryl-for-glycyl substitution at site 3 results in the greatest conformational effects. In Paper I (13) it was shown that the solution conformation of deferriferrichrome was consistent with a Schwyzer-type structure (1) which is largely maintained in the chelate (Fig. 1). If such a structure is accepted for the metal-free seryl peptides, the position of Ser^2 would be such that its sidechain hydroxyl would be quite exposed to the solvent. This would not be the case, however, for Ser^3. An inspection of a Corey-Pauling-Koltun model clearly shows that its sidechain, lying below the plane of the β-folded sheet, finds itself relatively more shielded from interaction with the solvent. The seryl sidechain will

tend to offer maximum exposure of its hydroxyl group for hydrogen bonding to the solvent and in so doing will perturb the basic deferriferrichrome backbone conformation. Thus the conformational pressure from the hydration energy of the serine might be larger at site 3 than at site 2. Steric hindrances due to bulk spatial interferences between the sidechains would also be expected to result in different conformational stabilities for these cyclohexapeptides. With synthetic cyclic peptides, there is evidence suggesting that the number of cis peptide bonds increases, with concomitant decreases in the cyclization yields, as a result of increased steric interference between sidechains (21,22).

In water, pH 5.14, where the temperature dependences of the different deferriferrichrome amide NH's are quite similar, the resonances labeled D_4 in deferriferricrocin and D_4 and D_5 in deferriferrichrysin show temperature dependences that are significantly weaker than any of the NH's in deferriferrichrome (13,16). Steric and hydration energy conformational pressures could be such that even in water some amide hydrogens now become internal in a conformation different from that of deferriferrichrome. In $(CD_3)_2SO$ D_1 and D_3 in deferriferricrocin (Fig. 5b) and D_2 and D_3 in deferriferrichrysin (Fig. 5d) show reduced temperature dependences, while D_5 in deferriferrichrysin is rather unique among the demetallopeptides in that it exhibits a positive slope at lower temperatures (linear region) and plateaus as the temperature is raised. As already conjectured for the case of alumichrome (13), the evidence supports the view that a non hydrogen-bonded, sterically buried amide NH can exhibit a positive slope in the chemical shift vs

temperature plot. The positive slope shown in $(CD_3)_2SO$ by the deferriferrichrysin D_5 amide NH (Fig. 5d) hence suggests that the conformation arising from the introduction of a second seryl at site 3 results in steric hindrance for this amide hydrogen (compare with equivalent plot for deferriferricrocin, Fig. 5b).

Since both seryl demetallopeptides show at least two amide NH resonances with a decreased temperature dependence in $(CD_3)_2SO$, it is possible that a Schwyzer conformation might be present in either of them. However, in the case of deferriferricrocin the particular conformation does not correspond to that for deferriferrichrome in $(CD_3)_2SO$. This would require antiparallel pairing of the site 3 residue with Orn^3 so that in deferriferricrocin at least one of the glycyl NH's, a triplet, should be in a transannular H-bond and hence manifest a reduced temperature dependence. This was not observed. Once again this points to the conformational influence of the seryl-for-glycyl substitution even in the less polar solvent.

The requirement that in a Schwyzer structure (1) the β-fold pairing be between Orn^i and Res^j, with i=j, implies that in deferriferricrocin the hydrogen bonds bridge Orn^2 with Ser^2. In deferriferrichrysin this same bonding might occur but the data do not exclude the alternate possible pairing of Orn^3 to Ser^3. It is interesting that, at sufficiently low temperature, those amides attributed to the paired residues in deferriferricrocin, namely, D_1 and D_3, resonate at relatively higher fields (Fig. 5b). This same correspondence has been found in deferriferrichrome (13) and in other cyclohexapeptides (4,5,7) and is attributable to anisotropic shielding by the peptidyl groups at each of the two neighbor β-turns.

Similarly, excluding D_5, which most likely represents a buried $\underline{NH}$, D_2 and D_3 in deferriferrichrysin (Fig. 5d) are also shifted to higher fields while exhibiting reduced slopes (Fig. 5d). No significant hydrogen-deuterium exchange differentiation between the amides was observed upon addition of a trace of D_2O to the $(CD_3)_2SO$ solutions of these peptides, thus suggesting fast conformational fluctuations that expose the protected, H-bonded amides to the solvent.

The failure to assign several of the resonances and the poor resolution of the aliphatic bands resulting, in part, from the lack of a simplifying structural symmetry, prevents, for the time being, a more refined analysis of the coupling constants in terms of the peptide conformation. Since no obvious correlation is apparent from comparison of the J_{NC} data for the amides of these two peptides (Table II) and those of deferriferrichrome (13) it is suggested that the conformation of the individual residues differs from one analogue to the other in agreement with the interpretation given above. This might be of biological relevance since iron transport mutants of Salmonella typhimurium have been isolated which show different extent of relative growth response to ferrichrome, ferricrocin and ferrichrysin (23).

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Table I

	Deferriferricrocin						Deferriferrichrysin					
	Gly_1	Gly_2	D_1	D_2	D_3	D_4	Gly	D_1	D_2	D_3	D_4	D_5
$N\underline{H}$	8.44	8.27	8.02	7.98	7.81	7.82	8.35	8.14	7.79	7.76	7.66	7.53
$C_\alpha\underline{H}$	3.88	3.75	4.21	4.02	4.18	3.97	3.77	4.11	4.03	4.06	4.17	4.14
$C_\beta\underline{H}$			3.83 (Ser)					3.81 (Ser)		3.79 (Ser)		
$C_\gamma\underline{H}$					1.67 (Orn)						1.57 (Orn)	
$C_\delta\underline{H}$					3.28 (Orn)						3.49 (Orn)	
$C_\beta O\underline{H}$			5.02 (Ser)					5.12 (Ser)				
$\underline{CH}_3$					2.00 (Orn)						1.99 (Orn)	
$N\underline{CH}$					9.63 (Orn)						9.45 (Orn)	

Chemical shifts, referred to TMS (ppm), of deferriferricrocin and deferriferrichrysin in $(CD_3)_2SO$, at 45°C and 220 MHz. Labeling of residues and their resonances follows the convention given in the text. D_i (i=1, ...,4) denotes an amide doublet and its spin-spin coupled $C_\alpha\underline{H}$ proton. The similar chemical shifts of those $C_\alpha\underline{H}$'s coupled to amide $N\underline{H}$ doublets made the establishment of the corresponding $C_\beta\underline{H}$'s ambiguous by double resonance experiments; hence their assignment to either ornithyl or seryl residues remains undetermined. Those resonances denoted by Ser or Orn were unequivocally assigned by double resonance and/or from their chemical shifts. In all cases, resonances ordered under the same column are assigned to the same residue(s).

Table II

	Deferriferricrocin						Deferriferrichrysin					
	Gly_1	Gly_2	D_1	D_2	D_3	D_4	Gly	D_1	D_2	D_3	D_4	D_5
H_2O	~6.5			~5.6	~6.3	~4.9	~5.8	~6.5	~5.7	~6.9	5.3	4.5
	±0.6			±0.2	±0.5	±0.5	±0.3	±0.1	±0.3	±0.5	±0.3	±0.2
$(CD_3)_2SO$	5.1	5.1	7.3	7.1	5.2	7.5	4.7	7.5	~6.7	~4.6	8.2	5.9
	±0.1	±0.1	±0.1	±0.1	±0.4	±0.2	±0.2	±0.1	±0.4	±0.6	±0.2	±0.1

Spin-spin coupling constants (J_{NC}) in Hz between amide N$\underline{H}$ and C$_\alpha\underline{H}$ protons for deferriferricrocin and deferriferrichrysin in water, at pH 5.14, and in $(CD_3)_2SO$. Residues are labeled as in Table I. Values are averages derived from determinations at different temperatures. The uncertainties are their standard deviations. Poorly resolved splittings are indicated by ~. Gly_2 and D_1 amide N$\underline{H}$'s could not be resolved for deferriferricrocin in water even at 220 MHz so their J_{NC} are not reported (see Fig. 3a). In general, the amide resonances for the deferripeptides in water appear quite broadened and the values for the spin-spin splittings are less accurate. D_i's denote amides that appear as doublets but whose assignment to seryl or ornithyl residues is uncertain.

FIGURE LEGENDS

Figure 1: Planar structure of deferriferrichrome, cyclo triglycyl-tri(δ-N-acetyl-δ-N-hydroxy-L-ornithyl). Evidence that in $(CD_3)_2SO$ the peptide backbone assumes the antiparallel β-pleated sheet (Schwyzer-type) structure shown here has been reported elsewhere (13). Deferriferricrocin and deferriferrichrysin can be generated from this structure by L-seryl-for-glycyl substitutions at site 2 and at sites 2 and 3 respectively. Evidence that these two seryl-containing peptides may also have two transannular H-bonds, which differ, however, from those in deferriferrichrome (denoted by dotted lines in this figure), is presented in the text.

Figure 2: The 220 MHz PMR spectra of (a) deferriferricrocin and (b) deferriferrichrysin at 45°C dissolved in $(CD_3)_2SO$. The peak marked "solvent" arises from the residual H in the solvent. The resonances at lowest field arise from the NOH protons of the three δ-N-acetyl-δ-N-hydroxy-L-ornithyl residues; the group centered at ∿8.0 ppm is the amide NH protons of the six residues. The peaks connected by light arrows are coupled by proton-proton spin-spin interactions and were determined by double resonance. The resonances at ∿5 ppm arise from the seryl hydroxy protons; these peaks are broadened, as is the hydroxamic NOH, due to exchange. Those peaks arising from exchangeable protons sharpen upon reducing the temperature to ∿20°C; the triplet nature of the seryl $C_\beta OH$ resonances then becomes clearly apparent. The spectra are referred to internal TMS.

FIGURE LEGENDS (Cont.)

Figure 3: The amide NH PMR region of deferriferricrocin in H_2O, pH 5.14, and in $(CD_3)_2SO$ is shown in (a) and (b) respectively. Dotted lines indicate temperature shifts of characteristic resonances. In dimethylsulfoxide the peaks are further separated than in water, where the cluster of one doublet and two triplets at lower fields allows only partial resolution of a triplet at intermediate temperatures, leaving the other two unresolved over the whole temperature range. The spectra are referred to internal TBA.

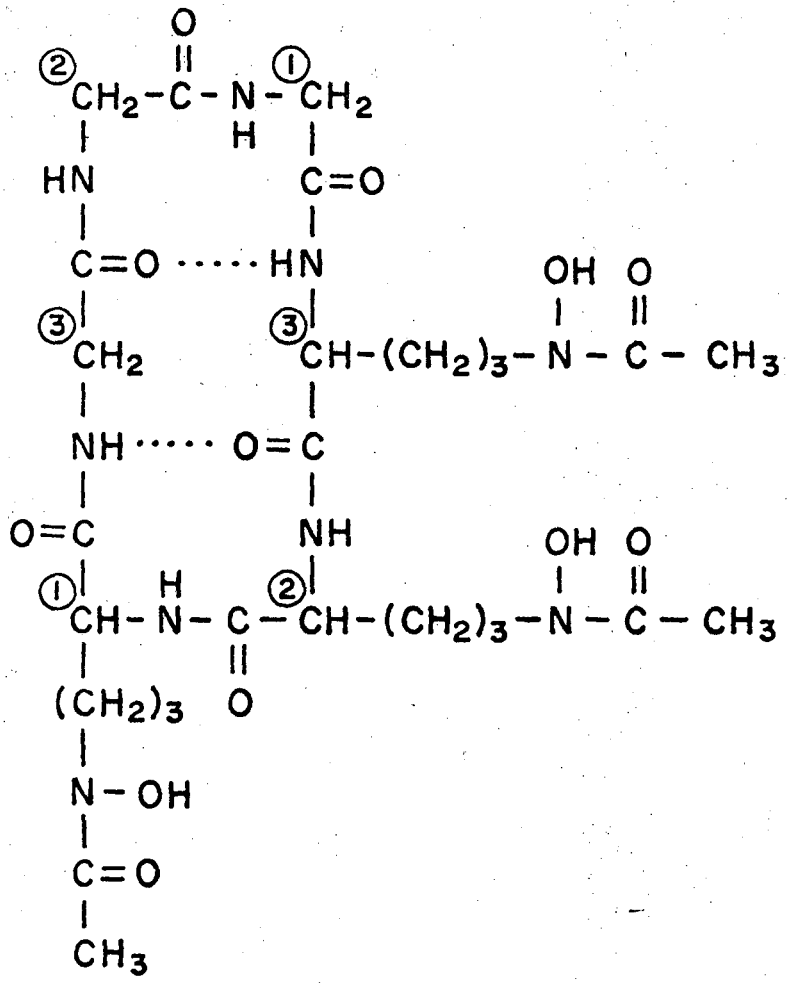
Figure 4: The amide NH PMR region of deferriferrichrysin in water, pH 5.14, and in $(CD_3)_2SO$ is shown in (a) and (b) respectively. Dotted lines indicate temperature shifts of characteristic resonances. Even though the peaks are less separated in H_2O than in $(CD_3)_2SO$, the different temperature dependence of the resonances and certain spectral details permit the peak drifts to be followed unequivocally even in that solvent. The spectra are referred to internal TBA.

Figure 5: The temperature dependence of the chemical shifts of the amide NH protons of deferriferricrocin and of deferriferrichrysin in H_2O at pH 5.14 and in $(CD_3)_2SO$. G denotes glycyl amide protons while D denotes an amide NH doublet assigned neither to seryl nor to ornithyl residues. The subindex refers to the order in which they resonate in scanning from low to high fields, at 45°C. The numbers in parentheses are 10^3 times the slope of the corresponding lines

FIGURE LEGENDS (Cont.)

Figure 5 (Cont.):

expressed in the graph units, i.e., -5.24 = -5.24×10^{-3} ppm/°C. In aqueous solution of deferriferricrocin Gly_2 and the first doublet (D_1) were never resolved and the line labeled G_2+D_1 refers to the center of the complex band (see Fig. 3a). The chemical shifts in both solvents were measured with respect to internal TBA.



XBL7111-5449

Fig. 1

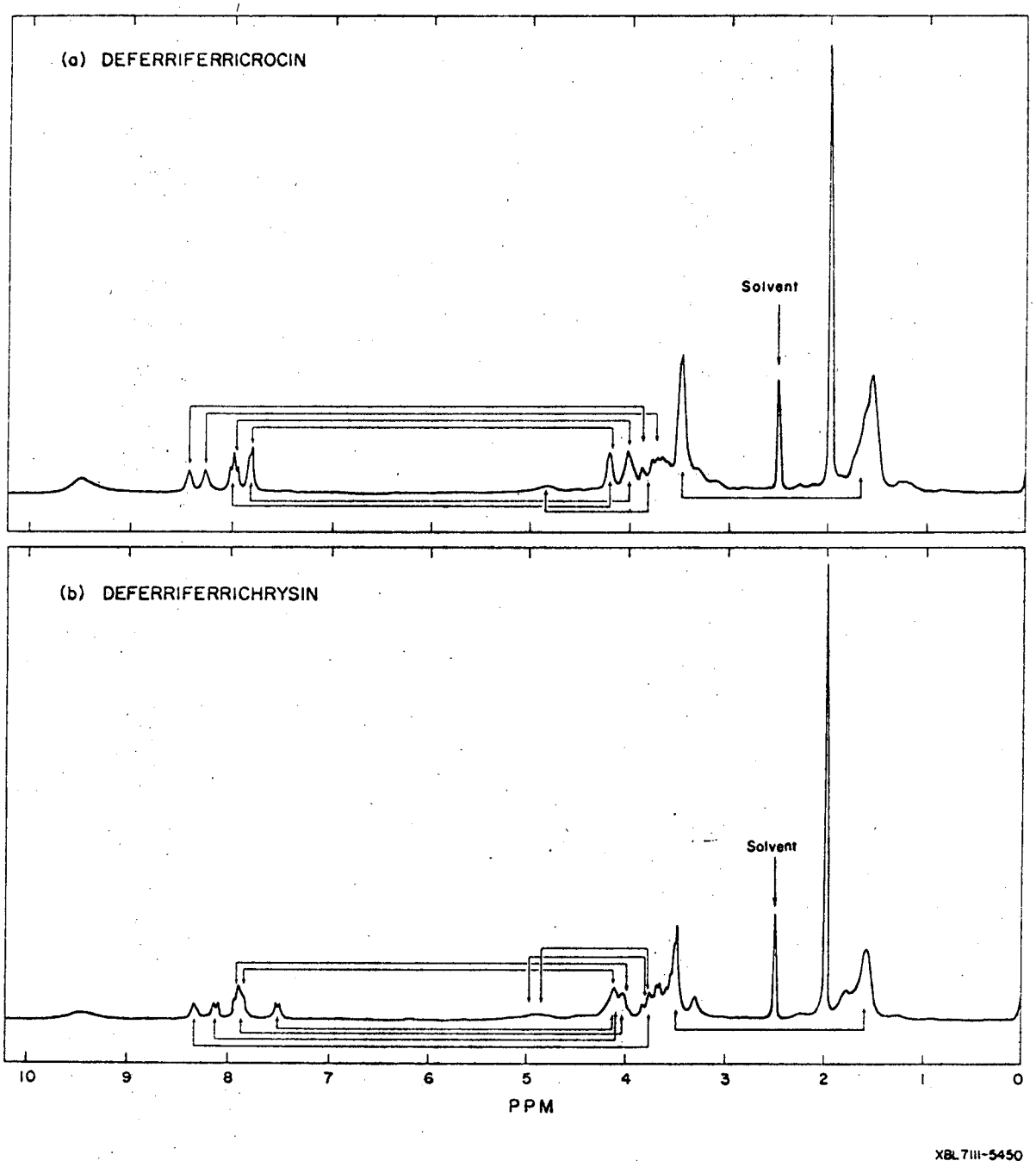
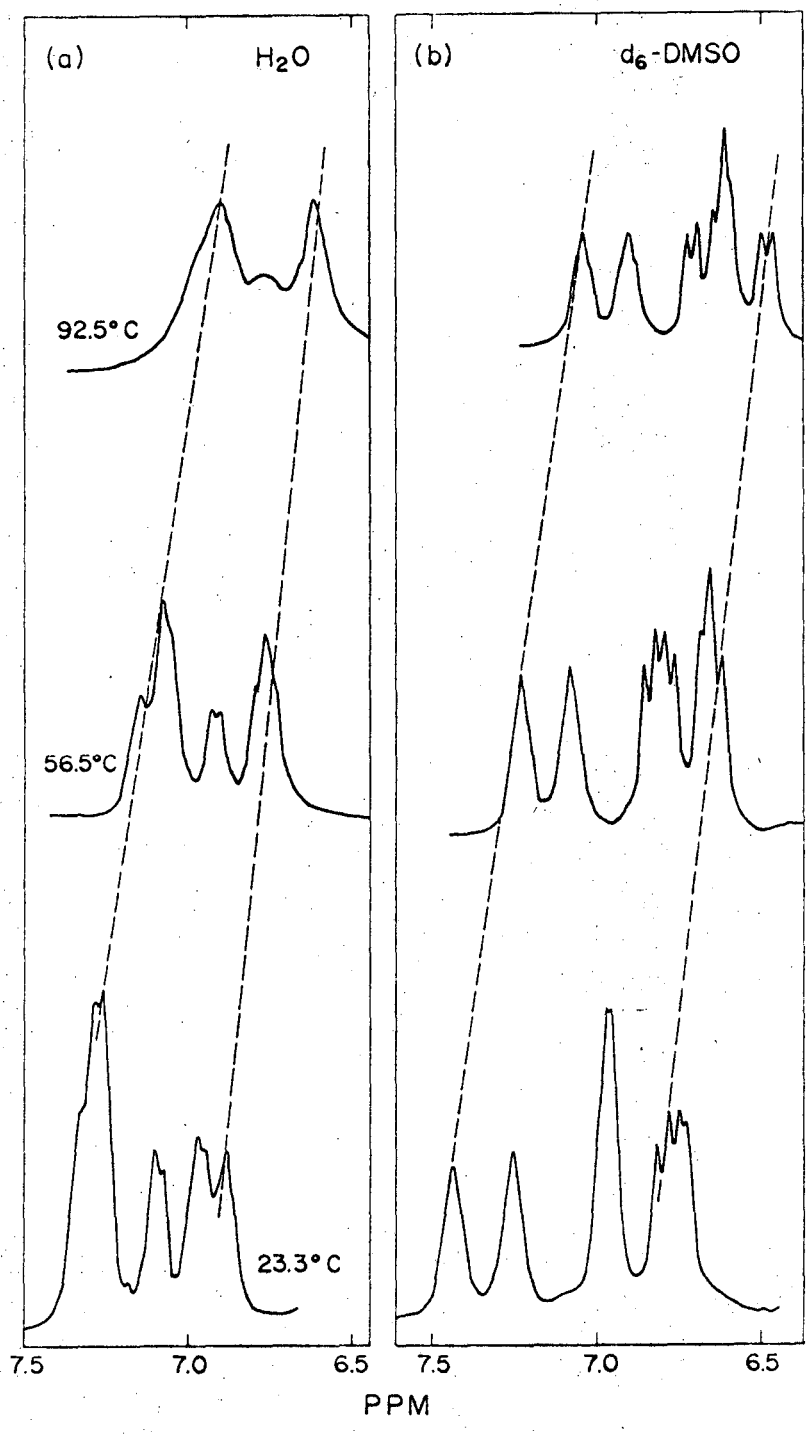

(a) DEFERRIFERRICROCIN
Solvent
(b) DEFERRIFERRICHRYSIN
Solvent
10
9
8
7
6
5
4
3
2
1
0
PPM
XBL7111-5450

Fig. 2

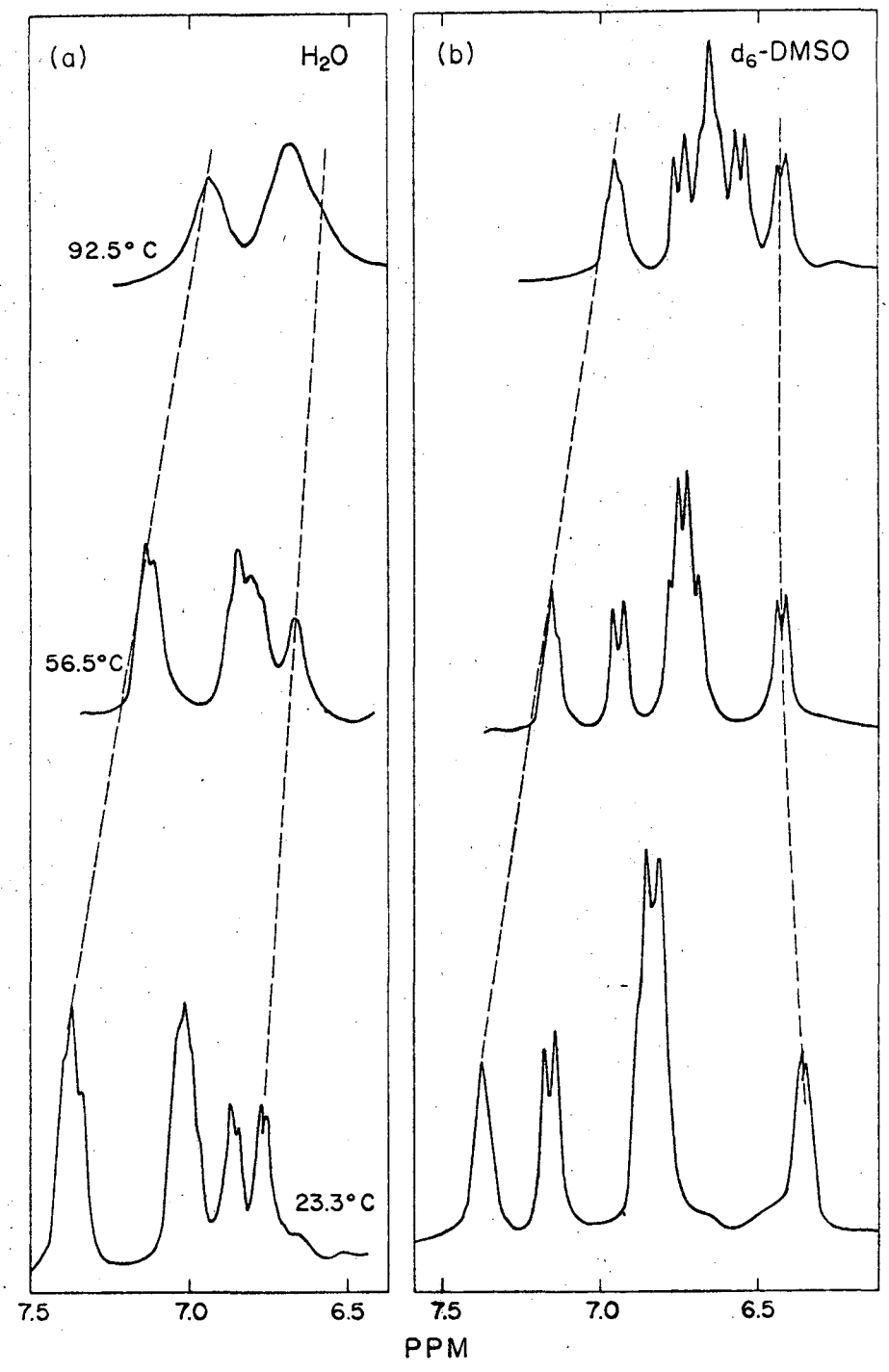
DEFERRIFERRICROCIN



XBL709-5437

Fig. 3

DEFERRIFERRICHRYSIN



XBL709-5438

Fig. 4

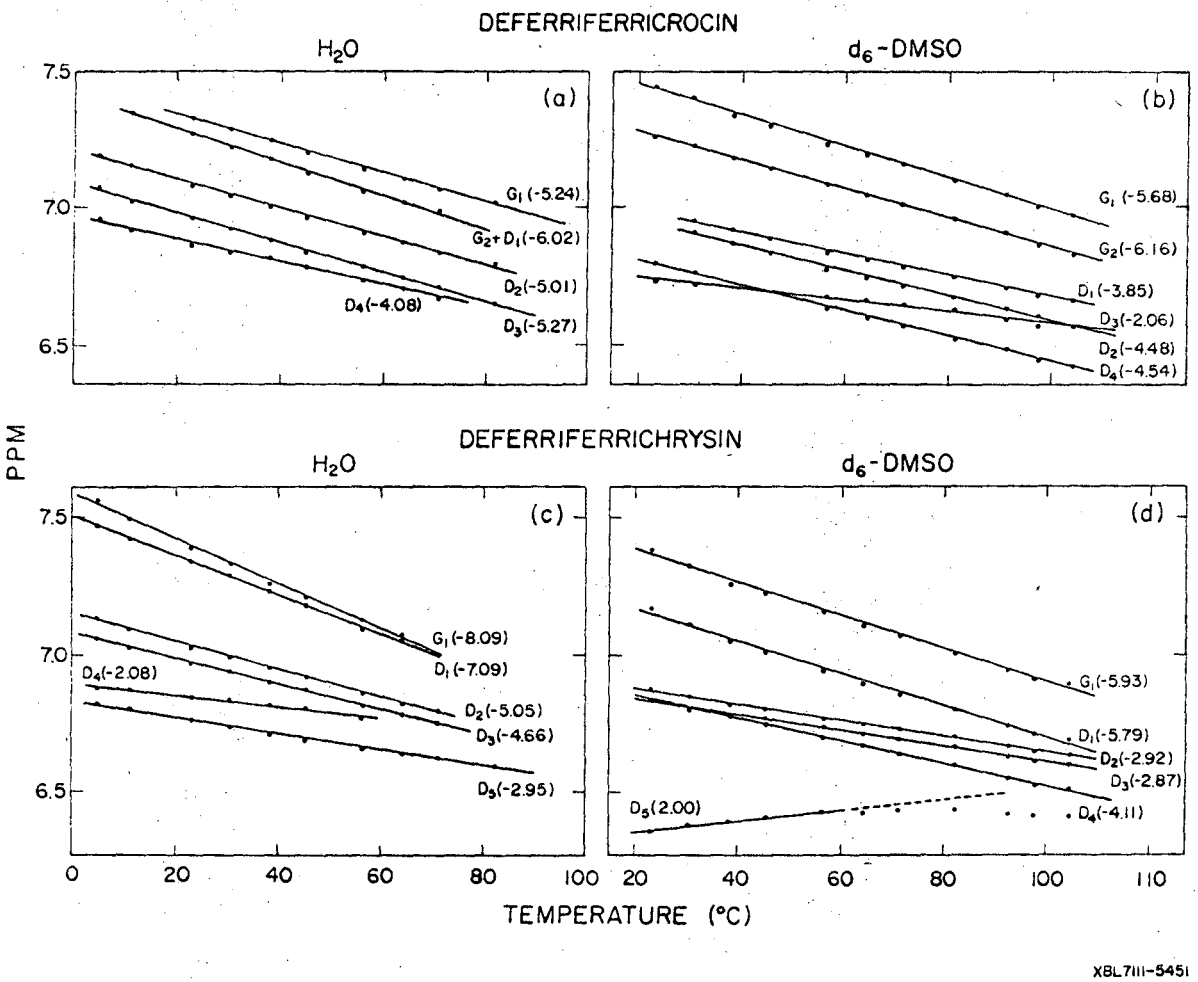

DEFERRIFERRICROCIN
H2O
d6-DMSO
(a)
(b)
G1 (-5.24)
G2+D1(-6.02)
D2(-5.01)
D4(-4.08)
D3(-5.27)
G1 (-5.68)
G2(-6.16)
D1(-3.85)
D3(-2.06)
D2(-4.48)
D4(-4.54)
DEFERRIFERRICHRYSIN
H2O
d6-DMSO
(c)
(d)
G1(-8.09)
D1(-7.09)
D4(-2.08)
D2(-5.05)
D3(-4.66)
D5(-2.95)
G1(-5.93)
D1(-5.79)
D2(-2.92)
D3(-2.87)
D5(2.00)
D4(-4.11)
PPM
7.5
7.0
6.5
0
20
40
60
80
100
110
TEMPERATURE (°C)
XBL7111-5451

Fig. 5

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