Ferrichrome-A Tetrahydrate. Determination of Crystal and Molecular Structure[1]

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Abstract: The crystal and molecular structure of ferrichrome-A tetrahydrate, $C_{41}H_{58}N_9O_{20}Fe \cdot 4H_2O$, has been determined with single-crystal X-ray diffraction techniques including use of anomalous dispersion to establish absolute configuration. The crystals are monoclinic, space group $P2_1$, with two molecules per unit cell with dimensions $a = 11.02$, $b = 13.26$, and $c = 18.22$ A, and $\beta = 99.48°$. The molecule contains a hexapeptide ring with the sequence of amino acid residues -Orn-Orn-Orn-Ser-Ser-Gly- and with a *trans* conformation at each peptide link. The iron atom is bound by three hydroxamate rings in the configuration of a left-handed propeller. Two hydrogen bonds are found within the molecule. Disorder is present in some of the side chains and in one of the four water molecules.

Ferrichrome-A is a metabolic product of the smut fungus *Ustilago sphaerogena*, and it is related in structure to several substances which are growth factors for certain microorganisms.[2-4] The molecule (Figure 1) contains a ferric atom bound by three hydroxamate rings; it includes a hexapeptide ring made up of one glycine, two serine, and three ornithine amino acid residues; and it has three *trans* (β-methyl) glutaconic acid residues. This molecular structure (except for the hydrogen bonding) has been established by chemical methods[5-7] and is fully confirmed by the X-ray crystallographic study described in this paper.[8]

Ferrichrome-A crystallizes from water as the tetrahydrate.[5] We studied these crystals to confirm the chemical results, to determine those details of structure not obtainable by chemical methods, and to seek clues to the structures of proteins. We also made a preliminary study of ferrichrome,[9] a closely related substance which can be crystallized from methanol, but did not determine the structure.

Experimental Section

Many batches of ferrichrome-A crystals, grown from water, were provided us by Professor J. B. Neilands. The diffraction data were obtained from a dark red and opaque crystal with dimensions approximately $0.1 \times 0.1 \times 0.2$ mm. The crystal was glued to a thin glass fiber and handled throughout the course of the experiment in air. After 20 to 30 hr of exposure to X-rays over a period of 2 months it showed no deterioration.

The data were taken on a General Electric XRD-5 unit equipped with a goniostat, a pulse height discriminator, and a scintillation counter. Iron Kα radiation was used, and cell dimensions were based on $\lambda(\alpha_1) = 1.93597$ and $\lambda(\alpha_2) = 1.93991$ A. The tube was operated at 40 kv and 20 ma. The diffracted beam was filtered through a Mn metal film that absorbed 75% of the Kβ and 25% of the Kα radiation.

The background was plotted as a function of θ, the Bragg angle. Where the background was larger due to streaking along a central lattice row, a minimum background was sought on the small-angle side of the reflection (2θ scan); otherwise, background was taken from the plot. Above $2\theta = 50°$ the background was about 2 counts/sec.

Each independent reflection with $\sin \theta/\lambda < 0.51$ ($2\theta < 165°$) was measured. Of these 3115 reflections, 56 were recorded as zero, while the others ranged from 1 count/sec to 17,000 counts/sec. Each reflection was counted for 10 sec using a stationary counter and stationary crystal technique.

The data were corrected for the effects of α_1–α_2 splitting on the basis of a curve derived by comparison of measurements of a set of strong reflections with Kα with measurements using Kβ, which has no splitting. No such correction was necessary up to $2\theta = 100°$. Correction for the Lorentz polarization factor was made by the standard formula.[10]

For copper Kα radiation ($\lambda = 1.54$ A) the out-of-phase amplitude for Fe is $\Delta f'' = 3.4$ electrons. If $k \neq 0$, the intensities of hkl and $h\bar{k}l$ are different in general because of this phase shift. Our crystal was set with the b axis parallel to the ϕ axis, and the settings χ, ϕ, and θ are the same for hkl and $h\bar{k}l$ except that the sign of χ is negative. We found that our General Electric goniostat could be operated as far as $\chi = -20°$, even though the scale extends only to $-10°$, by counting revolutions of the setting wheel. We measured intensities of 71 pairs of reflections with copper Kα radiation and found 40 with considerable intensity differences.

Humidity Effects. The cell dimensions were observed to expand in an anisotropic manner with changes in the humidity. Between 30 and 40% relative humidity, the a, b, and c axes expanded 0.08 (0.008 A), 0.17 (0.022 A), and 0.03% (0.005 A), respectively. The changes in dimensions occurred rapidly and reversibly. The room was not controlled for humidity, and the changes were sufficient to cause the goniostat settings to be off. Three sets of goniostat settings were computed which covered a low, medium, and high range of humidity. By using the appropriate set, the intensities could be accurately and rapidly measured. The relative humidity ranged from a low of 24 to a high of 47% during the measurements.

Computations. The calculations were performed on an IBM 7044 computer equipped with a 32K memory. Least-squares refinements were made with a modified version of the Sparks, Gantzell, and Trueblood program (unpublished). As modified to work under the Fortran IV-IBSYS system, this full-matrix program could only handle 161 parameters. Since over 300 parameters were being adjusted, the refinement was done piecemeal in blocks of 161 parameters or less at a time, each block requiring about 70 min per cycle. Each cycle the assignment of atoms to blocks was changed, but the iron atom was included in every block, and most atoms were in the same block as their immediate neighbors. The function minimized was $\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2$. The weighting factors w were taken as unity. Programs of our own design (unpublished) were used for data processing, Fourier functions, and distances and angles.

The "unreliability index" referred to in this paper is defined as

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[1] Work done under the auspices of the U. S. Atomic Energy Commission.

[2] J. B. Neilands, *Bacteriol. Rev.*, **21**, 101 (1957).

[3] V. Prelog, *Pure Appl. Chem.*, **6**, 327 (1963).

[4] O. Mikeš and J. Turková, *Chem. Listy*, **58**, 65 (1964).

[5] T. Emery and J. B. Neilands, *J. Am. Chem. Soc.*, **83**, 1626 (1961).

[6] S. J. Rogers, R. A. J. Warren, and J. B. Neilands, *Nature*, **200**, 167 (1963).

[7] S. Rogers and J. B. Neilands, *Biochemistry*, **3**, 1850 (1964).

[8] A. Zalkin, J. D. Forrester, and D. H. Templeton, *Science*, **146**, 261 (1964).

[9] Ferrichrome, $C_{27}H_{42}N_9O_{12}Fe$, differs from ferrichrome-A (Figure 1) in that H replaces each $HOCH_2$ (*i.e.*, residues of glycine instead of serine) and CH_3 replaces each $CHC(CH_3)CH_2COOH$ (*i.e.*, residues of acetic acid instead of methylglutaconic acid).[5]

[10] H. Lipson, "International Tables for X-Ray Crystallography," Vol. II, J. S. Kasper and K. Lonsdale, Ed., Kynoch Press, Birmingham, England, 1962, p 265.

$$R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$$

Crystal Data for Ferrichrome. A tiny crystal of ferrichrome[8] about 0.1 mm long and a few hundredths of a millimeter in diameter was studied by the Weissenberg technique with Cr Kα radiation (λ = 2.291 A). The orthorhombic cell has dimensions a = 16.1, b = 29.6, and c = 8.95 A with four molecules per cell. Absent reflections correspond to space group $P2_12_12_1$. The density is estimated to be 1.40 ± 0.05 g/ml by flotation in a mixture of ethylene dichloride and chloroform. This density with the above cell dimensions indicates a molecular weight of about 900, whereas the analysis[5] indicates 741. The discrepancy may result from methanol incorporated in the crystals, but removed by drying prior to analysis; if so, it corresponds to five molecules of methanol per molecule of ferrichrome. Because better crystals of ferrichrome-A became available, no further work was done on ferrichrome.

Unit Cell and Space Group of Ferrichrome-A Tetrahydrate. The crystals are monoclinic with the cell dimensions (at 22° and 36% relative humidity): a = 11.02 ± 0.03 A, b = 13.26 ± 0.03 A, c = 18.22 ± 0.01 A, β = 99.48 ± 0.08°. The precision of our measurements is considerably better than the errors indicated, which include the variations in cell dimensions due to the fluctuations in humidity during the data-taking period.

The cell volume and measured density (1.45 ± 0.06 g/ml)[2] dictate two molecules per unit cell. The density calculated from the X-ray data is 1.42 ± 0.01 g/ml. Reflections of the type $0k0$ are observed only if $k = 2n$. The space group $P2_1$ is the only one consistent with this extinction rule in the observed point group (2). It lacks reflection and inversion symmetry as required for an optically active substance. The twofold general position: (x, y, z; $-x, \frac{1}{2} + y, -z$) accommodates one complete molecule in the asymmetric unit. Because this space group is polar, the origin must be defined with respect to the b direction; the y parameter of Fe was set at zero for this purpose.

Structure Determination

The structure was derived by Fourier methods combined with least-squares calculations in which atoms were judged by the behavior of their individual isotropic thermal parameters. A three-dimensional Patterson function was calculated and studied. Three large peaks appeared: the origin peak and two peaks in the Harker section at $z = \frac{1}{2}$. The Harker peaks were about 12 and 10% of the height of the origin peak. We gambled on the assumption that the largest of the two Harker peaks was the Fe–Fe vector, though it was too large to be a single peak. A least-squares calculation using all the data and the Fe atom alone yielded R = 0.54. Six oxygen atoms forming the anticipated octahedron about Fe were derived from the Patterson function and tested by least squares. The Fe and four of these oxygens refined to R = 0.51. An electron density Fourier was calculated, and the next seven biggest peaks were included in the next series of refinements. Four of these did not refine and were removed. Another Fourier and least-squares procedure similar to the above one was tried.

We were very uncertain of our progress and then calculated a Patterson superposition of the origin peak on the "Fe–Fe" peak. From this pattern the three hydroxamate rings at the iron atom and a scattering of other atoms were guessed at, and the procedure was continued. At this point we had 27 atoms in the refinement, but could not recognize any structure besides the hydroxamate system.

Dispersion Effect with Copper Radiation. Slow progress stimulated us to make the measurements with copper radiation to get more direct evidence of the iron coordinates. Inspection of the results showed immediately that the large effects were associated with large values of $\cos 2\pi[(h/24) + (k/4) + (l/8)]$, corresponding to $x \sim 0.04$ and $z \sim 0.12$ for Fe in agreement with our choice from the Patterson function.

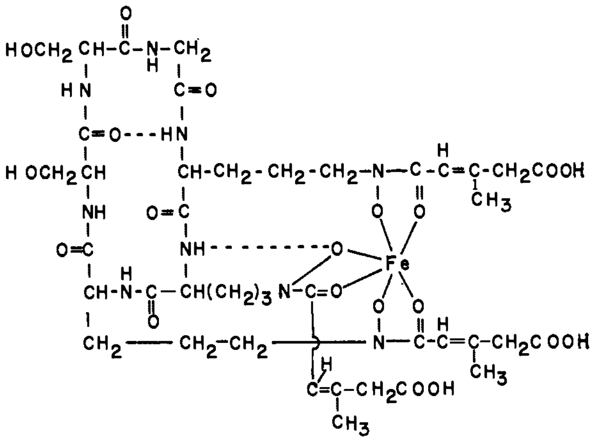

Figure 1. Ferrichrome-A.

Because the refinement began to improve, we made no use of the phase information which can be extracted from these data, but we used them to check the absolute configuration of the structure and as a confirmation of the correctness of our structure determination. After the entire structure is known, one can calculate the magnitude of the effect for any pair of reflections. In Table I we list the intensity difference as a percentage of the average of the two intensities for the 26 cases in which the absolute difference was largest. The agreement is good, and the sign of the effect is correct in each case, as it is also for the next 14 pairs in order of decreasing effect. For the remaining 31 pairs, the effects are small or negligible, and the sign is wrong in 12 cases. If the absolute configuration were reversed, the numerical effects would be the same, but all the signs would be reversed.

Table I. Dispersion Effect for hkl–$h\bar{k}l$ Pairs with Cu Kα Radiation. Values of $200(|F_+|^2 - |F_-|^2)/(|F_+|^2 + |F_-|^2)$

hkl	Obsd	Calcd	*hkl*	Obsd	Calcd
210	−11	−11	0.3.11	−38	−47
015	+27	+32	014	+1	+1
$31\bar{2}$	−14	−16	0.1.11	+18	+13
$31\bar{1}$	−3	−1	0.1.14	+48	+47
312	−44	−55	420	−10	−23
016	−130	−121	$62\bar{2}$	−10	−11
410	+36	+38	634	−7	−3
027	−69	−81	028	−3	−2
0.1.10	−34	−41	019	+44	+37
510	+8	+19	0.3.14	+75	+93
610	−41	−48	$93\bar{6}$	−50	−59
614	+55	+60	0.2.11	−48	−64
520	−8	−8	029	−10	−21

Final Solution. Confident of the Fe position, we continued the procedure of adding new peaks from the Fourier, refining them by least squares, deleting those whose temperature factors became too large, calculating a new Fourier, and repeating the procedure. Occasionally atoms were included where we thought they might be required to link up the developing molecule. About half the atoms we added each time did not refine, but the list of acceptable atoms continued to grow. Finally the hexapeptide ring did resolve, and the

Table II. Atomic Parameters in Ferrichrome-A Tetrahydrate[a]

Atom[b]	*x/a*	*y/b*	*z/c*	B, A^2	Atom[b]	*x/a*	*y/b*	*z/c*	B, A^2
Fe	0.0444	0.0	0.126	3.1[c]	C1Gly	0.419	0.159	−0.294	4.0
O1Orn1	−0.238	0.453	−0.507	4.6	C2Gly	0.474	0.130	−0.362	4.2
O2Orn1	−0.204	0.462	−0.186	4.0	O1Mga1	0.135	0.133	0.133	3.8
N1Orn1	−0.171	0.319	−0.330	3.6	O2Mga1′	0.463	0.404	0.297	7.0[e]
N2Orn1	0.280	0.043	0.204	3.7	O2Mga1″	−0.419	0.289	0.227	9.6[e]
C1Orn1	−0.183	0.402	−0.453	3.6	O3Mga1′	−0.390	0.392	0.221	10.4[e]
C2Orn1	−0.249	0.372	−0.390	3.5	O3Mga1″	−0.430	0.445	0.261	11.2[e]
C3Orn1	−0.311	0.461	−0.360	3.8	C1Mga1	0.243	0.132	0.175	3.5
C4Orn1	−0.393	0.434	−0.304	4.0	C2Mga1	0.321	0.221	0.190	4.1
C5Orn1	0.400	0.014	0.246	4.1	C3Mga1	0.316	0.302	0.149	4.5
O1Orn2	−0.235	0.164	−0.365	4.3	C4Mga1	0.402	0.393	0.171	7.1
O2Orn2	0.008	0.359	−0.119	3.3	C5Mga1	−0.495	0.381	0.229	9.3
N1Orn2	0.021	0.214	−0.230	3.3	C6Mga1	0.231	0.319	0.076	6.6
N2Orn2	−0.026	0.310	−0.059	3.2	O1Mga2	−0.077	0.462	−0.023	3.8
C1Orn2	−0.176	0.222	−0.319	3.3	02Mga2	0.076	0.414	0.229	5.9
C2Orn2	−0.106	0.179	−0.247	3.2	03Mga2	0.027	0.304	0.309	7.5
C3Orn2	−0.180	0.199	−0.183	3.1	C1Mga2	−0.066	0.368	−0.009	3.6
C4Orn2	−0.127	0.149	−0.108	4.3	C2Mga2	−0.082	0.325	0.063	4.2
C5Orn2	−0.014	0.199	−0.063	3.8	C3Mga2	−0.119	0.370	0.119	4.0
O1Orn3	0.081	0.107	−0.310	4.1	C4Mga2	−0.106	0.313	0.192	4.5
O2Orn3	−0.041	0.009	0.214	3.7	C5Mga2	0.002	0.346	0.247	4.8
N1Orn3	0.303	0.188	−0.304	3.4	C6Mga2	−0.178	0.472	0.117	6.0
N2Orn3	−0.164	0.026	0.194	3.9	O1Mga3	−0.121	0.066	0.081	4.3
C1Orn3	0.106	0.176	−0.266	2.9	O2Mga3	−0.491	0.110	−0.093	7.7
C2Orn3	0.238	0.212	−0.242	3.3	O3Mga3	0.349	0.189	−0.067	9.8
C3Orn3	0.257	0.324	−0.218	3.8	C1Mga3	−0.199	0.059	0.124	5.2
C4Orn3	0.221	0.398	−0.280	3.7	C2Mga3′	−0.321	0.114	0.108	4.4[f]
C5Orn3	−0.235	0.009	0.253	4.8	C2Mga3″	−0.336	0.029	0.081	4.7[f]
O1Ser1	0.416	0.308	−0.439	5.1	C3Mga3′	−0.413	0.064	0.063	4.8[f]
O2Ser1′	0.455	0.172	0.397	5.0[d]	C3Mga3″	−0.411	0.106	0.058	3.4[f]
O2Ser1″	0.400	0.315	0.394	7.8[d]	C4Mga3′	0.453	0.085	0.030	5.2[f]
NSer1	0.186	0.304	0.457	4.5	C4Mga3″	0.465	0.130	0.040	3.4[f]
C1Ser1	0.369	0.230	0.535	3.5	C5Mga3	0.443	0.141	−0.046	6.5
C2Ser1	0.280	0.228	0.463	3.8	C6Mga3′	0.403	0.459	−0.041	8.2[f]
C3Ser1	0.355	0.239	0.397	6.5	C6Mga3″	−0.380	0.214	0.074	7.9[f]
O1Ser2	0.126	0.257	−0.436	4.6	W1	0.249	0.467	0.334	6.2
O2Ser2	−0.122	0.026	0.446	6.4	W2	0.030	0.105	0.352	7.4
NSer2	−0.068	0.370	−0.450	3.7	W3	−0.227	0.447	0.342	6.3
C1Ser2	0.110	0.311	0.508	3.8	W4′	−0.312	0.163	0.474	4.3[g]
C2Ser2	0.006	0.387	0.491	3.8	W4″	−0.347	0.216	0.441	10.0[g]
C3Ser2	0.048	0.492	0.487	4.9	W4‴	0.399	0.497	0.502	5.8[g]
OGly	0.489	0.155	−0.232	5.6	W4⁗	−0.370	0.023	0.451	5.2[g]
NGly	0.388	0.133	−0.432	4.5					

[a] Not including hydrogen atoms. [b] Identification is by a six-letter code. Orn stands for ornithyl, Ser for seryl, Gly for glycyl, Mga for β-methylglutaconic acid, W for water oyxgen, Fe for iron, O for oxygen, N for nitrogen, and C for carbon. [c] Value equivalent to the average motion described by the anisotropic set: B11 = 3.20, B22 = 3.67, B33 = 2.16, B12 = −0.11, B13 = −0.18, and B23 = 0.08 A^2. [d] Disordered hydroxyl in a seryl group. Each was refined as half an oxygen atom. [e] Disordered oxygens on the carboxyl of a methylglutaconic acid chain. The singly primed atoms form one conformation; the doubly primed atoms form the second. Each was refined as half an oxygen atom. [f] Disordered central portion of a methylglutaconic acid chain. Singly primed atoms form one conformation and were each refined as 0.6 of an atom. Doubly primed atoms form the minor conformation and were each refined as 0.4 of an atom. [g] Disordered water molecule. Each was refined as a quarter oxygen atom.

process was continued until the methylglutaconic acid side chains developed, and the molecule was fully determined.

Difficulties were encountered in resolving the structure of one seryl hydroxyl group, one terminal carboxyl group, the central portion of a methylglutaconic acid side chain, and one of the water molecules. Disorder was invoked to explain the difficulties. The disordered atoms were split into fractional atoms in separate locations. Seven of the 75 heavy atoms (hydrogen excluded) were given two locations each, and one water molecule was split into four quarter atoms. This increased the number of atomic positions refined to 85. Attempts to treat the disordered atoms with anisotropic thermal parameters (rather than splitting them into fractional atoms) were not successful. It is interesting that a similar kind of disorder was encountered by Alden, Stout, Kraut, and High.[11]

An electron-density difference function (all atoms except hydrogen subtracted out) gave peaks in approximately the positions expected for 42 of the 68 hydrogen atoms. These hydrogen peaks were found only for parts of the molecule not involved in disorder. None of the hydrogen atoms of water could be found even when anisotropic thermal parameters were used for oxygen. The difference function showed many other peaks similar in height to the hydrogen peaks, or smaller, which may result from errors in the data or from anisotropic motion of other atoms. These 42 hydrogen atoms were included in the final calculations, but the resulting thermal parameters and bond lengths indicate that the resulting positions are not accurate enough to justify reporting them here.

(11) R. A. Alden, G. H. Stout, J. Kraut, and D. F. High, *Acta Cryst.*, 17, 109 (1964).

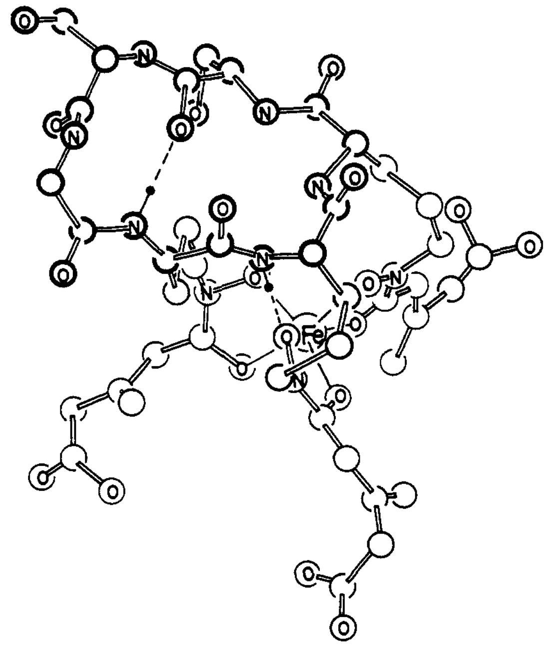

Figure 2. Projection of ferrichrome-A molecule down the crystallographic *b* axis.

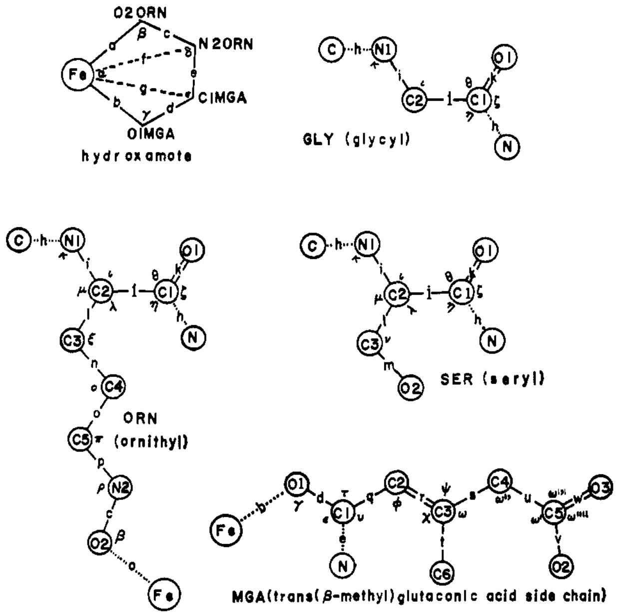

Figure 3. Schematic molecular formulas of the subgroups in ferrichrome-A, to be used in identifying the distances and angles in Tables III, IV, and V.

In the final calculations, with anisotropic thermal parameters only for iron, R was 0.090 for the 3115 reflections. Shifts of coordinates of (nonhydrogen) atoms not involved in disorder were less than the estimated standard deviations by factors that in most cases exceeded 10. Further refinement might lead to a slightly better fit, especially with a larger computer which could refine all the atoms simultaneously. But our lack of progress in the later cycles convinced us that the structure is refined as far as is justified for the present experimental data.[12]

Results

The atomic coordinates and isotropic thermal parameters are shown in Table II. As the full matrix was too large to fit into the computer memory, standard deviations of the parameters could not be calculated properly. From calculations based on as much of the matrix as could fit into the memory, the standard deviations of atomic coordinates are estimated to correspond to 0.002 for Fe and 0.015 A for C, N, and O atoms which are not disordered. A projection of the molecule is shown in Figure 2.

Tables III, IV, and V itemize the distances and angles as designated in Figure 3. The standard deviations of these distances are estimated to be about 0.02 A and of the angles about 1°, except near disordered atoms. The average distances and angles for the planar peptide groups agree well with those given by Cory and Pauling.[13]

There are two intramolecular hydrogen bonds, indicated as dotted lines in Figures 1 and 2. One is across the hexapeptide ring between the carbonyl oxygen of a seryl group (O1Ser2) and the nitrogen of an ornithyl group (N1Orn3). The second hydrogen bond is between the two ends of an ornithyl group (N1Orn2) to (O2Orn2). The two hydrogen-bond distances are 2.98 and 2.80 A, respectively. Several other oxygen atoms are hydrogen bonded to the four water molecules which

Table III. Distances and Angles in the Three Hydroxamate Rings about Fe in Ferrichrome-A[a]

Code	Ring 1	Ring 2	Ring 3	Av
a	1.97	1.96	2.00	1.98
b	2.02	2.03	2.06	2.04
c	1.37	1.39	1.37	1.38
d	1.30	1.28	1.27	1.28
e	1.32	1.31	1.33	1.32
f	2.81	2.79	2.80	2.80
g	2.83	2.79	2.79	2.80
α	78	79	78	78
β	113	112	111	112
γ	115	113	112	113
δ	117	116	116	116
ϵ	116	118	119	118

[a] Italic letter codes are given in A units, Greek letter codes are given in degrees.

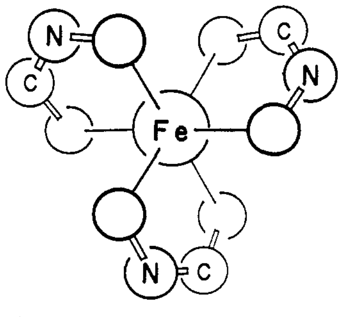

Figure 4. Absolute configuration about the iron atom in ferrichrome-A.

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(12) A table of observed and calculated structure factors has been deposited as Document No. 8701 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the document number and by remitting $1.25 for photoprints, or $1.25 for 35-mm microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

(13) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, p 498.

Table IV. Distances and Angles in the Six Amino Acids in Ferrichrome-A[a]

	Code	Gly	Ser1	Ser2	Orn1	Orn2	Orn3	Av
N–C1	*h*	1.41	1.35	1.33	1.30	1.33	1.32	1.34
C2–N1	*i*	1.47	1.44	1.47	1.45	1.46	1.47	1.46
C1–C2	*j*	1.52	1.51	1.52	1.51	1.52	1.53	1.52
C1–O1	*k*	1.25	1.22	1.23	1.27	1.24	1.22	1.24
C2–C3	*l*		1.57	1.47	1.51	1.54	1.56	1.53
C3–O2	*m*		*b*	1.42				
C3–C4	*n*				1.51	1.55	1.50	1.52
C4–C5	*o*				1.51	1.53	1.54	1.53
C5–N2	*p*				1.47	1.48	1.46	1.47
N–C1–O1	ζ	125	126	121	123	122	121	123
N–C1–C2	η	117	113	116	117	119	117	117
O1–C1–C2	θ	117	121	123	120	119	122	120
C1–C2–N1	ι	115	114	104	113	113	106	111
C2–N1–C	κ	115	121	124	124	120	122	121
C1–C2–C3	λ		108	114	112	109	117	112
N1–C2–C3	μ		111	114	111	114	111	112
C2–C3–O2	ν		*b*	113				
C2–C3–C4	ξ				114	115	114	114
C3–C4–C5	ο				114	117	113	115
C4–C5–N2	π				114	113	110	112
C5–N2–O2	ρ				113	113	114	113
C–N2–C5	σ				130	131	130	130

[a] Italic letter codes are given in A units, Greek letter codes are given in degrees. [b] Disordered serine oxygen. The corresponding two distances and angles (*m* and ν) are 1.12 A, 1.42 A, 117°, and 114°.

Table V. Distances and Angles in the Three Glutaconic Acid Side Chains in Ferrichrome-A[a]

Atoms[a]	Code[a]	Mga1	Mga2	Mga3	Ave
Fe–O1	*b*	2.02	2.03	2.06	2.03
O1–C1	*d*	1.30	1.28	1.27	1.28
C1–N2(Orn)	*e*	1.32	1.31	1.33	1.32
C1–C2	*q*	1.47	1.47	(y)	1.47
C2–C3	*r*	1.30	1.30	(y)	1.30
C3–C4	*s*	1.54	1.51	(y)	1.53
C3–C6	*t*	1.51	1.50	(y)	1.51
C4–C5	*u*	(xx)	1.50	(y)	1.50
C5–O2	*v*	(xx)	1.29	1.26	1.28
C5–O3	*w*	(xx)	1.25	1.22	1.24
Fe–O1–C1	γ	115	113	113	114
O1–C1–N2(Orn)	ε	116	118	118	117
O1–C1–C2	τ	123	122	(y)	123
C2–C1–N2(Orn)	ν	121	120	(y)	121
C1–C2–C3	φ	127	129	(y)	128
C2–C3–C6	χ	126	125	(y)	126
C2–C3–C4	ψ	122	118	(y)	120
C6–C3–C4	ω	112	117	(y)	115
C3–C4–C5	ω''	119	113	(y)	116
C4–C5–O2	ω'	(xx)	120	(y)	120
C4–C5–O3	ω'''	(xx)	121	(y)	121
O2–C5–O3	ω''''	(xx)	120	121	121

[a] Codes are labeled according to Figure 3. Italic letter codes are given in A units, Greek letter codes are given in degrees. (xx) signifies a disordered carboxyl group. (y) signifies disordered C2, C3, C4, and C6 atoms.

in turn are hydrogen bonded to oxygens in adjacent ferrichrome-A molecules. There is disorder in these bonds which involve atoms in alternate sites.

The anomalous dispersion experiment enabled us to determine the absolute configuration of the molecule. The five asymmetric amino acid residues are all found to have the expected L configuration. The three rings at the iron atom have the shape of a left-handed propeller as shown in Figure 4.

At the suggestion of Dr. J. Kraut we calculated the angular twists of adjacent peptides in the hexapeptide ring, in order to compare some actual values of these angles with those postulated by Ramachandran, Ramakrishnan, and Sasisekharan.[14] These authors calculated the range of twisting allowed in terms of two angles ϕ and ϕ' which represent right-handed rotations of the peptide NCO planes as viewed from the α carbon. The observed angles are listed in Table VI.

Table VI. Peptide Twist Angles in Ferrichrome-A

Peptides	ϕ, deg	ϕ', deg
Gly-Orn3-Orn2	35	200[a]
Orn3-Orn2-Orn1	103	311
Orn2-Orn1-Ser2	76	5[a]
Orn1-Ser2-Ser1	17	174
Ser2-Ser1-Gly	123	131
Ser1-Gly-Orn3	262	358[a]

[a] Angles outside the "permissible" limits.

The three values that lie outside the limits suggested by Ramachandran, *et al.*, involve either glycine or hydrogen bonding. These values are not very far outside the outer limits, and an extension of the ϕ' limits by about 20° would include all of these values.

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(14) G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, *J. Mol. Biol.*, 7, 95 (1963).