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Hydroxamic Acids in Nature

Sophisticated ligands play a role in iron metabolism and possibly in other processes in microorganisms.

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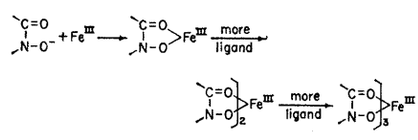
During the past decade a substantial number of natural products which contain one or more oxidized peptide (amide) bonds, —CON(OH)— have been found mainly from microbial sources. These substances, the hydroxamic acids, act variously as potent growth factors, antibiotics, antibiotic antagonists, tumor inhibitors, or cell-division factors. While the particular physiological activity cannot in each case be assigned to the hydroxamic acid bond, this bond is the outstanding chemical feature of these molecules, and it can be expected to play an important role in their biological action. For this reason the properties and characteristics of the natural hydroxamic acids are surveyed here as a group, irrespective of source or alleged function (*1*).

In 1869 H. Lossen treated hydroxylamine with diethyloxalate and obtained a derivative which he called oxalohydroxamic acid (*2*). The capacity of these compounds to yield isocyanates on heating and to form highly colored complexes with iron was early recognized. However, the special affinity of hydroxamate anion for ferric ion seems to have been discovered independently, and many millions of years earlier, by living cells—especially those of microorganisms—which found a need for such agents in their metabolism of iron. The source and biological activity of natural hydroxamates are shown in Table 1. Most of these products reported are from fungi and actinomycetes but they occur also in yeast, bacteria, and green plants (Table 1). Ferrichrysin, and probably also ferrichrome, are found in Japanese sake and account for part of the yellow color (and perhaps the special properties as well) of that beverage (*3*).

Physical and Chemical Properties

Certainly the outstanding property of hydroxamic acids is their ability to dissociate a proton in slightly alkaline media ($pK_a \sim 9$). This allows for attachment of a metal ion in a stable, five-membered ring. Binding occurs in a stepwise manner as the *p*H is raised. Complexes with ferric ion are



the best known, and in this case the 1:1 structure formed at low *p*H is transformed into a 3:1 complex as the *p*H approaches neutrality. The stability constants of some ferric hydroxamates are given in Table 2. A ferric hydroxamate may be distinguished from a ferric enolate by the fact that the former survives decomposition in slightly alkaline solution. The typical 3:1 ferric hydroxamate is a water-soluble, neutral, high-spin complex. The characteristic deep reddish-brown color found in concentrated solution can be diluted to a yellow hue. The absorption band in the visible range is very wide, the maximum is located at 420 to 450 mμ, and the millimolar absorbancy is approximately 3 to 4. At low *p*H a simple ferric trihydroxamate, such as ferric triacethydroxamate, cannot resist competition from protons in the medium, and as a result it is degraded to a 1:1 complex and ultimately dissociated entirely. As the complex passes through the 1:1 stage it assumes a purple color, the absorption maximum is around 510 mμ, and the absorbancy coefficient drops to about one-third of the value for the 3:1 form. When three hydroxamate functions are present in the same molecule, as in the case of ferrichrome, the complex tends to retain the 3:1 structure even at low *p*H. Typical spectra of simple ferric hydroxamates are shown in Fig. 1.

The hydroxamic acid bond may be made to undergo both oxidation and reduction. Reagents which are very efficient for these purposes are Raney nickel and hydrogen gas, hydriodic acid, performic acid, and periodic acid. The last-named is especially useful for characterization of the natural hydroxamic acids. This reagent selectively cleaves the hydroxamic acid linkage while amide and other even more sensitive bonds remain unaffected. The fragments from the "hydrolysis by oxidation" are, apart from the acyl moiety, oxides of nitrogen or dimerization products of nitroxyl derivatives (from primary and secondary hydroxamic acids, respectively). For purposes of detection, it is a fortunate circumstance that virtually all naturally occurring hydroxamic acids are of the secondary variety, that is, they are lacking a hydrogen atom on the nitrogen. On periodate oxidation these compounds yield *cis*-nitrosoalkane dimers which have a millimolar absorbancy coefficient of about 10 at 267 mμ. The periodate oxidation proceeds at an appreciable rate only on the metal-free hydroxamates. It is also necessary to remove iron before the hydroxamate is subjected to hydrolysis in mineral acids, otherwise the hydroxyl-

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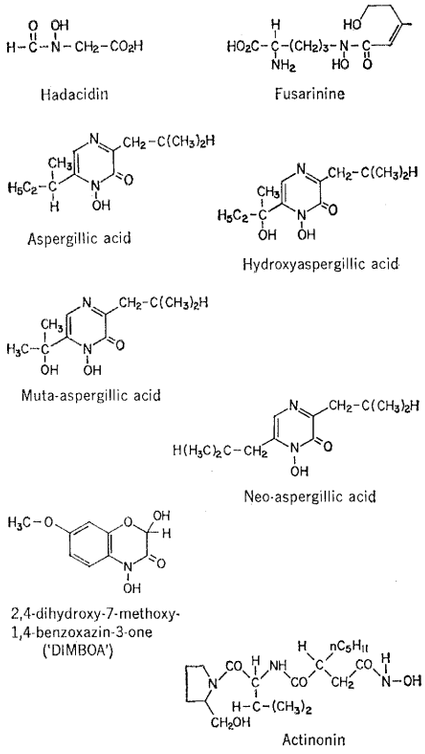
amino group will decompose, producing a bewildering variety of erroneous clues to structure elucidation. Iron can be leached out of ferric hydroxamates with dilute strong base, by extraction with a great excess of a powerful chelating agent such as 8-hydroxy-quinoline or by reduction and trapping with cyanide. Simple hydroxamic acids can be prepared from active acyl parent compounds, such as esters, but thus far no structure containing optically active subunits has been obtained by chemical synthesis.

Chemical Structures

It is convenient to classify the natural hydroxamic acids according to the number of —CON(OH)— groups which they contain. Monohydroxamic acids will occur mainly in the uncomplexed form whereas trihydroxamates may very well be attached to ferric ion. As one may see from the accompanying formulas, a lone hydroxamic acid group occurs in both aliphatic and cyclic structures, of which most are related in one way or another to conventional amino acids.



The unique amino acid, N^{δ}-hydroxyornithine, occurs in fusarinine and in the siderochromes. The aspergillic acids are closely related to amino acid anhydrides or diketopiperazines. Actinonin is the only primary hydroxamic acid in the group but *N*-hydroxyaspartic acid can be synthesized from hydroxylamine and fumaric acid in the presence of aspartase. All of these products are from microbial sources except 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which is present in certain plants as the glycoside.

Among the dihydroxamic acids, both mycobactins P and T contain the next higher homolog of N^{δ}-hydroxyornithine, namely, N^{ϵ}-hydroxylysine. Obviously, the μ-amino-ω-hydroxyamino acids constitute a favorite means of introducing the hydroxamic acid bond.

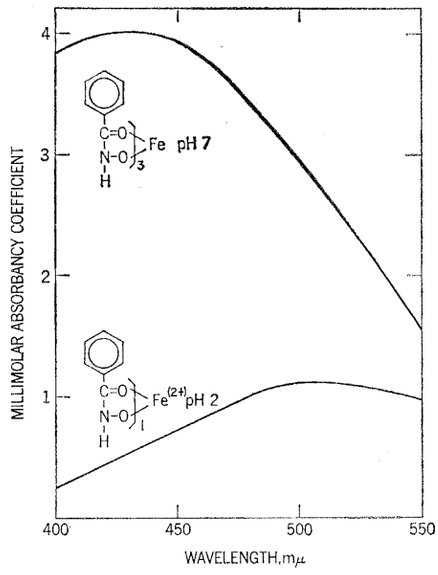


Fig. 1. Spectra of ferric benzhydroxamate at *p*H 2 and ferric tribenzhydroxamate at *p*H 7.

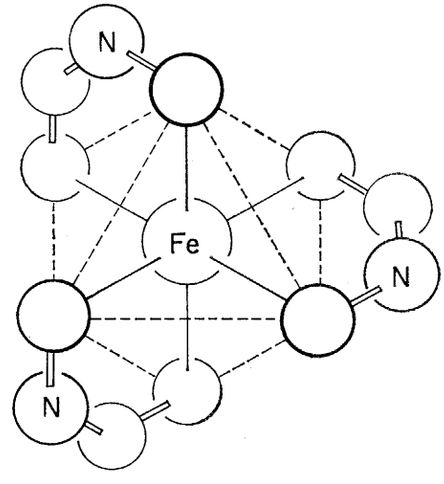
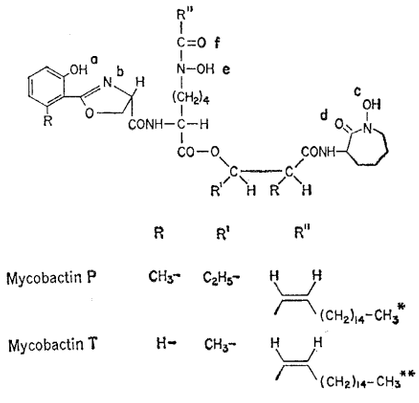


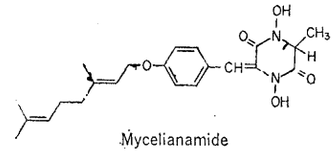
Fig. 2. Absolute configuration and octahedral arrangement of the six oxygen ligands coordinated to the iron atom in ferrichrome A.

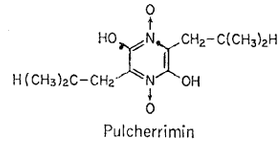
These products form powerful complexes with ferric iron, the third set of coordinating ligand atoms being provided by the hydroxyphenyloxazoline nucleus. Mycelianamide, at least in the nitrogenous moiety, is closely related to the aspergillic acids. In pulcherrimin the cyclic bis-acylhydroxylamine nucleus (of mycelianamide) is raised to a higher state of oxidation. A structure formula can be written indicating that the species contains one hydroxamic acid linkage, but the fully conjugated ring is probably more likely, and this latter structure affords centers in each molecule where ferric ion complexes could form.



The Mycobactins
(letters refer to metal binding sites)

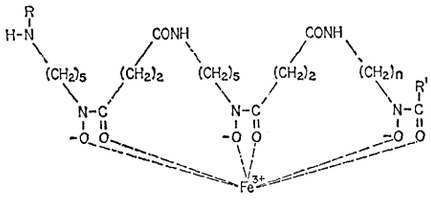
**This is a major substituent at R''; the major C_{14}, C_{16} and *trans*-Δ^2-acids are also present.
**This is a major substituent at R''; the major substituents are unidentified.


Mycelianamide


Pulcherrimin

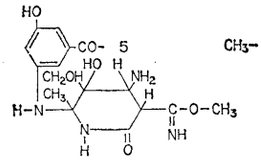
The trihydroxamates form a class comprised of many known members. They occur, at least partially, as the ferric derivatives and may be divided into two groups, the ferrioxamines and the ferrichromes. All natural products with a ferric trihydroxamate center are designated at siderochromes. The ferrioxamines are made up of repeating units of 1-amino-ω-hydroxyaminoalkane and succinic or acetic acid. In the ferrichrome series the basic structural feature is a cyclic hexapeptide with the hydroxamic acid linkages provided by

acyl-N^{δ}-hydroxyornithine. Within the cyclic hexapeptide there is a tripeptide sequence composed of this substituted ornithine; the remaining three residues are made up of glycine, serine, or a combination of these two amino acids. The acyl moiety varies from acetic acid (as in ferrichrome, ferrichrysin, ferricrocin, albomycin) to higher, unsaturated acids such as *trans*-β-methylglutaconic acid (as in ferrichrome A) or the corresponding *cis* and *trans* alcohol analogs of this acid (as in ferrirhodin, ferrirubin). It seems characteristic of the acyl substituent that it is related to mevalonic acid.

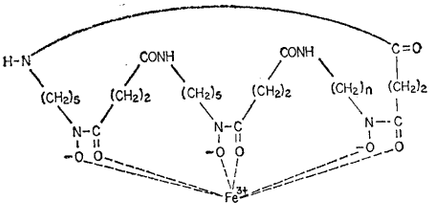


	R	n	R'
Ferrioxamine B	H	5	CH_3–
Ferrioxamine D_1	CH_3CO–	5	CH_3–
Ferrioxamine G	H	5	$HO_2C(CH_2)_2$–.
Ferrioxamine A_1	H	4	$HO_2C(CH_2)_2$–

Ferrimycin A_1*



Metabolite "C" A derivative of ferrioxamine B in which the terminal amino group is oxidized to COOH.

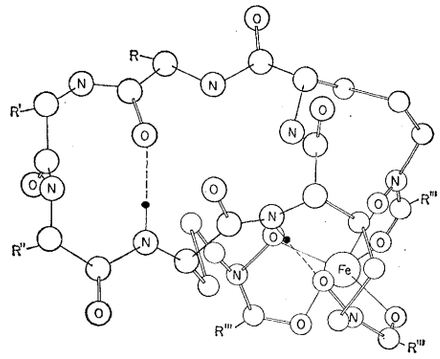


	n
Ferrioxamine E	5
Ferrioxamine D_2	4

Ferrioxamine A_2 contains one residue of acetic acid, two of succinic acid, one of 1-amino-5-hydroxyaminopentane and two of 1-amino-4-hydroxyaminobutane. Components C and F are basic ferrioxamines. *See ref. 13.

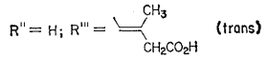
Ferrioxamines

The simpler members of the ferrioxamine series may be prepared in the chemical laboratory. Deferrideoxyferrichrome has been synthesized and shown to be identical with the Raney-nickel and hydrogen gas reduction product of ferrichrome. In an elegant investigation, Zalkin *et al.* (*4*) achieved a complete crystallographic analysis of ferrichrome A. This revealed two hydrogen bridges and established absolutely the configuration around the iron atom (Fig. 2). In the three ferric hydroxamate rings of ferrichrome A the hydroxylamino-oxygen bonds have lengths of 1.97, 1.96, and 2.00 Å; while in the same three rings the distances for the carbonyl-oxygen bonds are 2.02, 2.03, and 2.06 Å, respectively. Thus, all three hydroxamates can penetrate to the octahedrally directed bonds of the central metal ion. The stability is enhanced by anchorage of the three hydroxamates, by way of the N^{δ}-hydroxyornithine side chains, to the cyclic hexapeptide platform.



Ferrichrome: R = R' = R'' = H; R''' = CH_3–

Ferrichrome A: R = R' = $HOCH_2$–;

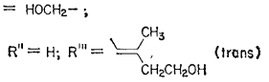


(trans)

Ferrichrysin: R = R' = $HOCH_2$–; R'' = H; R''' = CH_3–

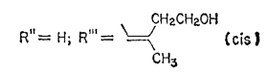
Ferricrocin: R, R', R'' = 2 H, 1 $HOCH_2$–; R''' = CH_3–

Ferrirubin: R = R' = $HOCH_2$–;

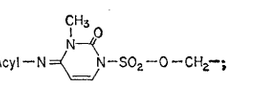


(trans)

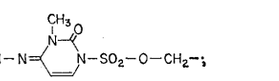
Ferrirhodin: R = R' = $HOCH_2$–;



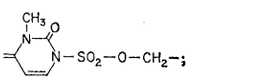
(cis)

Albomycin δ_2: R = Acyl–N= 

R' = R'' = $HOCH_2$–; R''' = CH_3–

Albomycin ϵ: R = H–N= 

R' = R'' = $HOCH_2$–; R''' = CH_3–

Albomycin δ_1: R = O= 

R' = R'' = $HOCH_2$–; R''' = CH_3–

Coprogen: This compound apparently contains the trihydroxamate peptide moiety of ferrirubin, substituted with acetic acid on the amino end of the tripeptide and with "X" on the carboxyl end.

Compound XFe: This substance appears to be closely related to coprogen.

Grisein: This antibiotic may be identical with one of the components of albomycin (11).

Ferrichromes

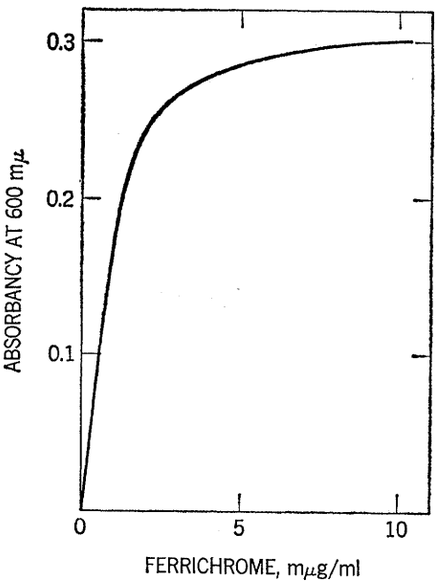


Fig. 3. Growth response of *Arthrobacter terregens* to ferrichrome.

The ferrimycin antibiotics are related to the ferrioxamines but, in view of a certain inherent instability, their characterization has presented special problems.

Biological Function

While the various naturally occurring hydroxamic acids act as growth factors, antibiotics, cell division agents, or tumor inhibitors, it is not known if these effects are related to a single fundamental physiological event and, furthermore, in no case is it possible to account for the action on a molecular level.

Figure 3 shows that ferrichrome is one of the most potent growth fac-

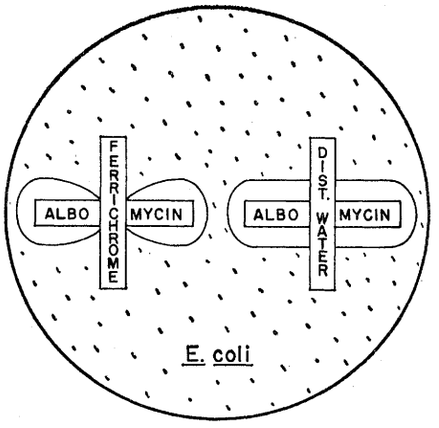


Fig. 4. Inhibition of the growth of *Escherichia coli* by albomycin and its reversal by ferrichrome.

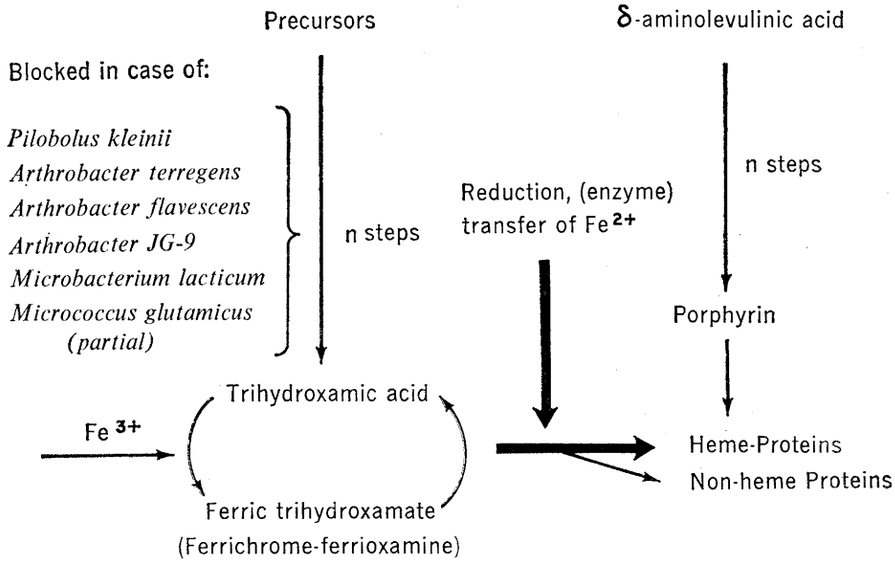


Fig. 5. Hypothesis for the function of ferrichrome in microbial iron metabolism.

Table 1. Source and biological activity of some naturally occurring hydroxamic acids.

Products	Source*	Activity
	Monohydroxamic acids	
Hadacidin†	*Penicillium aurantioviolaceum*	Antitumor agent
Fusarinine	*Fusarium roseum*	
Aspergillic acid	*Aspergillus flavus*	Antibiotic
Hydroxyaspergillic acid	*Aspergillus flavus*	Antibiotic
Muta-aspergillic acid	*Aspergillus oryzae*	Antibiotic
Neoaspergillic-acid	*Aspergillus sclerotiorum*	Antibiotic
Neohydroxyaspergillic acid	*Aspergillus sclerotiorum*	Antibiotic
"DIMBOA"	Plant seedlings	Fungistatic agent
Actinonin	*Streptomyces* sp.	Antibiotic
Schizokinen	*Bacillus megaterium*	Cell division factor
Unidentified compound‡	Unidentified yeast sp.	Growth factor
	Dihydroxamic acids	
Mycobactin P	*Mycobacterium phlei*	Growth factor
Mycobactin T	*Mycobacterium tuberculosis*	Growth factor
Mycelianamide	*Penicillium griseofulvum*	Antibiotic
	Trihydroxamic acids	
Ferrioxamine A_1, A_2, B, C, D_1, D_2, E, F, and G	*Streptomyces* sp.	Growth factor
Ferrimycins	*Streptomyces* sp.	Antibiotic
Succinamycin	*Streptomyces olivochromogenes*	Antibiotic
Danomycin§	*Streptomcyes albaduncus*	Antibiotic
Ferrichrome	*Ustilago sphaerogena*	Growth factor
Ferrichrome A	*Ustilago sphaerogena*	
Ferrichrysin	*Aspergillus melleus*	Antibiotic antagonist
Ferricrocin	*Aspergillus fumigatus*	Antibiotic antagonist
Ferrirhodin	*Aspergillus versicolor*	Antibiotic antagonist
Ferrirubin	*Penicillium variable*	Antibiotic antagonist
Albomycin ‖	*Actinomyces subtropicus*	Antibiotic
Grisein ‖	*Streptomyces griseus*	Antibiotic
Coprogen	*Penicillium* sp.	Growth factor
Terregens factor	*Arthrobacter pascens*	Growth factor
Compound XFe	*Neurospora crassa*	

*Only one source is given here. Ferrichrome, for example, may be obtained from *Ustilago sphaerogena, U. maydis, Aspergillus niger, A. oryzae* and *Penicillium resticulosum*. †Identical with asymmetrin, a plant growth inhibitor of fungal origin (*9*). ‡ Unknown structure, Atkin and Neilands (*10*). § Probably a ferric trihydroxamate. ‖ Albomycin and grisein are probably identical (*11*).

tors known. A typical test organism, *Arthrobacter terregens*, exhibits half-maximum growth at a concentration of about 1 mμg/ml. This corresponds to only a few dozen molecules of ferrichrome per cell. It is not possible to test deferriferrichrome in these systems since contaminating iron readily enters the complex.

A striking property of the ferric trihydroxamate growth factors is their power to antagonize the toxicity of the related antibiotics. Thus, ferrichrome completely reverses the toxicity of albomycin δ_2 for organisms such as *Escherichia coli* or *Bacillus subtilis* (Fig. 4). Among the antibiotics listed in Table 1, albomycin, grisein, and the aspergillic acids are of the broad spectrum type while others, such as ferrimycin and succinamycin, act only on Gram-positive organisms.

For the past decade it has been assumed that the ferrichrome compounds act as iron transfer agents as illustrated in Fig. 5. This role is compatible with the following observations. The ferric complex of ferrichrome forms rapidly and is remarkably stable relative to the ferrous complex. This affords a mechanism for pickup and delivery of trivalent iron, the form encountered by aerobic organisms in nature. At the same time the large discrepancy in stability between the two oxidation states rules out an electron-transfer function, in the manner of heme. The growth-factor activity of ferrichrome is commonly replaced by much higher concentrations of heme, and sometimes by the proper concentration of a synthetic chelating agent, but not by protoporphyrin. All of this suggests that the only role of ferrichrome is to insert iron into porphyrin. Cells of *Arthrobacter* sp. which have been starved for ferrichrome are low in catalase even though the growth medium contains large amounts of inorganic iron. Finally, production of deferrisiderochromes is greatly augmented in iron deficiency. The latter phenomenon runs through bacterial, fungal, and even plant species (*5*) and applies to such diverse structures as mycobactin and fusarinine. The overproduction of the ligand in iron deficiency may be an adaptation which enables the cell to scavenge the metal from inaccessible sources in the environment.

If ferrichrome is required for the insertion of iron into protoporphyrin, and if ferric hydroxamate antibiotics block this reaction, an organism which is

auxotrophic for (and hence permeable to) iron protoporphyrin should be able to grow when supplied with this factor even though the antibiotics may be present. But this has not been found to be true in at least some cases. This does not deny the iron transfer role of ferrichrome but merely indicates that the siderochrome-type antibiotics do not act by interfering with the insertion of iron into porphyrin.

Of one thing we can be certain—in low-iron fermentations with *Ustilago sphaerogena*, all of the metal must pass through the trihydroxamates since the latter are present in enormous excess. Ferrichromes hence play a role in iron transfer in this situation, but at this time it cannot be certain that it is an obligatory function.

Ferrichrome activity can be demonstrated in soil, and this is not surprising in view of the roster of organisms which excrete such compounds (Table 1). It is interesting to speculate on the extent to which green plants may depend on these products for their iron supply. Tomato plants growing in nutrient solution can use the iron from ferrichrome and ferrichrome A for this purpose, and the latter is superior to ethylenediaminetetraacetic acid in alkaline media (*6*).

Hydroxamates apparently do not occur in animal tissues, and this has prompted the thought that it may be possible to build chemotherapeutic agents around such structures, the theory being that this would not interfere with natural processes in the host. This proposal is particularly attractive for the mycobacteria, since such organisms appear to be absolutely specific for the mycobactins, that is, the ferrichromes and related substances are quite unable to satisfy their growth requirements. At least in some cases, specifically that of danomycin, the ferric hydroxamate antibiotics are not highly toxic, and they are effective against pathogenic bacteria in vivo. The usefulness of many of these antibiotics is unfortunately limited by the rapid appearance of resistant strains.

An iron-free trihydroxamate preparation (*7*) has been given to children on a few occasions to combat accidental iron poisoning.

It has already been noted that iron-deficiency conditions favor the production of the hydroxamic acids in many species. At higher levels of iron the presence of substantial amounts of cobalt causes a ferrichromosis. Tracer studies indicate that alternate pathways may exist for the origin of the —CON(OH)— bond. In aspergillic acid the route is by the oxidation of the amide linkage. But in an aliphatic hydroxamic acid such as hadacidin or ferrichrome the mechanism is that of oxidation of the amino group with molecular oxygen (*8*). Such studies have not yet been applied to enzyme degradation or synthesis.

A soil organism, designated *Pseudomonas* Fc-1, is capable of growing on the ferrichromes as sole course of carbon and nitrogen. The initial attack is by way of a peptidase which cleaves the ring at the carboxy terminal position of the acyl-N^{δ}-hydroxyornithyl sequence in either ferrichrome or ferrichrome A. For activity, the hydroxamates must be bonded to a metal such as ferric or aluminum ion. Cyclic peptides in general are not attacked, and it seems the enzyme is relatively specific for the following structure:

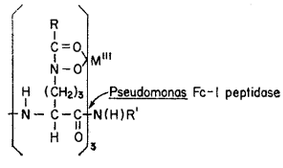


Table 2. Stability constants of some ferric hydroxamates compared with ferric ethylenediaminetetraacetate (*12*).

Ligand	log K	Increment over triacethydroxamate*
Deferriferrichrome	29.1	0.8
Deferriferrichrysin	30.0	1.7
Deferriferrioxamine B	30.5	2.2
Deferriferrioxamine D_1	30.8	2.5
Deferriferrichrome A	~ 32.0	3.7
Deferriferrioxamine E	32.4	4.1
Ethylenediaminetetraacetate	25.1	

*The stability series for triacethydroxamates is $Fe^{3+}>Al^{3+}>Yb^{3+}>La^{3+}$.

Summary

The hydroxamic acid bond occurs in products from fungi, yeast, bacteria, and plants. The —CON(OH)— bond arises by oxidation of a free or bound amino group in a unit structure which is often closely related to conventional amino acids. Products are known with one, two, or three hydroxamic acid groups per molecule. The chemistry of the ferrichrome type compounds, which are ferric trihydroxamate-containing peptides, has been worked out in detail and includes a complete crystallographic analysis of the ferrichrome A molecule. The trihydroxamates form potent complexes with ferric ion, called siderochromes, and these are believed to play a role in the metabolism of the metal ion in microorganisms. The actual physiological activity observed ranges from that of growth factor, antibiotic, antibiotic antagonist, tumor inhibitor or cell-division factor. The precise molecular mechanism whereby these substances exert their potent biological activity remains to be elucidated.

References and Notes

1. Citations of the original literature may, in most cases, be found in the following reviews: J. B. Neilands, *Structure and Bonding* (Springer-Verlag, New York, 1966), vol. 1, p. 59; W. Keller-Schierlein and H. Zahner, *Progr. Chem. Org. Natur. Prod.* **22**, 279 (1964); O. Mikes, J. Turkova, *Chem. Listy* **58**, 65 (1964).
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