triple bond.[30] In **5**, pπ–dπ bonds perpendicular to and in the pseudo-trigonal plane are possible, but the latter interaction may be weak due to the disparity in energy between the nitrogen 2p orbital and the Zr dp-hybrid that is characteristically σ*. The resulting electron density on N combined with the electrophilicity of a three-coordinate zirconium center enables the polarization of a C–H bond, rendering it susceptible to activation. Ground-state steric arguments provide an explanation for CyH vs MeH extrusion rates, but the relatively rapid and reversible PhH loss from **3** may be a consequence of transition-state stabilization by the Ph group. Theoretical investigations of **5** and further substrate and mechanistic studies focusing on the relationship of these activations to related heterogeneous processes utilizing metal oxides,[10] such as the ammoxidation of propylene,[31] are ongoing.

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Supplementary Material Available: Synthetic procedures for amido complexes **1–4** and full analytical data (2 pages). Ordering information is given on any current masthead page.

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Isolation and Structure of the Novel Dihydroxamate Siderophore Alcaligin

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Iron is an essential element for the growth of microorganisms. In an oxidative environment, iron exists mainly as colloidal aggregates of ferric hydroxide, which microorganisms cannot take up. Many bacteria, fungi, and phytoplankton living in aerobic environments are known to excrete siderophores to chelate insoluble iron.[1] Siderophores are virtually specific for ferric iron, have low affinity for ferrous iron, and are not produced when iron is available to the microorganisms. Such chelators are generally classified into two main groups from their structures, e.g., secondary hydroxamic acids and catechols.[1]

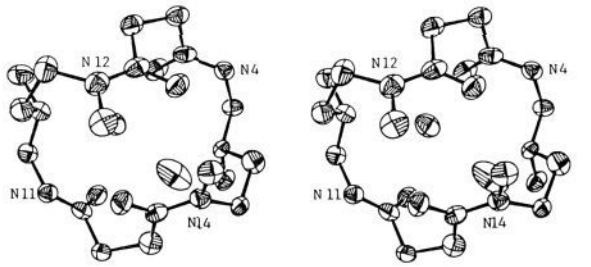

Figure 1. Stereoview of alcaligin. Selected bond lengths (Å) and angles (deg), errors in last digit shown in parentheses. Lengths: O3–C21, 1.235 (7); O9–C18, 1.241 (8); O5–C16, 1.424 (7); O1–N14, 1.383 (6); N4–C18, 1.335 (8); N4–C25, 1.486 (8); N14–C17, 1.346 (7), N14–C13, 1.458 (7). Angles: O2–C17–N14, 121.42 (50); O1–N14–C17, 118.12 (43); C14–C13–C15, 111.34 (45); C25–N4–C18, 121.35 (49); O5–C16–C25, 109.75 (44); O9–C18–N4, 121.17 (59); O3–C21–C30, 119.69 (54); O1–N14–C13, 113.65 (41).

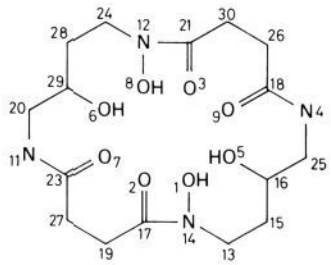

Figure 2. Structure of alcalignin.

Among hundreds of heterotrophic bacteria isolated from sediments of a lagoon near lake Biwa, Japan, a bacterium, *Alcaligenes denitrificans* subsp. *xylosoxydans* KN 3-1, giving a positive reaction for a bioassay for hydroxamate siderophore[3] was selected. *A. denitrificans* KN 3-1 excreted the siderophore into the culture fluid of TTG medium (contained 5 g of Tripticase peptone (BBL), 0.5 g of yeast extract (Difco), and 20 g of glucose (Nakarai) in 1 L of tap water) after its mid-logarithmic growth phase over a 2-week period. The final yield of the siderophore was ca. 1.3 mM in a 10-day-old culture fluid. A siderophore designated alcaligin was isolated and purified from 7- to 10-day-old culture fluid by the following procedure: the culture fluid was applied on Dowex 1×4 (base form) and eluted with 2 M of NaCl. The eluate was adjusted to pH 7, saturated with ammonium sulfate, and extracted with the benzyl alcohol–ether procedure,[4] and gel permeation chromatography on a BioGel P-2 column (2.6 × 90 cm) was performed with aqueous concentrate. The alcaligin fractions detected by adding $FeCl_3$ solution were pooled, and the alcaligin was crystallized from water. The recovery of alcaligin through an overall procedure of isolation was about 20%, corresponding to about 100 mg of alcaligin from 1 L of the culture fluid. Contaminating iron was removed by a treatment with 8-hydroxyquinoline,[5] and the product was recrystallized from water.

The results of FABMS spectrometry[6] and elemental analysis[7] indicated that a crystal of alcaligin contains two molecules of H_2O and leads to an empirical formula of $C_{16}H_{28}N_4O_8 \cdot 2H_2O$. Absorption spectrum of aqueous solutions of alcaligin shows max-

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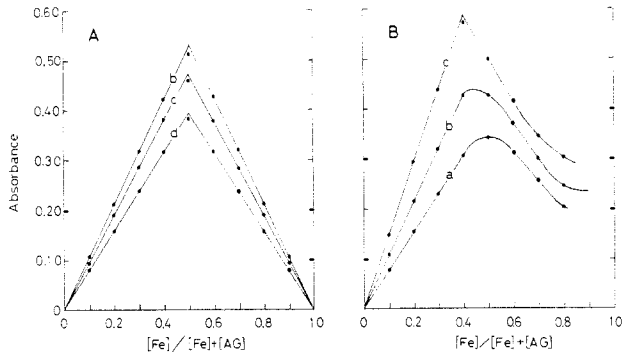

Figure 3. Job's plots of continuous variation method measured at 500 (a), 480 (b), 440 (c), and 420 (d) nm. Experiments were performed with 0.5 mM of alcaligin (AG) and $FeCl_3$ (Fe) at pH 2.0 (A) and 6.0 (B).

imum at 206 nm, while those of ferric alcaligin exhibits maxima at 206 and 426 nm at pH 6.0, which is a typical absorption spectrum for a ferric hydroxamate. As the pH is lowered from 6.0 to 2.0 with HCl, the wavelength of the absorption maximum moves from 426 (ϵ 2432) to 472 (ϵ 2128) nm, and an isosbestic point appears at 450 nm. The red shift at lower pH[8] and the content of bound hydroxylamine[9] indicates that alcaligin has two hydroxamate groups per molecule [IR(KBr) 3420.0 s, 3304.0 vs, 3120.0 s, 2940.0 s, 2832.0 s, 1648.0 vs, 1585.6 s, 1553.6 s, 1478.4 s, 1420.0 s, 1215.7 m, 1174.7 m, 1109.2 m, 1000.1 s, 703.7, 501.0, and 407.4 cm^{-1}]. See ref 10 and 11 for the ^{13}C and ^1H NMR assignments. The chemical shifts were assigned by using results of ^1H homonuclear decoupling, HETCOR, and DEPT experiments. The complete structure determination was given by a single-crystal X-ray crystallography[12] (Figure 1). As shown in Figure 2, alcaligin has a novel structure with a ring dihydroxamate. Hydroxamate siderophores have rarely been found in bacterial species, and ring form trihydroxamates, such as ferrichromes[13] and fusarinines,[14] are all of fungal origin while ferrioxamines[15] occur in actinomycetes. The closest known relative of alcaligin is the very recently described bisucaberin,[16] a cyclic dihydroxamate from the salt water bacterium *Alteromonas haloplanktis*. In alcaligin, the two residues of *N*-hydroxycadaverine of bisucaberin have been replaced by 2 mol of *N*-hydroxyputrescine additionally substituted with an alcohol function in the carbon chain. This type of linker has not been reported previously in the siderophore series.

Analysis of Job's plots of continuous variation method demonstrated that alcaligin chelates ferric iron at a molar ratio of 3:2 at pH 6.0 and 1:1 at pH 2.0. A molecular model shows that intramolecular coordination with iron is sterically capable when two carbonyl oxygens of amide groups other than two hydroxamate groups coordinate to the ferric iron. Unfortunately, we have been unable to crystallize the ferric complex, and elucidation of coordinating atoms remained uncertain. The apparent stability constants were determined by displacement method with EDTA[17] and found them smaller than those of EDTA or known dihydroxamate siderophores.[18] The approximate values of apparent stability constants defined as $K_{Fe_2AG_3} = [Fe_2AG_3]/[Fe]^2[AG]^3$ at pH 6.0 and $K_{FeAG} = [FeAG]/[Fe][AG]$ at pH 2.0 are calculated to be 10^{37} and 10^9, respectively.

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Supplementary Material Available: Precise data for X-ray crystallography, bond angles and distances, and torsion angles and ^1H and ^{13}C NMR spectra (9 pages). Ordering information is given on any current masthead page.

(6) The FABMS was measured on a JEOL JMS-DX303 mass spectrometer. Two major peaks, $[M + H]^+$ m/z 405 and $[M + H - 16]^+$ m/z 389, were obtained. The latter, which corresponds to the loss of an oxygen from the hydroxylamine group, is typical of a hydroxamate siderophore. Dell, A.; Hider, R. C.; Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N.; Neilands, J. B. *Biomed. Mass Spectrom.* **1982**, *9*, 158-161.

(7) Anal. Calcd for $C_{16}H_{28}N_4O_8 \cdot 2H_2O$: C, 43.62; H, 7.34; N, 12.72; O, 36.32. Found: C, 43.64; H, 7.29; N, 12.69; O, 36.23.

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(10) ^{13}C NMR (100 MHz, CD_3OD) δ 29.015 (C-15 and C-28); 31.497, 31.847, 32.328, and 32.620 (C-19, C-27, C-30, C-26); 45.946, 46.705, 46.938, and 48.033 (C-25, C-13, C-20, C-24); 64.818 and 68.408 (C-16 and C-29); 170.516, 174.953, 175.391, and 175.683 (C-17, C-23, C-21, C-18).

(11) ^1H NMR (400 MHz, CD_3OD, 10 °C) δ 1.24 (tm, 1 H, J = 12.8 Hz, 28-H), 1.47 (m, 1 H, 15-H), 1.83 (m, 1 H, 15-H), 2.15 (dt, 1 H, J = 13.5 and 3.9 Hz, 27-H), 2.22 (tm, 1 H, J = 12.9 Hz, 28-H), 2.36 (ddd, 1 H, J = 14.9, 7.1, and 3.7 Hz, 26-H), 2.44-2.58 (m, 4 H, 19-H, 27-H, 20-H, 26-H), 2.67 (ddd, 1 H, J = 16.9, 7.1 and 2.9 Hz, 30-H), 2.87-2.98 (m, 3 H, 25-H, 19-H, 30-H), 3.27-3.37 (m 3 H, 25-H, 13-H, 24-H), 3.49 (tm, 1 H, J = 10.5 Hz, 29-H), 3.59 (m, 1 H, 16-H), 3.70 (dd, 1 H, J = 12.7 and 3.4 Hz, 20-H), 3.98 (td, 1 H, J = 12.7 and 2.5 Hz, 24-H), 4.17 (ddd, 1 H, J = 14.3, 10.0, and 4.4 Hz, 13-H).

(12) Alcaligin was crystallized in the space group $P2_12_12_1$ with a = 11.706 (1) Å, b = 16.200 (2) Å, c = 11.030 (1) Å. Data collection was performed with an automated diffractometer. Reflections in the two octants were measured within 2θ = 120°, with the Cu Kα radiation. The R_{sym} of averaging the equivalent reflections was 0.015. The structure was solved and refined by the automatic structure analysis package for the microcomputer, based on MULTAN 78. The final R factor was 0.059, including 32 hydrogen atoms and two water molecules.

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Total Synthesis of (±)-Methyl Homosecodaphniphyllate: A Remarkable New Tetracyclization Reaction

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The *Daphniphyllum* alkaloids are a group of complex, squalene-derived natural products.[1] In this communication, we

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