Structure of Anguibactin, a Unique Plasmid-Related Bacterial Siderophore from the Fish Pathogen *Vibrio anguillarum*

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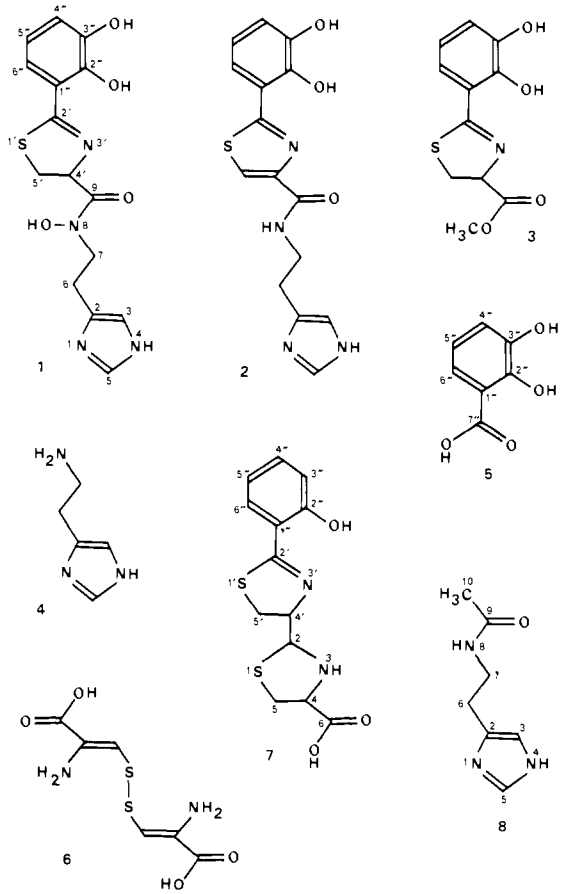
Abstract: Anguibactin (**1**), a novel siderophore isolated from the fish pathogen *Vibrio anguillarum* 775 (pJM1), has been identified as ω-*N*-hydroxy-ω-*N*-[[2′-(2″,3″-dihydroxyphenyl)thiazolin-4′-yl]carboxy]histamine. The structure determination was based on (i) single-crystal X-ray diffraction studies of its anhydro derivative (**2**), (ii) ^1H and ^{13}C NMR spectroscopy, (iii) FAB mass spectrometry, and (iv) chemical degradation. Anhydroanguibactin (**2**) crystallizes in the monoclinic space group $C2/c$, $a = 22.71$ (5) Å, $b = 8.052$ (6) Å, $c = 15.879$ (18) Å, $\beta = 95.72$ (9)°, $V = 2889.2$ Å^3, and $Z = 8$. The structure has been determined from 2029 diffractometer data and refined to a final $R = 0.048$. Spectroscopic results have shown that anguibactin (**1**) differs from **2** by having (i) a thiazoline ring instead of a thiazole ring and (ii) a hydroxyl group on the peptide N atom of the histamine residue. Anguibactin belongs to a unique structural class of its own, with its backbone derived from ω-*N*-hydroxyhistamine, cysteine, and 2,3-dihydroxybenzoic acid. The structure of another related compound, copurified with anguibactin, was identified as 2-(2′,3′-dihydroxyphenyl)thiazoline-4-carboxylic acid methyl ester (**3**).

A novel siderophore (microbial iron transport compound), named anguibactin (**1**), has been isolated recently[1,2] from the iron-deficient cultures of a fish pathogenic bacterium, *Vibrio anguillarum* 775. Preliminary investigations have revealed that anguibactin (**1**) is unique in several respects. The production and biological activity of anguibactin and the virulence of the bacteria are linked to the presence of a 65-kb plasmid, designated pJM1, in the organism.[2,3] Anguibactin is a very important factor of virulence as demonstrated by its ability to cross-feed a siderophore-deficient receptor-proficient mutant of *V. anguillarum*, which allows the establishment of this organism in the host vertebrate.[4] Anguibactin possesses an unusual molecular composition, $C_{15}H_{16}N_4O_4S$, and shows significant structural differences with all known classes of siderophores.[1] Moreover, the anguibactin receptor system has been shown to be highly specific for anguibactin and inert toward a range of bacterial, fungal, and synthetic iron chelators. Attempts to reveal its complete structure have been hindered due to problems associated with its purification in sufficient quantity. However, some aspects of its structure, such as the presence of a catechol group, have already been established.[1] We have achieved success in determining the chemical and molecular structure of one of its closely related derivatives, anhydroanguibactin (**2**), by single-crystal X-ray diffraction. On the basis of this structural information and on the results obtained with NMR and FAB mass spectrometry, we have determined the structure of anguibactin (**1**) and a related compound (**3**), which has been copurified from the culture medium. In this paper, we present the evidence for the structure of anguibactin.

Experimental Section

Organism Source and Culture. The bacteria *Vibrio anguillarum* 775 (pJM1) was grown as described previously.[3]

Purification of the Siderophore. Extraction and initial purification of anguibactin was reported before.[1] A sample (approximately 12 mg), obtained from the previously described Sephadex LH-20/methanol gel filtration, was separated in another Sephadex LH-20 column (20 × 1 cm) with absolute ethanol as the mobile phase. Fractions containing anguibactin were pooled, dried under vacuum, and rechromatographed in a silica gel column (10 × 1 cm; the stationary phase was silica gel 60 H, TLC grade; the mobile phase was chloroform–methanol–water 35:12:2, CMW). The column was deferated by passing 8-hydroxyquinoline through it prior to loading the sample. Two compounds, a slower moving major band (anguibactin, ca. 7 mg) and a faster moving minor band



(identified as **3**., ca. 1 mg), eluted out from the column. This last chromatographic step was repeated once more to separate the partially overlapped fractions. Anguibactin (**1**) was finally purified by gel filtration with ethanol in a Sephadex LH-20 column. Compound **3**, 2-

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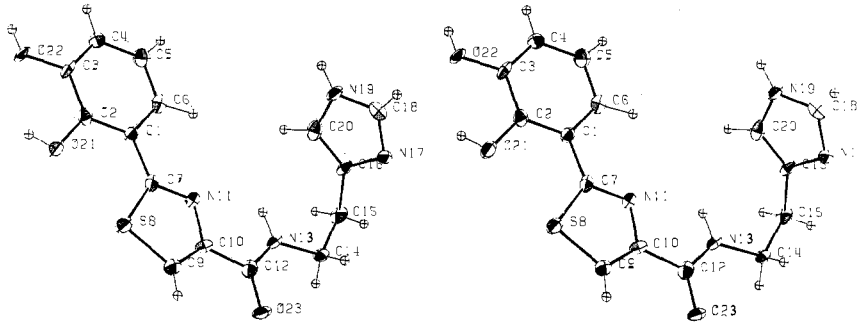

Figure 1. An ORTEP plot showing a stereoview of a single molecule of anhydroanguibactin. The atom-numbering scheme is different from that used in the schematic drawings.

(2′,3′-dihydroxyphenyl)thiazoline-4-carboxylic acid methyl ester: FAB mass spectroscopy, m/z 254 (61) MH^+; ^1H NMR, CH_3OD, δ 3.68 (m, CH_2), 3.82 (s, OCH_3), 5.45 (t, thiazole CH), 6.75 (t, *m*-CH), 6.95 (m, *o*- and *p*-CH).

Purification of Anhydroanguibactin. Anhydroanguibactin was produced during the preparation of acetylated anguibactin described previously,[1] with the exception that the concentration of acetic anhydride used in the reaction was 0.21 mL/μmol of anguibactin. The pooled peak fractions from the LH-20 column were analyzed by thin-layer chromatography (reverse-phase Supelco C-18 plates developed with methanol–water 40:15) and found to contain two components, triacetylanguibactin (R_f 0.55) and anhydroanguibactin (**2**) (R_f 0,45). The latter behaves similarly to anguibactin (**1**) on thin-layer chromatograms but elutes earlier on an LH-20 column.

Thin-Layer Chromatography. Detection of anguibactin was carried out with silica gel thin layers developed in CMW. Anguibactin and its derivatives formed compact spots with reproducible R_f values. With $FeCl_3$ spray (1% in 0.05 N HCl), anguibactin (**1**) (R_f 0.35) and compound **3** (R_f 0.8) produced a green color, while anhydroanguibactin (**2**) (R_f 0.36) produced a blue color, all turning brown with time.

Acid Hydrolysis. Hydrolysis of anguibactin (3.0 mg) was carried out with 6 N HCl at 100 °C for 24 h. The hydrolysate was dried over NaOH pellets in vacuum. The products were chromatographed on a Sephadex LH-20 column (10 × 1 cm) with absolute ethanol as the mobile phase. Fractions containing individual compounds histamine (**4**), 2,3-dihydroxybenzoic acid (**5**), and dehydrocystine (**6**) (detected by UV light and ninhydrin spray) were pooled, dried, and analyzed by NMR and FAB mass spectrometry. Histamine (**4**): ninhydrin positive; FAB mass spectrometry, m/z 134 (50) (MNa^+), 112 (100) (MH^+), 95 (35) ($M^+ - NH_2$); ^1H NMR (CD_3OD) δ 2.96 (t, CH_2), 3.21 (t, CH_2N), 7.09 (s, =CH), 7.94 (s, N=CHN). 2,3-Dihydroxybenzoic acid (**5**): FAB mass spectrometry, m/z 177 (18) (MNa^+), 176 (19) ($MNa^+ - H$), 155 (30) (MH^+), 137 (33) ($M^+ - OH$); ^1H NMR (CD_3OD) δ 6.7 (t, *m*-CH), 6.95 (d, *o*-CH), 7.34 (d, *p*-CH). Dehydrocystine (**6**): FAB mass spectrometry, m/z 259 (8) (MNa^+), 237 (84) (MH^+), strongly ninhydrin positive.

NMR Spectroscopy. NMR spectroscopy of anguibactin (**1**) and its derivatives was carried out at 300 MHz for ^1H and 75.4 MHz for ^{13}C nuclei at 23 °C on a Varian XL300 FT NMR spectrometer. Assignment of the NMR signals was based on selective decoupling and correlation with the results obtained with compounds containing similar structural groups (e.g., pyochelin (**7**),[5] obafluorin,[6] enterobactin, and ω-*N*-acetylhistamine (**8**). The ^1H chemical shifts and the coupling constants of complex spin systems were refined by an iterative spin-simulation program.[8]

FAB Mass Spectrometry. Positive-ion FAB mass spectrometry (nominal and accurate mass) was carried out on a VG ZAB-E FAB mass spectrometer with a magic bullet matrix. External calibration for nominal mass was done with CsI. High-resolution calibration was done with CsNaRbI.

Crystallization and X-ray Diffraction Data Collection. Pure anguibactin formed minute crystals from ethanol, methanol, and some other solvent systems. These crystals were not sufficiently large for X-ray diffraction. Anhydroanguibactin (**2**), purified from the reaction products of the acetylation trials of anguibactin, formed suitable well-shaped crystals from an ethanolic solution equilibrated with diethyl ether at 4 °C.

Structure Determination

Crystals of anhydroanguibactin showed some degree of instability at room temperature but were stable at low temperature. Preliminary investigation showed that the crystals in general diffract poorly. A spear-shaped crystal of approximate size, 0.38 × 0.16 × 0.07 mm, was used for unit-cell dimensions and complete data collection. The crystal was mounted while still in the mother liquor and quickly transferred into a stream of cold nitrogen gas. The monoclinic space group $C2/c$ was established from systematic absences, intensity statistics, and structure refinement. The unit-cell parameters were calculated by least-squares fit of $\pm 2\theta$ for 48 reflections measured at 138 ± 2 K with Mo $K\alpha_1$ radiation. The diffraction data were collected on an Enraf-Nonius CAD-4 counter diffractometer fitted with liquid N_2 low-temperature device using graphite monochromated Mo $K\bar{\alpha}$ radiation. The intensities of all independent reflections in the range $0.5 \leq 2\theta \leq 46°$ were measured at 138 ± 2 K in the θ–2θ scan mode with a variable scan speed and a scan width of $(0.80 + 0.25 \tan\theta)°$. Intensities of three standard reflections were monitored every 2 h of X-ray exposure and they showed no significant variation (~2%). Of the total 2029 reflections measured, 1317 were considered observed on the basis $I > 2\sigma(I)$, and these reflections were used in the structure refinements. Data were corrected for Lorentz and polarization effects but no absorption correction was made.

Crystal data: $C_{15}H_{14}N_4O_3S$, M_r = 330, monoclinic, $C2/c$, a = 22.71 (5) Å, b = 8.052 (6) Å, c = 15.879 (18) Å, β = 95.72 (9)°, V = 2889.2 Å^3, Z = 8, D_{calcd} = 1.519 g cm^{-3} at 138 K, F(000) = 1376, μ(Mo $K\bar{\alpha}$) = 2.5 cm^{-1}.

The structure was determined by direct methods with the program MULTAN[9] and refined by a full-matrix least-squares routine.[10] All the hydrogen atoms in the molecule were located from a difference Fourier map and they were refined isotropically. In the final stages of the refinement, all the non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.048, R_w = 0.046, S = 1.3 for 1317 reflections and 262 variable parameters. The maximum peak height in the final difference map ranged between ±0.3 e/Å^3.

Results and Discussion

(i) Structure of Anhydroanguibactin (2). A stereoview of the single molecule of anhydroanguibactin is shown in Figure 1, which also shows the atom numbering scheme used for the description of the crystal structure of **2**.

The molecule consists of two planar sections, one containing the phenyl–thiazole system with its substituents and the other the imidazole ring. The two planar sections are nearly perpendicular

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Table I. Hydrogen Bond Parameters of **2**

D	A	D···A, Å	D-H, Å	H···A, Å	D-H···A, deg
O(22)	N(17)[a]	2.592 (5)	0.83 (5)	1.77 (5)	167 (5)
O(21)	O(22)[b]	2.734 (5)	0.80 (6)	2.05 (6)	142 (6)
N(19)	O(23)[c]	2.758 (5)	0.86 (5)	1.93 (5)	164 (5)

[a] $[-^1/_2 + x, -^1/_2 + y, z]$. [b] $[^1/_2 - x, -^1/_2 - y, -z]$. [c] $[x, y - 1, z]$.

to each other. The dihedral angle between the plane of the imidazole ring and a least-squares plane through the 18 nonhydrogen atoms of the rest of the molecule is 78.7°. The coplanarity of the phenyl-thiazole system (dihedral angle between the two rings is 7.2°) contrasts with the biphenyl systems where the two rings are generally skewed, but is consistent with the analogous system in agrobactin,[11] where an oxazoline ring replaces the thiazole ring. Similar coplanarity has also been observed in the phenyl-bithiazole system present in PBCP [[3-(2′-phenyl-2,4′-bithiazole-4-carboxamido)propyl]dimethylsulfonium iodide], an analogue of the DNA-binding portion of the antibiotic bleomycin A_2.[12] The hydroxyl groups in the present structure lie on the side of the sulfur atom, giving a short nonbonded S···O contact of 2.707 Å. In agrobactin, the phenyl ring is rotated 180° about the C(1)-C(7) bond bringing the hydroxyl groups on the side of the nitrogen atom of the heterocyclic ring.

The only amide bond in the present structure [C(12)-N(13)] is trans; the ω torsion angle C(10)-C(12)-N(13)-C(14) is 172.7°. The torsion angle N(11)-C(10)-C(12)-N(13) is close to zero (2.8°), which brings the histamine amide group in the plane of the thiazole ring. The histamine moiety assumes a gauche conformation, [N(13)-C(14)-C(15)-C(16) = 59.1°] in contrast to a fully extended conformation observed in several histamine derivatives, as in histamine diphosphate[13] and 6-histaminopurine.[14]

The bond lengths and angles in the thiazole ring in general compare well with those observed in other thiazole derivatives.[15] The difference in the two C-S distances [S(8)-C(7) of 1.747 Å and S(8)-C(9) of 1.700 Å] is significant. The short S···O contact may have caused some steric strain as reflected in the enlarged angles, C(2)-C(1)-C(7) of 122.9° and C(1)-C(7)-S(8) of 123.9°.

The imidazole ring has the N(19)-H tautomeric form, established by the location of the N(19) hydrogen atom from the difference Fourier map. The similar tautomeric form has been observed in histamine imidazole ring in 6-histaminopurine[14] and other imidazole derivatives.[16,17] However, the difference in the endocyclic angles at C(19) (106.7°) and at N(17) (105.6°) in the present structure is not as large as seen in other imidazole compounds. This may be attributed in part to the fact that atom N(17) in the present structure acts as an acceptor in a very strong hydrogen bond. The near equivalence of the two bonds, N(17)-C(18) = 1.326 Å and N(19)-C(18) = 1.328 Å, is also noteworthy and indicates substansive charge delocalization in the imidazole ring.

With the exception of the thiazole nitrogen and the histamine amide group, both of which are buried in the inner side of the molecule, all the nitrogen and oxygen atoms in the molecule take part in the hydrogen bonding. Table I gives all the relevant parameters for the three independent hydrogen bonds in the present structure. The O(22)-H···N(17) bond of 2.592 Å is one of the shortest ever reported. A view of the crystal packing is shown in Figure 2. Centrosymmetrically related molecules are linked in a dimeric form through a pair of O-H···O hydrogen

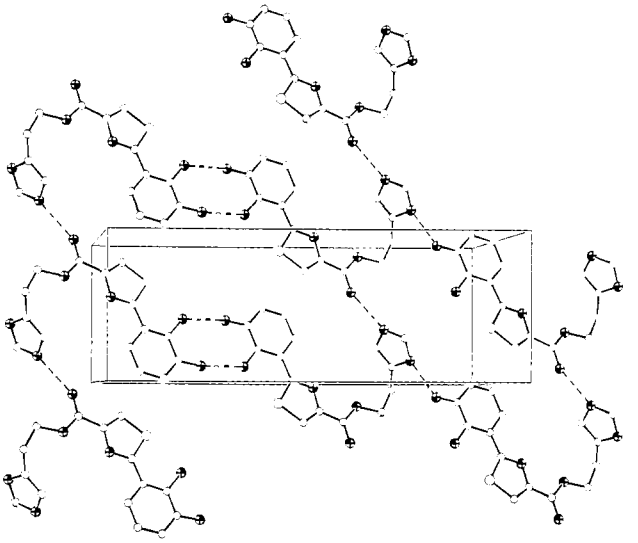
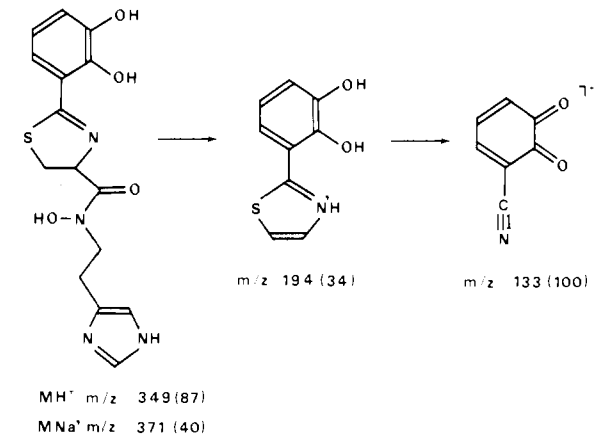


Figure 2. A view of the crystal packing down the *c* axis (*b* vertical, *a* horizontal). Hydrogen bonds are indicated by dashed lines.

Scheme I



bonds formed by the two benzylhydroxy groups. In addition, two very strong N···O hydrogen bonds involving both the imidazole nitrogen atoms pack the molecules is unending multilayered zigzag chains.

(ii) **Structure of Anguibactin (1).** The determination of the structure of anhydroanguibactin (**2**) by single-crystal X-ray diffraction facilitated the structure determination of anguibactin (**1**) by spectroscopic and chemical means. Acid hydrolysis of anguibactin with 6 N HCl produced 2,3-dihydroxybenzoic acid (**5**), dehydrocystine (**6**), and histamine (**4**). These results suggest that anguibactin is made of similar structural units as found in anhydroanguibactin.

FAB mass spectroscopy of **1** revealed four conspicuous ion peaks (Scheme I). The peaks at *m/z* 349 (87) and 371 (40) correspond to the MH^+ and MNa^+ ions, respectively. Peaks for MH^+ and MNa^+ are frequently observed in the FAB mass spectra of other microbial siderophores. The accurate mass of the MH^+ ion is 349.0944 (calculated for $C_{15}H_{17}N_4O_4S$, 349.0971) and that of the MNa^+ ion is 371.0751 (calculated for $C_{15}H_{16}N_4O_4S$, 371.0790). On the basis of these findings, it is believed that anhydroanguibactin is the dehydration product of this compound.

NMR spectroscopy has revealed that anguibactin (**1**) contains a thiazoline ring, instead of the thiazole ring found in anhydroanguibactin (**2**), and a hydroxyl group attached to the peptide N atom. ^1H NMR spectra of anguibactin (**1**) in CD_3OD (Table II) shows three spin systems, a three-proton system of the aromatic ring, a four-proton CH_2-CH_2 system of the histamine chain, and a three-proton CH_2-CH system of the thiazoline ring, in addition to two singlets produced by the CH protons of the imidazole ring. The major differences observed in the spectra of anhydroangui-

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Table II. ^{1}H Chemical Shifts (δ) of Anguibactin (**1**), Anhydroanguibactin (**2**), and ω-*N*-Acetylhistamine (**8**)[a]

position	group	1	2	8
3	imidazole CH	6.90 (s, 1 H)	6.93 (s, 1 H)	6.85 (s, 1 H)
5	imidazole CH	7.61 (s, 1 H)	7.67 (s, 1 H)	7.60 (s, 1 H)
6	CH_2	2.97 (t, 2 H) (J = 6.9 Hz)	2.93 (t, 2 H) (J = 7.1 Hz)	2.77 (t, 2 H) (J = 7.2 Hz)
7	CH_2	3.94 (m, 2 H) (J_{gem} = −11.0 Hz, J_{vic} = 6.5 Hz)	3.67 (t, 2 H) (J = 7.1 Hz)	3.42 (t, 2 H) (J = 7.2 Hz)
10	CH_3			1.93 (s, 3 H)
4′	thiazoline CH	5.88 (t, 1 H) (J = 9.1 Hz)		
5′	thiazoline CH_2	3.41 (q, 1 H) 3.66 (q, 1 H) (J_{gem} = −11.0 Hz, J_{vic} = 9.1 Hz)		
5′	thiazole CH		8.16 (s, 1 H)	
4″	aromatic CH	6.94 (d, 1 H)	7.51 (d, 1 H)	
6″	aromatic CH	6.92 (d, 1 H) (J_{ortho} = 7.9 Hz)	6.91 (d, 1 H) (J_{ortho} = 7.9 Hz)	
5″	aromatic CH	6.74 (t, 1 H)	6.80 (t, 1 H)	

[a] Solvent, CD_3OD.

Table III. ^{13}C NMR Chemical Shifts (δ) of Anguibactin (**1**) and Related Compounds with Similar Structural Moieties

position	group	1[a]	8[a]	7[b]	5[c]
2	imidazole C	136.6	137.5		
3	imidazole CH	120.2	119.6		
5	imidazole CH	136.8	137.7		
6	CH_2	25.8	29.4		
7	CH_2	48.3	42.1		
9	C=O	176.1	174.8		
10	CH_3		24.2		
2′	thiazoline C	172.4		172.2	
7″	COOH				172.8
6	COOH			173.5	
4′	thiazoline CH	75.9		73.6	
4	thiazolidine CH			75.9	
5′	thiazoline CH_2	34.4		32.1	
5	thiazolidine CH_2			32.7	
1″	aromatic C	117.3		116.0	113.1
2″	aromatic C	148.6		158.8	150.5
3″	aromatic C	146.8		116.3	145.7
4″,5″	aromatic CH	122.0		133.9	120.8
6″		120.5		119.6	118.6
		120.5		130.9	120.5

[a] Solvent, CD_3OD. [b] Reference 5. [c] Reference 21.

bactin is the absence of the three-proton CH_2–CH spin system of the thiazoline ring and the presence of a singlet (δ 8.16) corresponding to the CH proton of the thiazole ring (Table II). The C–C single bond in the thiazoline ring in **1** gives rise to the asymmetric center at the CH carbon atom and indicates the origin of this five-membered ring from conjugation of the amino acid cysteine with 2,3-dihydroxybenzoic acid. A similar ring system has been observed in another bacterial siderophore, pyochelin (**7**), isolated from *Pseudomonas aeruginosa*.[5] The absolute configuration of the cysteine residue in anguibactin has not been determined.

A comparison of the NMR spectra of anguibactin (**1**), anhydroanguibactin (**2**), and ω-*N*-acetylhistamine (**8**) (in CD_3OD) (Tables II and III) suggests that the OH group is present on the peptide N atom. The chemical shifts of the protons of the imidazole ring in all three compounds are similar. The ^{13}C chemical shifts of the imidazole ring in **1** and **8** are also similar. These observations indicate that the imidazole ring of anguibactin (**1**) does not contain a hydroxyl group. On the other hand, the NMR resonances arising from the protons of the CH_2 group (position 7) adjacent to the N atom of the peptide group in anguibactin (**1**) show significant downfield shift compared to that of anhydroanguibactin (**2**) ($\Delta\delta$ = 0.27) and ω-*N*-acetylhistamine (**8**) ($\Delta\delta$ = 0.52). The downfield shift of these CH_2 protons in (**2**) ($\Delta\delta$ = 0.24) compared to those of **8** is caused by the large conjugating system of **2**, which extends upto the peptide group. An upfield shift of these CH_2 protons is expected in **1**, in which conjugation of the peptide group is absent. Instead these proton signals shifted further downfield with strong indication that the adjacent N atom is hydroxylated and the peptide group is replaced by a hydroxamic acid group. The large downfield shift (6.2 ppm) of the ^{13}C signal of this CH_2 group (C7) in **1** compared to that in **8** is a further indication of the presence of a hydroxamic acid functionality adjacent to this methylene group. The splitting pattern of these methylene protons (in CD_3OD) in **1** is not similar to those observed in **2** and **8**. These CH_2 protons show chemical shift equivalence and produce a triplet in both **2** and **8**. In **1** this pattern changes to a symmetrical multiplet. Spin simulation shows that these two protons are chemical shift nonequivalent (δ 3.91 and 3.95, J_{gem} = −11.0 Hz, J_{vic} = 6.3 and 6.9 Hz). Presence of an OH group on the adjacent N atom is a likely cause of this nonequivalence. However ω-*N*-hydroxyhistamine could not be detected from the products of the acid hydrolysis of **1**. The presence of dehydrocystine (**6**) together with histamine (**4**) among the hydrolysis products indicates that the formation of anhydroanguibactin (**2**) from anguibactin precedes hydrolysis of the peptide bond. It is likely that anhydroanguibactin was similarly produced by weakly acidic conditions during the methanolysis of triacetylanguibactin.

The peptide NH proton of **2** (in $(CD_3)_2SO$) produces a triplet at δ 8.78, which is at the downfield end of the resonance range of such protons (peptide NH proton in **8** produces a triplet at δ 7.92). The increased deshielding of this proton in **2** is caused by the conjugation of the peptide group with the thiazole and the aromatic rings. The proton NMR spectrum of **1** (in $(CD_3)_2SO$) shows neither this NH signal nor any similar signal in the chemical shift range of δ 6.0–9.0 which may correspond to a peptide NH proton. On the other hand, the spectrum of **1** shows a broad band centered at δ 9.25. Hydroxamic N–OH protons in various siderophores produce a broad band in the δ 9.0–10.0 range.[18–20]

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Note Added in Proof. A recent single-crystal structure determination of the Ga(III) complex of racemized anguibactin shows a 1:1 metal to ligand stoichiometry, in which the O-hydroxy group, the nitrogen of the thiazoline ring, the hydroxamate (N–O) group, and the deprotonated nitrogen of the imidazole ring coordinate the metal ion. The crystal structure also confirms the chemical structure of anguibactin. (D. van der Helm, M. B. Hossain, M. A. F. Jalal and D. L. Eng-Wilmot, to be published.)

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Supplementary Material Available: Listing of atomic parameters (Table S1), bond distances and angles (Table S2), hydrogen atom parameters (Table S3), anisotropic thermal parameters (Table S4) (7 pages); table of structure factors (6 pages). Ordering information is given on any current masthead page.

Stereochemical Control in Hex-5-enyl Radical Cyclizations: Axial vs Equatorial 2-(1-But-3-enyl)cyclohexyl Radicals

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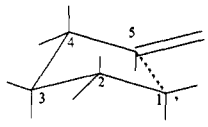
Abstract: Free radical cyclization reactions of 4-substituted *cis*- and *trans*-2-(1-but-3-enyl)cyclohexyl radicals and the analogous 3,5-dioxacyclohexyl radicals were investigated. Within the framework of hex-5-enyl radical cyclization, 1,5-cis or 1,5-trans product predominates if the butenyl group occupies an equatorial or axial position of the cyclohexane chair form, respectively. The results are rationalized by the transition-state model *originally* proposed by Beckwith for acyclic hex-5-enyl radical cyclizations.

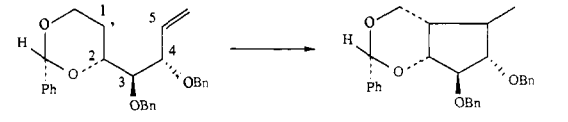
1,5-Ring closures of hex-5-enyl radicals followed by trapping of the resulting cyclopentylmethyl radicals have attracted considerable attention in both synthetic and physical organic chemistry.[1] Studies carried out by Beckwith and co-workers[2] with prototypical alkyl-substituted acyclic hexenyl radicals have provided general guidelines to predict the stereochemical outcome of the cyclization. It was proposed that the transition state resembles a cyclohexane chairlike conformation (**1**), in which



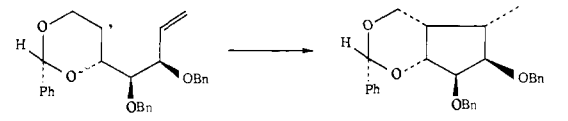
1

substituents preferentially occupy pseudoequatorial positions. Ring closures of cyclic radicals are not as predictable.[3] For example, 2-(1-but-3-enyl)cyclohexyl radicals generally cyclize to give predominantly 1,2-cis;1,5-cis products. (In this paper, atoms are numbered according to the hex-5-enyl radical numbering system to emphasize regio- and stereochemical relationships of the cyclizations, and other atoms such as those in the six-membered rings will be differentiated by primed numbers.) To account for the observed 1,2-cis;1,5-cis stereochemistry, Beckwith and co-workers[3b] concluded that the ring closure occurs through the cyclohexane conformer in which the butenyl substituent occupies an axial site, because in this conformation orbital overlap between the radical SOMO and the olefin π^* orbital was believed to be maximized. Irrespective of the mechanistic details, it is this 1,5-cis stereoselectivity that has found elegant uses in several natural product syntheses.[4] However, various other structural features also appear to influence the stereochemical outcome.

We have recently observed[5] that the stereochemistry of 2-butenyl-3,5-dioxacyclohexyl radical ring closure is critically influenced by the nature of the substituents at the 4-position. For example, as shown in eq 1 and 2, the glucose-derived hex-5-enyl radical cyclizes with unprecedented exclusive 1,5-trans selectivity (eq 1) whereas the corresponding radical in the manno series yields exclusively 1,5-cis product (eq 2). In these systems, the but-3-enyl



(1)



(2)

and phenyl groups are cis on the dioxane ring system and most likely occupy the equatorial sites. Therefore, the results indicate

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