Pseudoalterobactin A and B, New Siderophores Excreted by Marine Bacterium *Pseudoalteromonas* sp. KP20-4

KANEO KANOH*, KEI KAMINO, GUAN LELEO[†], KYOKO ADACHI and YOSHIKAZU SHIZURI

Marine Biotechnology Institute Co., Ltd. 3-75-1 Heita, Kamaishi-shi 026-0001, Japan * 410 Agriculture/Forestry Center, University of Alberta Edmonton, AB, Canada T6G 2P5

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Siderophores are relatively low-molecular-weight compounds that typically have a very high affinity constant $(10^{25} \sim 10^{50})^{11}$ for iron (Fe³⁺), which is an essential element for most microorganisms owing to its importance in a variety of biochemical reactions. The role of siderophores is to scavenge extracellular iron from the environment and transport it into microbial cells. Hundreds of siderophores have been isolated from terrestrial, especially pathogenic, microorganisms, and their biosyntheses and ironsequestering systems have been enthusiastically studied.^{2~4} In spite of the high abundance of iron in the earth's crust, the dissolved iron concentration is particularly low $(20 \text{ pM} \sim 1 \text{ nM})^{5}$ in the surface water of the open ocean. In such an iron-deficient environment, marine bacteria are thought to get iron by siderophore-based ironsequestering systems.⁶⁾ However, only a few studies concerning siderophores of marine bacteria have been done.^{$7 \sim 9,12$} In the course of screening for new siderophores from marine bacteria, we found that Pseudoalteromonas sp. KP20-4 produced new siderophores. In this note, the

isolation and structural determination of pseudoalterobactin A (1) and B (2), excreted by marine bacterium *Pseudoalteromonas* sp. KP20-4, are reported.

Pseudoalteromonas sp. KP20-4 was isolated from a marine sponge (Cinachyrella australiensis) obtained in the Republic of Palau. The bacterium was identified from the results of taxonomic studies and 16S rDNA sequence. All glassware used for the culture and for isolating the siderophore was washed with 6N HCl and then rinsed with milli Q water to avoid iron contamination. Pseudoalteromonas sp. KP20-4 was cultured in an ASG medium (iron free) containing casamino acid (5 g/liter), glycerol (3 g/liter), glycerophosphate (0.1 g/liter), NaCl (15.5 g/liter), KCl (0.8 g/liter), MgSO₄·7H₂O (12.4 g/liter), $CaCl_2 \cdot 2H_2O$ (2.9 g/liter), NH_4Cl (1.0 g/liter), HEPES (10 mM), and NaHCO₃ (2 mM) at pH 6.8 (before being autoclaved) for 72 hours at 30°C with rotary shaking at 100 rpm. The culture broth was centrifuged at $8,000 \times g$ for 15 minutes at 4°C. The collected supernatant was batch loaded onto a Diaion HP20 (Mitsubishi Chemical Co.) column. After washing the resin with acidic water (pH 2, adjusted with conc. HCl), the Fe-binding fraction was eluted with methanol, the chrome azurol S (CAS) assay¹⁰⁾ being used as the index. This methanol fraction was chromatographed on a reverse-phase open column (Wakogel[®] 100C18, Wako Pure Chemical Industries, Ltd.), using stepwise-elution with acidified water, 20% methanol, and 50% methanol. The 20% methanol fraction including CAS-positive substances was further chromatographed on an LH20 column, using 50% methanol - water (pH 2.0) as the mobile phase. The CAS-positive fractions were collected and evaporated in vacuo. The resulting fraction was loaded into a reverse-phase HPLC (column: TSK gel ODS 80Ts, i.d. 7.8×300 mm, Tosoh Co.), eluting with 15%



Pseudoalterobactin A (1): R=CH₂NH₂

Pseudoalterobactin B (2): R= NHC(NH)NH₂

^{*} Corresponding author: kaneo.kanoh@mbio.jp



Fig. 1. Structural determination of pseudoalterobactin A (1) by NMR data.

a) Partial structures determined from COSY, TOCSY and HMBC data. Numbers reveal ¹H chemical shifts, italic numbers reveal ¹³C chemical shifts. Bold lines reveal the connections obtained from COSY or TOCSY data. Arrows indicate HMBC signals. * Chemical shifts were obtained from the HSQC spectrum.

b) Connection of the partial structures and amino acids. Bold lines reveal the connections obtained from COSY or TOCSY data. Arrows indicate HMBC signals.

acetonitrile - 0.1% TFA - water at a flow rate of 2 ml/minute with detection at UV λ 250 nm. The CAS-positive fractions were purified twice by HPLC, finally yielding 1 (8 mg) and 2 (6 mg) from 40 liters of the culture broth.

The ESI-MS spectrum of 1 displayed an $[M+H]^+$ ion at m/z 1078.4. The molecular formula of 1 $(C_{41}H_{63}N_{11}O_{21}S)$ was established from ¹³C-NMR and HR-FAB-MS data: $(M+H)^+$ peak at m/z=1078.3986 [calcd. for $C_{41}H_{64}N_{11}O_{21}S$, 1078.3999]. The ¹H- and ¹³C-NMR spectra suggested that 1 had a peptide moiety. The results of an amino acid analysis indicated that 1 contained Asp (or Asn; HR-FAB-MS and MS/MS analyses described later indicate the presence of Asn) as well as Gly, Lys and an unusual amino acid. This unusual amino acid was clarified to be *threo-β*-hydroxy-Asp by an analysis of the NMR data (Figure 1a), and confirmed by its comparison with an authentic sample. Two partial structures, 4,8-diamino-3-hydroxyoctanoic acid and one-substituted (at the C-4 position) 2,3-dihydroxy benzoic acid, were constructed from 2D NMR spectra including COSY, TOCSY and HMBC (Figure 2a). The sequences of these two parts and the amino acids were determined by the correlation signals from the amide proton and the α -proton of amino acid to the neighboring carbonyl carbon in the HMBC spectra (Figure 1b). The HMBC spectra also indicated the ring structure by amide bond formation between ε -NH of Lys

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Fig. 2. Characteristic fragmentation of pseudoalterobactin A (1) and B (2) in the ESI-MS/MS data.

The upper numbers correspond to 1, and the lower to 2.

(1) and the C-end of Gly. The ESI-MS/MS data for the protonated molecule exhibited the formation of Y-type ions (nomenclature according to ROEPSTORFF).¹¹⁾ The characteristic fragmentation of 1 is shown in Figure 2. The fragmentation of 1 reveals that the substitution group at the C-4 position was sulfonic acid, and the fragment ion [y3: 690.2-y4: 576.2=114.0] indicates that the amino acid residue at this position was Asn. The fragmentation in the ESI-MS/MS data also exhibits the complete sequence of the 2,3-dihydroxy-4-sulfobenzoic acid, 4,8-diamino-3hydroxyoctanoic acid, Asn, threo- β -hydroxy-Asp (1) and a cyclic peptide moiety composed of four amino acids (Lys (1), Lys (2), threo- β -hydroxy-Asp (2) and Gly), in agreement with the structure determined from the NMR data. These results established the planar structure of pseudoalterobactin A as 1, the assignment of 1 H and 13 C chemical shifts being summarized in the Table 1.

The structure of pseudoalterobactin B (2) was determined by the same procedure as that used for 1. The ESI-MS data for 2 displayed an $[M+H]^+$ ion at m/z 1106.5, and the molecular formula of 2 was determined to be $C_{41}H_{63}N_{13}O_{21}S$ (HR-FAB-MS data: $(M+H)^+$ peak at m/z=1106.4089; calcd. for $C_{41}H_{64}N_{13}O_{21}S$, 1106.4060). The amino acid analysis and NMR data for 2 indicate that 2 contained Asn (or Asp), *threo-β*-hydroxy-Asp, Lys, Arg and Gly. Two partial structures, 4,8-diamino-3-hydroxyoctanoic acid and 2,3-dihydroxy-4-sulfobenzoic acid, were constructed from the 2D NMR spectra. The connection of these two components and the amino acids

was determined by the HMBC spectra and confirmed by ESI-MS/MS data; the ESI-MS/MS data also indicated the presence of Asn [y3: 718.3-y4: 604.3=114.0], as in the case of 1 (Figure 2). These data determined the planar structure of pseudoalterobactin B to be 2, the assignment of ¹H and ¹³C chemical shifts being summarized in the Table 1. The structural difference between 1 and 2 is only in the replacement of Lys(2) in 1 by Arg in 2. An investigation of the absolute stereochemistry of 1 and 2 is now being undertaken.

REID et al. have reported the marine siderophores, alterobactins A and B, which had exceptionally high affinity for the ferric ion (affinity constant of $10^{49} \sim 10^{53}$).¹²⁾ They speculated that this high affinity was derived from the coordination by a catechol and two β -hydroxy-Asp residues with the ferric ion. The structural resemblance between these alterobactins and pseudoalterobactins 1 and 2, having a catechol and two β -hydroxy-Asp residues, led us to presume that the pseudoalterobactins would also have extraordinary affinity to the ferric ion. In fact, the psedoalterobactins exhibited strong activity comparable to that of enterobactin by a CAS assay, the affinity constant of enterobactin to the ferric ion being 10^{49} .¹³⁾ Both 1 and 2 exhibited an ED₅₀ value, the concentration that reduced the absorbance at 630 nm of the CAS solution by 50% in 2 hours, of 20 µM under our assay conditions. Enterobactin and desferrioxamine B (affinity constant of $10^{31})^{14)}$ exhibited ED₅₀ values of $60 \,\mu\text{M}$ and $500 \,\mu\text{M}$, respectively, under the same conditions. Detailed analysis of the binding

Table 1. 1 H (500 MHz) and 13 C (125 MHz) chemical shift of pseudoalterobactin A and B (DMSO- d_6).

jnshim "C (B pro) H (B pro) (matheway run) (matheway run)<	Pseudoalterobactin A (1)				Pseudoalterobactin B (2)				
23-dim/totavel/section 23-dim/totavel/section 23-dim/totavel/section C1 1154 C1 1155 C2 1155 C2 1157 727 1158 C2 C4 C1 1155 725 (C5 C1 C5 <	position	¹³ C (δ ppm)	¹ Η (δ ppm) (multiplicity J (Hz))	HMBC(H→C)	position	¹³ C (δ ppm)	'Η (δ ppm)	(multiplicity J (Hz)	HMBC(H→C)
C1 115.41	2,3-dihydrox	y-4-sulfobenzoi	c acid		2,3-dihydrox	y-4-sulfobenzoid	acid		
C2 149.40 12.51(-CH) (br.9) C2 149.38 12.52(-CH) (br.9) C3 142.81 10.56(-CH) (br.9) C3 142.81 10.56(-CH) (br.9) C5 115.37 5.24 (d.5.5) C2, C4, C7 C5 115.38 6.55 (d.5.5) C3 C6 115.37 7.27 (d.5.5) C2, C4, C7 C7 188.54 C3 142.55 C7.65 C1 28.58 1.356 (m) C3 143.54 MI 6.75 (d.5.5) C7.75 C1 28.58 1.357 (m) C7 188.54 MI 7.76 (b.5) C7.75, C7.75 C1 28.28 1.354 (m) C1 24.52 1.354 (m) C1 24.52 1.354 (m) C1 1.454 (m) C1 C1 1.454 (m) C1 C1 C1 C1 C1 C1 C1	C1	115.41			C1	115.45			
C3 142.63 10.58(-OH) Dr st C3 142.83 10.58(-OH) Dr st C4 142.83 C3 C3 <thc3< th=""> <thc3< th=""> <thc3< th=""></thc3<></thc3<></thc3<>	C2	149.40	12.51(-OH) (br s)		C2	149.35	12.52(-OH)	(brs)	
C4 132.83	C3	142.63	10.55(-OH) (br s)		C3	142.63	10.56(-OH)	(brs)	
C6 115.77 C3 C3 C5 115.36 9.56 (d 8.5) C3 C4 4.8-demins-5-hydrogramma and marked	C4	132.93			C4	132.93		()	
C6 115.77 7.27 (el.5.5) C2, C4, C7 C6 115.75 7.25 (el.5.5) C2, C4, C7 4.4-demine-3-hydrogradmole add Admine-3-hydrogradmole add Admine-3-hydrogradmole add Admine-3-hydrogradmole add C7 168.54" C7 C8 23.3 (el.5.5) C7, C8	C5	115.37	6.94 (d 8.5)	C3	C5	115.38	6.95	(d 8 5)	C3
C7 18.85	C6	115.77	7.27 (d 8.5)	C2. C4. C7	C6	115.75	7 25	(d 8 5)	C2 C4 C7
4.4-dimits ->hydronycetinole add 4.4-dimits ->hydronycetinole add 7.7 (th 5.5) C.7 (cb 1.55) C.7 (cb 1.55) <thc.7 (cb="" 1.55)<="" th=""> <thc 1.55<="" th=""> C.7 (cb 1.</thc></thc.7>	C7	168.58		,,	C7	168.54**		(0.0)	02, 04, 07
N1 $$ 8.73 0.53 0.77 N1 $$ 8.73 0.53 0.77 C, G, Cio C6 38.73 1.59 (m) C9 C10 C2 33.11 (m) C7, G, Cio C10 22.35 1.314 (m) C10 22.35 C13.141 (m) C11 4.438 (m) C12 64.32 3.07 (m) C10 22.35 C13.141 (m) C11 4.438 (m) C11 4.438 (m) C11 4.439 C11 4.412 (m) C11 4.439 C11 4.421 C11 4.451 C12 C13 C13 <thc13< th=""> <thc13< th=""> <thc13< th=""></thc13<></thc13<></thc13<>	4,8-diamino-:	3-hydroxyoctan	oic acid		4.8-diamino-:	3-hydroxyoctano	dc acid		
Ces 38.73 3.25 (m) C.9. C10 Ces 3.31 (m) C7, C5, C10 C9 22.56 1.55 (m) C7, C5, C10 22.55 1.31 (m) C7, C5, C10 C11 22.37 1.48 (m) C10 22.35 1.31 (m) C11 2.43 (m) C11 C12 C12, C13, C15 C13 C13 C12, C13, C15 C14 4.53 (b12, C13, C15 C14 4.53 (b12, C13, C15 C15 C15 C15, C16, C16 C11 C12 C12, C13, C15 <td< td=""><td>N1</td><td></td><td>8.75 (bt 5.5)</td><td>C7</td><td>N1</td><td></td><td>8.73</td><td>(bt 5.5)</td><td>C7. C8</td></td<>	N1		8.75 (bt 5.5)	C7	N1		8.73	(bt 5.5)	C7. C8
C6 28.56 1.558 (m) C9 28.60 1.558 (m) C10 22.55 1.318 (m) C11 22.55 1.318 (m) C11 22.55 C11 C12 C13 G17 G17 G18 G17 G18 <td>C8</td> <td>38.73</td> <td>3.29 (m)</td> <td>C9, C10</td> <td>C8</td> <td>38.72</td> <td>3.31</td> <td>(m)</td> <td>C7 C9 C10</td>	C8	38.73	3.29 (m)	C9, C10	C8	38.72	3.31	(m)	C7 C9 C10
C10 22.38 1.31# (m) C10 22.35 1.33# (m) C11 26.31**** 1.439 (m) C11 24.42 1.439 (m) C12 26.32**** 7.74 (bit) C11 24.62 1.439 (m) C12 57.3********** 7.74 (bit) C11 24.62 1.439 (m) C13 57.3************************************	C9	28.58	1.55# (m)		C9	28.60	1 55#	(m)	07, 00, 010
C11 26.31** 1.40e (m) C11 24.32** 1.40e (m) C12 54.32** 3.07 (m) C12 54.27 3.08*** (pr d) C11 N2 -7.78 (bs) N2 -7.78 (bs) N2 -7.74 (bs) C11 24.62*** 3.08*** (pr d) C11 24.62*** 3.08**** (pr d) C11 24.62*** 3.08**** (pr d) C11 24.62*** 3.08**** (pr d) C11 24.5**** C11 24.65 C12 C13 67.18 4.12**** (m) C12 C13 C14 3.85 C15 C15 C15 C15 C15 C16	C10	22.36	1.31# (m)		C10	22.35	1 31#	(m)	
C11 26.31* 1.434* (m) C11 24.42 1.434* (m) C12 54.32 3.07* (m) C13 67.19 4.12** (m) C11 24.42 3.08**** (m) C11 C13 67.19 4.12**** (m) C12.C13.C15 C13 67.16 4.12 (m) C12.C13.C15 C15 198.51 C14 38.50 2.36 (d17.0) C12.C13.C15 C15 198.51 C15 C15 C15 C15 C16 C16.C16 C16 C16 C16 C17 C17 C16 C17 C17 C16			1.40# (m)		•.•	22.00	1.01#	(m)	
Cit2 Size Cit2 Cit2 Size Cit2 Cit2 <th< td=""><td>C11</td><td>26 31**</td><td>1.49# (m)</td><td></td><td>C11</td><td>24 62</td><td>1.40#</td><td>(m)</td><td></td></th<>	C11	26 31**	1.49# (m)		C11	24 62	1.40#	(m)	
Lybric Crit Sign of the component of th	C12	54 32	3.07 (m)		C12	E4.02	2 00***	(11) (br.d)	011
cr3 cr1 4 cr3 cr1 4 design of the second seco	N2		7.78 (bs)		NO	54.27	3.08	(bra)	CII
Cita Dial Dial <thdial< th=""> Dial Dial <thd< td=""><td>C12</td><td>67 10</td><td>1 12*** (m)</td><td></td><td>NZ 012</td><td>67.40</td><td>1.14</td><td>(DS)</td><td></td></thd<></thdial<>	C12	67 10	1 12*** (m)		NZ 012	67.40	1.14	(DS)	
C14 38.50 2.30 (b5.0) C12, C13, C15 C14 38.50 2.36 (d7.0) C12, C13, C15 Aspensition C15, C16, C16, C19 C15, C16, C17, C19 C16, C16, C19 C16, C17, C19 C16, C17, C19 C16, C17, C19 C16, C17, C19 C16, C19 C16 <thc16, c19<="" th=""> C16 C16</thc16,>	014	67.19	4.12 (11)	040 040 045	C13	67.18	4.12	(m)	
C15 169.52 C15 169.51 Asparagine C17 3.22 (bd.8.0) 2.5* C15 C15 C16 C17 3.33 (bd.7.5) 2.5* C15, C16 2.5* C15, C17 C15, C17 C15, C17 C15 C17 3.633 2.5* C15, C17, C19 C16, C18, C19 C16 C16, C18, C19 C16 C16 C17 3.636 C16, C18, C19 C16 C17 3.63 C16, C18, C19 C16 C18 T17.73 ND C16, C18 C19 C19 T7.084 ND C18 C19 ND ND C19 ND C19 ND C19 ND C19 ND C19 R0 C19 R0 C19 C22 C21 69.57 C43 G43.64.2.5 C22	015	38.50	2.37 (0.6.0)	012, 013, 015	C14	38.50	2.36	(d 7.0)	C12, C13, C15
Appartagine N3 Assaragine (1) Assarag	015	169.52			C15	169.51			
No. Ass. (bit 80./) C15 C17 C18 Ass. (bit 80./) C15 C17 C18 C17 C16 C18 C17 C18 C18 C18 C18 C18 C18 C18 C18 C18 C17 C18 C17 C19 C18 C12 C12 C12 C12 C12 C12 C12 C12 C22 C23 C24 C44 C40 C04 C10 C23 C24<	Asparagine		9 22 /bd 9 0)	015	Asparagine			<i>(</i> ,) – –)	0.0
C17 36.83 2.5° C16 C18 C17 36.87 2.6° C16 C16 <thc16< th=""> C16 C16 C1</thc16<>	143 C16	40.00		015	NJ		8.33	(DCI /.5)	015, 016
C17 36.39 2.5 C16 C18 C17 36.87 2.5 C16 C16 C18 C19 C12 C13 C13<	017	49.38	4.66 (00 14.0, 7.0)	015, 017, 018, 019	C16	49.32	4.66	(dd 7.0, 6.5)	C15. C17, C19
C18 171.80 ND C18 171.73 ND PHydroxyseparite scid (1) NS 8.03 (bd 8.5) C19 NS SC20 55.72 4.63 (dd 8.5, 3.0) C22 C22 C22 55.72 4.63 (dd 8.5, 3.0) C22 C23 168.54 C23 C44 A06 (bd 7.0) C23 C23 C24 FA48 4.06 (bd 7.0) C23 C24 FA48 A06 FA48 A06 (bd 7.5) C25 C29 C24 FA48 A06 FA48	017	36.93	2.5" 2.65 (dd 15.5, 7.0)	C16, C18, C19 C16, C18, C19	C17	36.87	2.5* 2.65	(dd 16.0. 6.5)	C16, C18 C16, C18
C19 TO P1 P	C18	171.80			C18	171.73			-,
P+tydrozysespartic acid (1) P-tydrozysespartic acid (1) N5 B.03 (bd 7.5) C19 NS - 4.53 (dd 8.5.0) C23 C20 55.66 4.52 (dd 8.0.2) C23 C21 69.97 4.54 (d 3.0.0) C22 C21 69.98 4.53 (d 2.5) C23 C23 168.42 - - C23 168.54** - Lysine (1) - - C24 54.48 4.08 (bd 7.0) C23 C24 54.51 4.07 (bdd 12.5, 7.5) C25, C29 C24 54.48 4.08 (bd 13.0, 7.5) C25, C29 C24 54.48 (m) C25 30.44 1.584 (m) 1.644 (m) 1.294 (m) - 1.294 (m) C27 27.40 1.364 (m) 1.284 (m) C28 37.27 2.86 (m) C28 37.31 2.91 (m) 3.22 (m) C29 171.21 - C19 K - 2.91 (m) C29, C41 C29 172.19 - C29 1	C19	170.83			C19	170.84			
NS NS C19 NS C20 55.72 4.63 (dd 8.5, 3.0) C23 C20 55.66 4.62 (dd 8.0, 2.5) C23 C21 69.97 4.54 (d 3.0) C22 C21 69.98 4.63 (dd 2.5) C23 C22 172.46	8-Hydroxyasi	partic acid (1)			8-Hydroxyae	partic acid (1)			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	N5		8 05 (bd 8 5)	C19	ME		0.00	(6476)	010
C21 G337 Test (04.0.5, 0.5) C22 C21 G3360 4.62 (03.6.0, 2.5) C22 C22 Test (d.0.) C22 C22 Test (d.2.5) C22 C22 Test (d.2.5) C22 C22 Test (d.2.5) C22 C22 Test (d.2.5) C23 C22 Test (d.2.5) C23 C22 Test (d.2.5) C23 C22 Test (d.2.5) C23 C24 53 (d.2.5) C23 C23 Test (d.2.5) C23 C24 54.48 C33 (d.2.5) C23 C23 C24 54.44 C33 (d.1.5) C23 C24 54.44 C33 (d.1.5) C25. C29 C23 C34.41 C33 (d.1.5) C25. C29 C23 C34.41 C33 (d.1.5) C23 C24 54.44 C33 (d.1.5) C23 C24 54.44 C33 (d.1.5) C23 C24 54.44 C33 (d.1.5) C23 C23 C33 C33.44 C33 (d.1.5) C23 C33 C33.12 C31 (C16.0) C28, C41 C33 <thc33< th=""> C33 C33 C</thc33<>	C20	55 72	4.63 (dd 8.5.3.0)	C23	C20	55.00	8.03		019
C22 03.9 4.5 (0.5.0) C22 C21 03.96 4.5 (0.2.5) C22 C23 168.42	C21	60.07	4.54 (d 3.0)	C23	C20	50.00	4.62	(00 8.0, 2.5)	C23
C23 112-30 (C23 C23 168.42 C23 168.54** Lysine (1) Lysine (C24 54.51 A.07 (bdd 12.5, 7.5) C25, C29 C24 54.48 A.08 (bd 7.0) C23 C25 30.58 1.51# (m) C25, C29 C24 54.48 (m) C23 0.64# (m) C26 21.20 1.29# (m) C26 21.18 1.64# (m) C23 0.44 (m) C24 54.48 (m) C23 0.64# (m) C23 0.29 (m) C23 0.29 (m) C23 0.29 (m) C24 54.48 (m) 0.29 (m) 0.28 (m) 0.29 (m) 0.29 (m) 0.29 (m) 0.29 (m) 0.29 (m) 0.29 (m) 0.28 (m) 0.29 (m) 0.28 (m) <th0.28 (m)<="" th=""> 0.28 (m)</th0.28>	C22	172 /6	4:54 (0 5:0)	022	021	170 55	4.53	(a 2.5)	C22
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NS 7.99 (bd 7.5) C23 N6 7.98 (bd 7.0) C23 C24 64.51 4.07 (bdd 12.5,7.5) C25, C29 C24 54.48 4.08 (bd 7.0) C23 C25 30.58 (cd 13.0.7.5) C25, C29 C25 30.44 1.53# (m) C25 30.44 1.53# (m) 1.64# (m) 1.65# (m)	l veine (1)				Lunina				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	N6		799 (bd 75)	C23	NG		7 00	(hd 7 0)	C02
C25 34.51 4.08 (03.12.5, 7.5) C25 C24 54.83 4.08 (03.12.7, 5) C25, (29) C25 30.58 1.51# (m) C25 30.44 1.53# (m) 1.64# (m) C26 21.20# (m) C26 21.18 1.18# (m) 1.28# (m) C27 27.40 1.33# (m) C27 27.43 1.34# (m) 1.28# (m) C28 37.27 2.88 (m) C28 37.31 2.91 (m) N7	C24	54 51	4 07 (bdd 12 5 7 5)	C25 C20	004	54.40	7.98		023
C23 30.36 1.544 (m) 1.544 (m) C26 21.20 1.204 (m) C26 21.18 1.194 (m) C27 27.40 1.354 (m) C27 27.43 1.344 (m) C28 37.27 2.86 (m) C28 37.31 2.91 (m) 3.26 (m) 3.26 (m) 3.22 (m) 3.22 (m) Variable 7.17 (bt 5.5) C41 N7 7.75 (bt 6.0) C28, C41 Variable 7.65 (bt 10.5) C29 N8 7.69 (bd 7.5) C29 C30 53.22 4.16*** (m) C31, C35 C30 53.14 4.17 (m) C35 C31 30.58 1.61# (m) C32 28.25 1.73# (m) C32 21.96 1.30# (m) C32 28.25 1.73# (m) C34 C33 26.38* 1.50# (m) C34 1.55# (m) C34 C34 C34	C25	20.50	4.07 (buu 12.5, 7.5)	025, 029	024	54.48	4.08	(bad 13.0 7.5)	C25, C29
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	025	30.56	1.51# (m)		C25	30.44	1.53#	(m)	
C28 21.20 1.29# (m) C28 21.13 1.18# (m) 1.29# (m) 1.35# (m) 1.28# (m) 1.28# (m) 1.28# (m) C28 37.27 2.88 (m) C28 37.31 2.91 (m) 3.26 (m) 3.26 (m) 3.22 (m) 3.29 (m) 3.29 (m) N7 7.17 (bt 5.5) C41 N7 7.16 (bt 6.0) C28, C41 C29 171.21 C29 171.23 Lysine (2) Arginine N8 7.65 (bd 10.5) C29 N8 7.69 (bd 7.5) C29 C31 30.58 1.61# (m) C31, C35 C30 53.14 4.17 (m) C35 C33 26.38** 1.50# (m) C32 22.82.5 1.73# (m) C34 C33 26.38** 1.50# (m) C33 40.19 3.07*** (br d) C34 C34 38.58 2.77 (bd 6.0) N9 7.35 (bt 5.0) C34 C35 172.60 ND C34 156.32 ND C35 172.60 ND C35	0.06	01.00	1.64# (m)		÷	• · · ·	1.64#	(m)	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	628	21.20	1.20# (m)		C26	21.18	1.18#	(m)	
C27 27.43 1.35# (m) 1.36# (m) 1.38# (m) 1.36# (m) 1.36# (m) 1.38# (m) 1.36# (m) 1.36# (m) C28 37.27 2.88 (m) 2.91 (m) 3.26 (m) 3.22 (m) 3.22 (m) N7 7.15 (b15.5) C41 N7 7.16 (b16.0) C28, C41 C29 171.23 7.16 (b16.0) C29, C41 C29 7.16 (b16.0) C28, C41 Lysine (2) 7.65 (bd 10.5) C29 N8 7.69 (bd 7.5) C29 C30 53.22 4.16** (m) C31, C35 C31 26.32 1.48# (m) C35 C31 30.58 1.61# (m) C31, C35 C33 40.19 3.07*** (br d) C34 C32 21.96 1.30# (m) C32 28.25 1.73# (m) C34 C33 26.38** 1.50# (m) C33 40.19 3.07*** (br d) C34 C34 38.58 2.77 (bd 6.0) N9 7.35 (bt 5.0) C34 16.32 C35 172.60 M0 C34 156.55	0.07		1.29# (m)				1.28#	(m)	
1.395 (m) 1.395 (m) 1.395 (m) C28 37.27 2.88 (m) 2.91 (m) 3.26 (m) 3.22 (m) 3.22 (m) N7 7.17 (bt 5.5) C41 N7 C29 171.23 7.16 (bt 6.0) C28, C41 Lysine (2) C29 171.23 N8 7.65 (bd 10.5) C29 N8 7.69 (bd 7.5) C29 C30 53.22 4.16*** (m) C31, C35 C30 53.14 4.17 (m) C35 C31 30.58 1.61# (m) C31, C35 C30 53.14 4.17 (m) C35 C32 21.96 1.30# (m) C32 28.25 1.73# (m) C34 C33 26.38** 1.50# (m) C33 40.19 3.07*** (br d) C34 C34 38.58 2.77 (bd 6.0) N9 7.35 (bf 5.0) C37 C38, C39 C36 55.56 4.29 (dd 7.0, 6.0) C37, C38, C39 C35 172.28 ND C35 11 ND C36	C27	27.40	1.35# (m)		C27	27.43	1.34#	(m)	
C28 37.27 2.88 (m) 3.26 (m) 3.22 (m)			1.38# (m)				1.36#	(m)	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	C28	37.27	2.88 (m)		C28	37.31	2.91	(m)	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			3.26 (m)				3.22	(m)	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	N7		7.17 (bt 5.5)	C41	N7		7.16	(bt 6.0)	C28, C41
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	C29	171.21			C29	171.23			
N8	Lysine (2)				Arginine				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	N8		7.65 (bd 10.5)	C29	N8		7.69	(bd 7.5)	C29
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C30	53.22	4.16*** (m)	C31, C35	C30	53.14	4.17	(m)	C35
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C31	30.58	1.61# (m)		C31	26.32	1.48#	(m)	
C32 21.96 1.30# (m) C32 28.25 1.73# (m) C33 26.38** 1.50# (m) C33 40.19 3.07*** (br d) C34 C34 38.58 2.77 (bd 6.0) N9 7.35 (bt 5.0) C34 156.32 - C35 172.60 - - C34 156.32 - ND C35 172.60 - - N10 - ND ND C36 55.56 4.27 (bt 7.5) C37, C38, C39 C36 55.56 4.29 (dd 7.0, 6.0) C37, C38, C39 C37 68.47 4.41 (d 6.0) C36, C38, C39 C37 68.50 4.41 (d 6.0) C36, C38, C39 C39 168.72 - - - C39 168.68 - Glycine - - - C39 168.68 - - C41 168.11 - - - - - - C39 168.72 - - - - - - - C41 168.10 -			1.68# (m)				1.55#	(m)	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C32	21.96	1.30# (m)		C32	28.25	1.73#	(m)	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C33	26.38**	1.50# (m)		C33	40.19	3.07***	(br d)	C34
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	C34	38.58	2.77 (bd 6.0)		N9		7.35	(bt 5.0)	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	N9		ND		C34	156 32		()	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	C35	172.60			N10		ND		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$					N11		ND		
β-Hydroxysapartic acid (2) β-Hydroxysapartic acid (2) N10 - 8.08 (bd 7.5) C35 N12 - 8.01 (bd 7.0) C35 C36 55.56 4.27 (bt 7.5) C37, C38, C39 C36 55.56 4.29 (dd 7.0, 6.0) C37, C38, C39 C37 68.47 4.41 (d 6.0) C36, C38, C39 C37 68.50 4.41 (d 6.0) C36, C38, C39 C38 172.56 - - C39 168.72 - - Glycine - Glycine Glycine - - - C39 168.68 -					C35	172.28			
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	β-Hydroxyas	partic acid (2)			8-Hydroxyaar	artic acid (2)			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	N10		8.08 (bd 7.5)	C35	N12		8.01	(bd 7 0)	C35
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C36	55 56	4 27 (bt 7 5)	C37 C38 C20	C26	EE EE	4.00		
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C37	69.47	4.41 (den)	Cae Cae Cae	030	00.00	4.29	(40.7.0, 6.0)	037, 038, 039
C30 172.30 C38 172.49	C29	170.50	4.41 (0 0.0)	030, 038, 039	037	68.50	4.41	(a 6.0)	C36, C38, C39
Glycine Glycine Glycine Glycine Scale Glycine Glycine <thglycine< th=""> <thglycine< th=""> <thgly< td=""><td>C20</td><td>1/2.50</td><td></td><td></td><td>C38</td><td>1/2.49</td><td></td><td></td><td></td></thgly<></thglycine<></thglycine<>	C20	1/2.50			C38	1/2.49			
Glycine Glycine Glycine Glycine Glycine Glycine N13 8.21 (bt 6.0) C39 C40 42.95 3.32 (dd 16.0, 6.5) C41 C40 42.99 3.34 (dd 16.5, 5.5) C39, C41 3.91 (dd 16.0, 6.5) C41 C41 168.10 3.91 (dd 16.5, 6.0) C41	639	168.72	_		C39	168.68			
N11	Glycine				Glycine				
C40 42.95 3.32 (dd 16.0, 6.5) C41 C40 42.99 3.34 (dd 16.5, 5.5) C39, C41 3.91 (dd 16.0, 6.5) C41 3.91 (dd 16.5, 6.0) C41 3.91 (dd 16.5, 6.0) C41 168.11 C41 168.10	N11		8.18 (bt 6.5)	C39	N13		8.21	(bt 6.0)	C39
C41 168.11 C41 168.10	C40	42.95	3.32 (dd 16.0, 6.5)	C41	C40	42.99	3.34	(dd 16.5, 5.5)	C39, C41
	C41	168.11			C41	168.10	3.91	(uu 10.5, 6.0)	C41

ND: not determined.

*: overlapping with DMSO. **, ***: overlapping with each other, interchangable.

#: Overapping. Chemical shifts were determined from HSQC and TOCSY spectra.

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affinity between the ferric ion and pseudoalterobactins should be done.

The only current application of a siderophore to pharmaceuticals is desferrioxamine B (desferalTM). However, the recent application of a siderophore as the carrier for drug derivery systems is being studied,¹⁵⁾ and the immunosuppressive activity of the siderophores, IC202A, B and C, has been reported.^{16~18)} The detailed biological activities of pseudoalterobactins and their application to pharmaceutical uses should be examined.

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