ISOLATION AND STRUCTURAL DETERMINATION OF SIDEROCHELIN C, A FERMENTATION PRODUCT OF AN UNUSUAL *ACTINOMYCETES* SP.

LESTER A. MITSCHER, THOMAS HÖGBERG, STEVEN D. DRAKE, ALBERT W. BURGSTAHLER,† MARIANNA JACKSON,†† BYUNGKOOK LEE†, ROBERT I. SHELDON†, H. EUGENE GRACEY††, WILLIAM KOHL†† and R. J. THERIAULT††

Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045, U.S.A.
†Department of Chemistry, University of Kansas, Lawrence, Kansas 66045, U.S.A.
††Abbott Laboratories, Scientific Divisions, Abbott Park, Illinois 60064, U.S.A.

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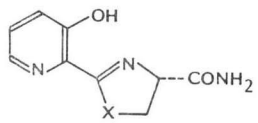
From the fermentation products of a new actinomycete (Abbott strain 69-209B-89), isolated from a Philippine soil sample, we have isolated phenylacetamide (**1**), the previously known antibiotics siderochelins A (**2**) and B (**3**),[1,2] and a new component; $C_{12}H_{15}N_3O_3$, mp 168~171°C, designated siderochelin C (**4**), which is shown here to be (2*S*,4*R*)-3,4-dihydro-4-ethyl-4-hydroxy-5-(3-hydroxy-2-pyridyl)-2*H*-pyrrole-2-carboxamide (**4**), a higher homologue of siderochelin A. In the course of this work we have also confirmed the structure and established the solid state conformation of siderochelin B by single crystal X-ray analysis.

The microorganism forms white to yellowish white aerial mycelia. It grows well at 28°C and 50°C. A pale reddish purple pigment, which is not a pH indicator, is formed on some media. Melanoid pigments are not produced. Spores occur in chains on the aerial mycelium. The conidiospores are oval and have a smooth surface. Sclerotia are also produced in the aerial mycelium. The substrate mycelium does not fragment. Strain 69-209B-89 contains *meso*-diaminopimelic acid and belongs to cell wall type IIIC.[3] Whole cell hydrolysates contain galactose as the only major diagnostic sugar. This strain is notably different from the organism described by LIU *et al.*[1] and excluded from the genus *Nocardia* by the absence of arabinose in cell hydrolysates and the lack of fragmentation. The whole cell sugar pattern, the formation of abundant aerial mycelium with sclerotia and the ability to grow at 50°C suggest *Streptoalloteichus* as a possible genus for this organism.[4] Definitive taxonomic studies are in progress and will be published later.



Siderochelin	X
A (**2**)	CH_3---C—OH
B (**3**)	CH_3—C---OH
C (**4**)	C_2H_5---C—OH

Submerged culture was carried out for 5 days at 30°C in 500-ml shake flasks containing 100 ml each of the following medium: lactalbumin 1%, primary NF yeast 0.5%, $CaCO_3$ 0.3%, KCl 0.03%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and Cerelose 1% (added post sterilization), pH 7.0 before sterilization.

The filtered fermentation broth (18 liters, pH 8.3) was passed through an Amberlite XAD-2 column and the eluate discarded. The resin was rinsed with 2-liter MeOH and the eluates were concentrated to an aqueous phase and partitioned between EtOAc and H_2O. During concentration of the dried organic solution, a precipitate formed. Filtration, further concentration and another filtration produced a total of 2.3 g of combined bioactive precipitate. The relative amounts of siderochelins A, B, and C were 10: 3: 1 based upon integration of the ^1H NMR methyl signals of the mixture. Using a gradient from 1% to 50% MeOH in $CHCl_3$, chromatography of 0.89 g of this material over a small Silica gel 60 column afforded, first, 0.48 g of pure phenylacetamide (**1**) (mixture mp, MS, ^1H NMR, IR) followed by 0.35 g of the siderochelins mixed with phenylacetamide. Repeated flash chromatography (silica gel; Et_2O - MeOH - H_2O, 40: 2: 1) followed by fractional crystallizations of the appropriate fractions from EtOAc - pentane by slow vapor transfer gave pure siderochelin A (**2**) (39 mg), B (**3**) (112 mg), and C (**4**) (8 mg). Siderochelins A and B were identified by coincidence of their physical and spectroscopic properties with those reported in

Table 1. Comparisons of prominent physical and spectroscopic properties of siderochelins A, B and C.

	Siderochelin A	Siderochelin B	Siderochelin C
MP °C	161~163 (Ref.[1]165~168)	186~186.5 (Ref.[1]200~201)*	168~171
UV (MeOH) λ_{max}, nm(ε)	316 (8,350)	315 (8,500)	316 (7,340)
IR ($CHCl_3$), cm^{-1}	3550, 3440, 3020, 1700, 1615, 1580, 1455, 1290, 1150	3550, 3440, 3020, 1700, 1615, 1580, 1455, 1300, 1160	3550, 3440, 2960, 1700, 1615, 1580, 1455, 1300, 1260
^1H NMR ($CDCl_3$): δ, ppm	1.70 (s), 4.96 (X of ABX, J=4.4 and 7.9 Hz)	1.63 (s), 4.61 (X of ABX, J=7.3 and 9.7 Hz)	0.97 (t, J=7.3 Hz), 4.98 (X of ABX, J=3.0 and 9.9 Hz)
Circular dichroism (MeOH): λ, nm ($\Delta\varepsilon$)	311 (−3.01)	312 (−0.66)	313 (−1.32)
	254 (−4.65)	254 (−7.07)	257 (−1.74)
		241 (+2.74)	
	227 (+10.2)	226 (−3.21)	227 (+7.65)
TLC (SiO_2)** Rf	0.51***	0.58***	0.62

* The discrepancy is not easily rationalized as the same solvent system (CH_3CN) was used for crystallization in both laboratories and the structures have been established in each case by independent X-ray analyses on different derivatives.

** Solvent: EtOAc - MeOH - H_2O, 8: 3: 1.

*** Previous work did not indicate separability of siderochelins A and B by TLC.[1] Use of our system greatly facilitates the analytical problem.

the literature (mp, IR, UV, ^1H NMR; Table 1) or those to be expected for such structures (^{13}C NMR, EIMS and CI(NH_3)MS).[1,2] Based upon its spectroscopic properties, the new antibiotic, siderochelin C (**4**), was clearly a homologue belonging to the siderochelin family. Chemical ionization mass spectrometry (CIMS-NH_3) suggested a molecular formula of $C_{12}H_{15}N_3O_3$ (MH^+ 250, 100%) which was confirmed by a peak matched electron impact (EIMS) molecule ion at 249.1108. The fragmentation pattern, in comparison with that of siderochelin A (**2**) (Table 2), demonstrated homology through ions *m/z* 249 *vs* 235, 205 *vs* 191, 187 *vs* 173, and 129 *vs* 115 whereas important ions at *m/z* 220, 203, 177, 149, 121, 115, 95, 94 and 72 were identical (Table 2), obviously each having lost the differentiating alkyl moiety. The IR and UV spectra (Table 1) of **2** and **4** were closely similar, as expected, while the C-CH_3 singlet at 1.70 ppm in the ^1H NMR of **2** was missing.[1] Instead, there was a 3 proton triplet at 0.97 ppm (J=7.3 Hz). The chemical shift (4.98 ppm) of the methine on the carboxamide-bearing carbon (X of ABX) was closer to that of the corresponding proton in A (4.96 ppm) than that of B (4.61 ppm) suggesting that the absolute configuration of C was that of **A**.[2] This inference was confirmed by circular dichroism comparisons of the three siderochelins (Table 1). These findings leave no reasonable doubt that siderochelin C possesses the biogenetically plausible structure **4**.[5]

Table 2. Mass spectral comparison of siderochelins A and C.

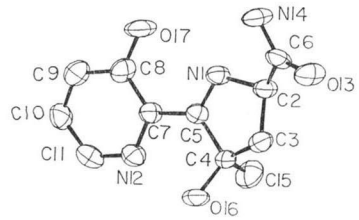
Ion	A		C	
	m/z	%	*m/z*	%
M^+	235	18	249	15
M^+-15	—		234	4
M^+-R	220	14	220	22
M^+-R-NH_3	203	12	203	23
M^+-CONH_2	191	8	205	8
—	—		189	7
$M^+-CONH_2-H_2O$	173	21	187	15
$M^+-CH_2CHCONH_2$	—		178	16
M^+-CONH_2-R	177	23	177	39
—	—		150	14
$M^+-R-HNCO-CO$	149	20	149	13
$M^+-R-HNCO-CH_2CH_2CO$	121	100	121	63
$RCOCH_2CH_2CONH_2$	115	92	129	13
—	115	92	115	9
3-Hydroxypyridinium	95	21	95	14
m/z 95−H	94	14	94	12
$[CH_2CHC(OH)NH_2]^+$	72	91	72	100

From the *in vitro* data in Table 3, it is interesting to note that siderochelin C, like siderochelin B, is dramatically narrower in anti-

Table 3. Antimicrobial activity of siderochelins *in vitro*.

Organism	MIC (μg/ml)		
	A	B	C
Staphylococcus aureus ATCC 6538P	>50	>50	>50
S. epidermidis 3519	50	>50	>50
Micrococcus luteus 9341	50	>50	>50
Escherichia coli Juhl	50	>50	>50
E. coli H560	50	50	50
Enterobacter aerogenes ATCC 13048	>50	>50	>50
Klebsiella pneumoniae 8045	50	>50	>50
Pseudomonas aeruginosa BMH 10	>50	>50	>50
P. aeruginosa K799/61	50	>50	>50
Acinetobacter sp. CMX 669	50	>50	>50

Fig. 1. Crystal structure of siderochelin B (enantiomer).



microbial spectrum than siderochelin A. The poor activity of C is unexpected in view of the relatively close structural relationship of C to A. The biological activity of all three siderochelins was destroyed by incorporation of ferrous iron in the medium.[1]

We have also confirmed in detail the structure and crystal conformation of siderochelin B by an independent X-ray crystallographic study (Fig. 1) and present these finding because of their biological and mechanistic value.

Precession photography showed that the crystals were orthorhombic and belonged to the space group $P2_12_12_1$ with four molecules per unit cell. Cell parameters were $a=10.443(3)$, $b=16.170(2)$ and $c=6.899(1)$Å. Reflection intensities were measured on a Syntex P1 automatic diffractometer using Ni-filtered CuKα radiation, $\lambda=1.5418$Å. In all, 763 reflections were collected from a needle shaped crystal ($0.45\times0.08\times0.05$ mm) using a $\theta-2\theta$ variable scan rate technique. Lorentz and polarization corrections were applied, but no absorption or extinction corrections were made.

The structure was solved by direct methods and all non-hydrogen atom coordinates and anisotropic temperature factors were refined by full matrix least squares. The final discrepancy factors were 0.053 for R and 0.064 for the weighted R calculated for 693 observed reflections with $I>2\sigma(I)$. The methyl and the carboxamide hydrogens were located from difference electron density maps. The remaining hydrogen positions were calculated except for the hydroxyl hydrogens which were not located.

The structure and atom numbering scheme for the molecule are shown in Fig. 1.*

In agreement with the reported structure of siderochelin A,[1] atoms N1, C2, C4 and C5 of siderochelin B lie essentially on a plane with the rms (root mean square) deviation of these atoms from their mean plane being 0.004Å. The fifth atom, C3, of the pyrroline ring is 0.49Å from the mean plane. As was found for siderochelin A, the torsion angle N1-C2-C6-N14 for this diastereoisomer has an eclipsed orientation of the C6-N14 and C2-N1 bonds. One primary amide hydrogen is hydrogen bonded to O16′ of a symmetry-related molecule (N14-O16′ intermolecular distance=3.021(6)Å, O16′-H1(N14) intermolecular distance =2.083(4)Å). The other primary amide hydrogen, just as was found for siderochelin A, is intermolecularly hydrogen bonded to O13′ of a symmetry-related molecule (N14-O13′ intermolecular distance=2.919(6)Å, O13′-H2(N14) intermolecular distance=1.842(4)Å).

Thus, the conformational differences of the hydroxypyridylpyrrole skeletons of the siderochelins appear to be insufficient to account for the significant differences in biological potency that they exhibit. Steric factors would, then, appear to be more important.

Acknowledgments

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