ISOLATION, RACEMIZATION AND ABSOLUTE CONFIGURATION OF SIDEROCHELIN A

Sir:

During our screening program of new antibiotics, we have isolated siderochelin A which was recently reported by Liu *et al.* in this journal¹⁾. According to those authors, siderochelins A and B were isolated from fermentation broths of *Nocardia* sp. SC 11,340 by solvent extraction and recrystallization, and siderochelin A was shown to be (*trans*)-3,4-dihydro-4-hydroxy-5-(3-hydroxy-2-pyridyl)-4-methyl-2*H*-pyrrole-2-carboxamide (Fig. 1) by X-ray crystallography and siderochelin B was assigned as a diastereoisomer of siderochelin A. However, the absolute configuration was not determined for either diastereoisomer. We have isolated siderochelin A by a resin adsorption method from a fermentation broth of our strain which belonged to *Nocardia*. Siderochelin B was not obtained by the process. We found that siderochelin B is a epimerization product of siderochelin A. We have also determined the absolute configuration of siderochelin A by X-ray crystallographic analysis of the *N*-bromoacetyl derivative of dihydrosiderochelin A. In this communication, isolation, racemization and absolute configuration of siderochelin A are reported.

Our siderochelin-producing strain, MG254-CF5, was isolated from a soil sample collected in our compound. Submerged culture was carried out at 27°C for 13 days in 500-ml flasks containing 100 ml of liquid medium of the following composition: glycerol 2%, dextrin 2%, Bactosoytone (Difco) 1%, yeast extract 0.3%, (NH₄)₂SO₄ 0.2%, CaCO₃ 0.2%, pH at 7.4 before sterilization.

The filtrate of the fermentation broth (2.1 liters) was passed through a column of Diaion HP-20, an adsorption resin manufactured by Mitsubishi Kasei Co., Japan, (40 × 300 mm). The adsorbed siderochelin was eluted by a linear gradient of aqueous acetone (0~50%) to give 432 mg of the crude material. The methanol extract of the crude material was chromatographed on a Sephadex LH-20 column (25 × 1,200 mm) developed with methanol. The bioactive fractions of the eluate were collected and dried under reduced pressure to give 159 mg of siderochelin. At this stage of purification, the

Fig. 1. Structure of siderochelin A¹⁾.

The absolute configuration was determined by the present study.

content of siderochelin A and B was analyzed by ¹H NMR spectrometry. The ¹H NMR spectrum in CDCl₃ containing 14% MeOH-*d*₄ showed a strong C-CH₃ signal for siderochelin A at δ 1.64, but the methyl signal of siderochelin B, which should appear at δ 1.58 (*vide post*), was not observed*. Pure crystalline siderochelin A was obtained from the crude siderochelin by crystallization with MeOH-Et₂O (1:5) (118 mg). The physico-chemical properties including IR and ¹H NMR spectra were the same as those previously reported¹⁾.

We found that siderochelin A is easily epimerized to afford siderochelin B. Siderochelin A (20 mg) was dissolved in 4 ml of 10% MeOH containing 0.1 N NaOH and the solution was kept for 16 hours at room temperature. After neutralization the reaction product (16 mg) was obtained by Diaion HP-20 adsorption followed by elution with methanol. This reaction product was found to contain siderochelin A and B in the molar ratio of 2.2:1 on the basis of the intensities of their C-CH₃ signals [δ 1.64 (A), 1.58 (B)] of the ¹H NMR spectrum. Siderochelin B was isolated from this reaction mixture and identified¹⁾. We observed further that the signal of the methine bearing the carboxamide function disappeared when the epimerization reaction proceeded in the deuterated solvent. Therefore, siderochelin B contained in the siderochelin isolated from the fermentation broth¹⁾ appears to be formed by epimerization of siderochelin A during the fermentation and isolation processes.

In order to determine the absolute configura-

* The siderochelin was hardly soluble in CDCl₃. In order to detect a small amount of siderochelin B, CDCl₃ containing 14% MeOH-*d*₄ was used as the solvent. The slight difference in the chemical shifts from those in reference 1 is due to the difference of the solvent.

Table 1. Crystal data.

<i>N</i> -Bromoacetyl dihydrosiderochelin A monohydrate			
$C_{18}H_{16}N_2O_4Br \cdot H_2O$ F. W. = 376.2			
Orthorhombic, space group $P2_12_12_1$, $Z=4$			
$D_{cal}=1.628$ g/cm ³			
$a=10.950$ (6)	$b=17.890$ (9)	$c=7.838$ (4) Å	
$U=1535.5$ Å ³			

tion of siderochelin A by X-ray crystallographic analysis, a crystalline derivative of siderochelin A containing a heavy atom was prepared. Siderochelin A (207 mg) was first reduced by catalytic hydrogenation with 10% Pd-C (20.7 mg) in 50% acetic acid (6.0 ml) at 1 atmospheric pressure of hydrogen at room temperature overnight. The resulting diastereoisomeric mixture of dihydrosiderochelin A (EIMS, m/z 237) was separated by column chromatography of silica gel (10 × 300 mm) developed with $CHCl_3$ - MeOH (15:1). The major isomer (140 mg) thus isolated showed the R_f value of 0.11 on a silica gel TLC developed with $CHCl_3$ - MeOH (4:1), and the minor isomer (51.3 mg) showed 0.36. The major isomer was treated with $BrCH_2COCl$ in MeOH at $-40^\circ C$ for 30 minutes to give the *N*-bromoacetyl derivative (quantitative). It was crystallized from acetonitrile - methanol (5:1) (colorless plates, mp $191^\circ C$) and used for the X-ray analysis.

The X-ray specimen (size $0.4 \times 0.2 \times 0.05$ mm) was cut from the plate. The cell dimensions and intensity data were measured on a Philips PW 1100 X-ray diffractometer using $CuK\alpha$ radiation monochromated by a graphite plate. Crystal data are given in Table 1. Intensities of 1679 reflections were measured to be above the 2σ (I) level within the θ angle of 3° through 78° . Below the θ angle of 50° , Friedel reflections ($\bar{h}kl$ vs. hkl) were measured. The number of $\bar{h}kl$ and hkl pairs was 468 and the R_F value

$$\left(\frac{\sum |F_o(\bar{h}kl)| - |F_o(hkl)|}{\sum |F_o(hkl)|} \right)$$

was 0.057 for this set of reflections.

The crystal structure was solved by the heavy atom method and refined by the block diagonal least-squares method. The R factor was dropped to 0.114 including anisotropic thermal parameters for all atoms. Hydrogen atoms were not included. The absolute configuration was determined by the anomalous dispersion method.

Fig. 2. Crystal structure of *N*-bromoacetyl dihydrosiderocheline A monohydrate.

The dispersion correction terms f' and f'' of bromine atoms for $CuK\alpha$ radiation were -0.767 and 1.283 . Comparison of $|F_o(hkl)|/|F_o(\bar{h}kl)|$ values with $|F_c(hkl)|/|F_c(\bar{h}kl)|$ clearly indicated that the structure should be described by a coordinate system inverted from that adopted for the intensity measurement. The least-squares refinement was then continued by taking into account the dispersion effect which yielded the final R value of 0.102 for 1679 hkl reflections. The weighting system was: $\sqrt{w}=1$ for $F_o \leq 20$, $\sqrt{w}=20/|F_o|$ for $F_o > 20^*$.

Fig. 2 illustrates the absolute configuration determined by the present study. Thus, the structure was shown to be (2*S*, 4*R*, 5*S*)-1-bromoacetyl-4-hydroxy-5-(3-hydroxy-2-pyridyl)-4-methylpyrrolidine-2-carboxamide. The bond lengths and angles were in reasonable overall agreement with the expected values for the chemical structure, and there was no intra-molecular hydrogen bond in the present structure whereas in siderochelin A each of the two potential ligand sites was connected by an intra-molecular $OH \cdots N$ hydrogen bond¹⁾.

Accordingly, the structure of siderochelin A was determined to be (2*S*, 4*R*)-3,4-dihydro-4-hydroxy-5-(3-hydroxy-2-pyridyl)-4-methyl-2*H*-pyrrole-2-carboxamide (Fig. 1). The predominant formation of the 5*S*-isomer of the dihydro-derivative by catalytic hydrogenation of siderochelin A may be explained by ready access of the catalyst from the less stereo-crowded site.

* The final atomic parameters have been sent to Cambridge Crystallographic Data Center.

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