## Communications to the editor

CHEMISTRY OF BLEOMYCIN. XV
ABSOLUTE CONFIGURATION OF β-AMINO-β-(4-AMINO-6-CARBOXY-5-METHYLPYRIMIDIN-2-YL)-PROPIONIC ACID, AN AMINE COMPONENT OF BLEOMYCIN

Sir:

β-Amino-β-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)-propionic acid, designated compound **II** in the former report,[1] is produced by degradation of an amino acid residue containing β-lactam in the bleomycin molecule.[2~4] The absolute configuration of the asymmetric carbon in this part of the bleomycin molecule has been determined by X-ray analysis of **II**-diamide hydrobromide.
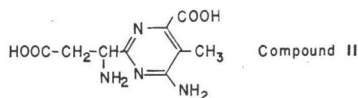
Attempts to determine the absolute configuration of **II** by forming an optically active aspartic acid by oxidative and reductive degradations of **II** have been unsuccessful due to racemization.

SETO et al.[5] recently proposed an experimental rule which can be applied to the determination of configuration of β-amino acids. If the molecular rotation of an L*-β-amino acid is measured in aqueous media at 250~350 nm, it is found that the values for rotations at various pH levels are in the following order: neutral>acidic>alkaline. Thus, the optical rotation of **II** was measured in 1 N HCl, 1 N NaOH and pH 6.81 phosphate buffer (0.125 M) at various wave lengths. The results are shown

Table 1. Optical rotation of compound **II**

Wave length nm	1 N HCl	1 N NaOH	pH 6.81 phosphate buffer (0.125 M)
380	+35*	+12.5	− 77.5
365	+47.5	+17.5	− 85
350	+67.5	+25	− 95
330	+150	+45	−110
320		+70	−120

* $[\alpha]^{20}$ $c=0.2$

in Table 1. The order of the value of the molecular rotation of **II** at different pH levels was: acidic>alkaline>neutral, a result which did not indicate a definite configuration, but rather suggested the $D(S)$-configuration.

An X-ray crystal analysis of **II**-diamide hydrobromide was undertaken in order to determine the absolute configuration of **II** unambiguously. A single crystal in the shape of a thin plate with approximate dimensions $0.05 \times 0.08 \times 0.2$ mm was used for the X-ray diffraction work. Reflection data were collected on a Philips PW 1100 four-circle X-ray diffractometer with graphite monochromated CuKα radiation. Crystal data are given in Table 2. Intensities were measured by a $\theta-2\theta$ scan method with scan speed of $4°\theta$ min^{-1}. The background was measured at each end of the scan for half the scan time. A total of 1,471 reflections was measured in the range of $2\theta \leq 156°$; of this total, 1,337 reflections having net intensities greater than the 2σ level were used for the following structure determination. For a total of 425 reflections, the intensities of both the hkl and $h\bar{k}l$ reflections

Table 2. Crystal data

β-Amino-β-(4-amino-6-carboxamido-5-methylpyrimidin-2-yl)-propionamide hydrobromide
M.W.=319.16
Monoclinic, Space group $P2_1$, $Z=2$
$a=10.213(5)$, $b=8.024(4)$, $c=7.813(4)$Å, $\beta=96.5(1)°$
$U=636.2$Å^3, $D_x=1.667$ gcm^{-3}

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* Designation of the absolute configuration at the β-carbon atom of β-amino acid in reference 5 is related to the α-carbon atom of D- or L-serine.

were measured. No absorption correction was applied for the intensity data.

The structure was solved by the heavy atom method. Ambiguity in the phase angle determination was removed by applying the anomalous dispersion method.[6] The result showed the locations of 17 light atoms with correct absolute configuration. Refinement by least-squares methods yielded a final R value of 0.048 for the 1337 observed reflections. In this refinement, unit weight was given to each reflection and no dispersion correction was applied for the atomic scattering factor of bromine. The absolute configuration was confirmed by comparing the values of $|F_c(hkl)|/|F_c(h\bar{k}l)|$ with those of $|F_0(hkl)|/|F_0(h\bar{k}l)|$. 111 Reflections* clearly favored the S-configuration. The final atomic parameters are listed in Table 3. The bond lengths and angles are given in Fig. 1. The values are consistent with those expected for the chemical structure. The standard

deviations are estimated to be about 0.013Å for C-C bond lengths and 0.8° for C-C-C bond angles. The amide groups, $C(9) \begin{smallmatrix} N(11)H_2 \\ O(10) \end{smallmatrix}$ and the amino nitrogen atom, N(13) are slightly twisted out from the plane of the pyrimidine ring, consisting of the atoms N1, C2, N3, C4, C5 and C6. The torsion angles about the C(4)-C(9) and C(2)-C(12) bonds are calculated to be $-11°$ for the former and $-13°$ for the latter. The conformation about the C(12)-C(14) bond is gauche and that about the C(14)-C(15) bond is planar; the torsion angles C(2)-C(12)-C(14)-C(15) and C(12)-C(14)-C(15)-O(16) are $-59°$ and $4°$, respectively. As a result of such conformations, the O(16) atom comes in proximity to N(13) and N(3). Electrostatic forces may stabilize the conformation. The amino nitrogen atom N(13) is protonated and forms three hydrogen bonds in tetrahedral directions.

Table 3. Final atomic parameters ($\times 10^4$)

The temperature factors are of the form: $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. Absolute configuration is represented by the right-hand coordinate system.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br	1194(1)	−2500(0)	4624(1)	58(1)	116(1)	152(1)	15(1)	24(1)	4(2)
N(1)	5561(7)	−3495(9)	7414(9)	48(6)	61(12)	105(12)	− 5(7)	21(7)	4(10)
C(2)	6326(8)	−2301(15)	6855(11)	53(7)	73(18)	103(13)	2(11)	− 3(7)	−21(14)
N(3)	6086(7)	− 674(9)	6721(9)	43(6)	58(11)	104(12)	2(7)	13(7)	− 7(10)
C(4)	4956(8)	− 160(11)	7364(11)	44(7)	64(13)	95(14)	− 4(8)	9(8)	−11(11)
C(5)	4068(8)	−1192(12)	8033(11)	43(7)	78(14)	89(13)	− 3(9)	11(8)	−13(12)
C(6)	4427(8)	−2977(11)	7992(10)	46(7)	82(16)	85(13)	6(8)	10(8)	− 2(10)
N(7)	3637(8)	−4129(10)	8583(10)	63(8)	78(13)	152(15)	− 3(9)	38(9)	8(12)
C(8)	2842(9)	− 730(12)	8795(13)	60(9)	82(15)	154(18)	6(10)	52(10)	−11(14)
C(9)	4783(8)	1697(12)	7286(11)	58(8)	71(14)	101(15)	2(9)	2(9)	2(12)
O(10)	3737(5)	2407(13)	7563(8)	56(5)	81(10)	177(11)	4(10)	29(6)	− 2(15)
N(11)	5830(6)	2582(16)	6898(9)	60(6)	74(11)	164(13)	− 1(13)	27(7)	−18(19)
C(12)	7615(8)	−2921(11)	6262(11)	44(7)	90(19)	101(13)	0(8)	16(8)	− 4(11)
N(13)	8167(7)	−1534(10)	5258(10)	61(7)	83(14)	132(14)	− 7(8)	30(8)	13(11)
C(14)	8613(8)	−3575(12)	7742(11)	50(8)	88(15)	101(14)	13(9)	3(8)	− 6(12)
C(15)	9027(8)	−2320(19)	9148(11)	49(7)	117(20)	139(15)	−10(13)	7(8)	6(18)
O(16)	8557(8)	− 905(11)	9188(10)	108(9)	127(14)	212(16)	21(10)	−42(10)	−52(13)
N(17)	9960(9)	−2939(12)	10408(11)	91(9)	146(22)	177(18)	0(10)	−22(10)	22(14)

* Among the 425 reflections for which the intensities of both hkl and $h\bar{k}l$ were measured, 111 reflections satisfied the following conditions and they were used for the determinations of the absolute configuration.

a) The difference in F_0's between the Friedel pair exceeds $3\sigma[F_0(hkl)]$.

b) The difference in F_c's between the Friedel pair exceeds $0.03\ F_c(hkl)$. The dispersion corrections for the bromine scattering factor used in the calculation of F_c were, $\Delta f' = -0.767$ and $\Delta f'' = 1.283$.

Fig. 1. The bond lengths and angles
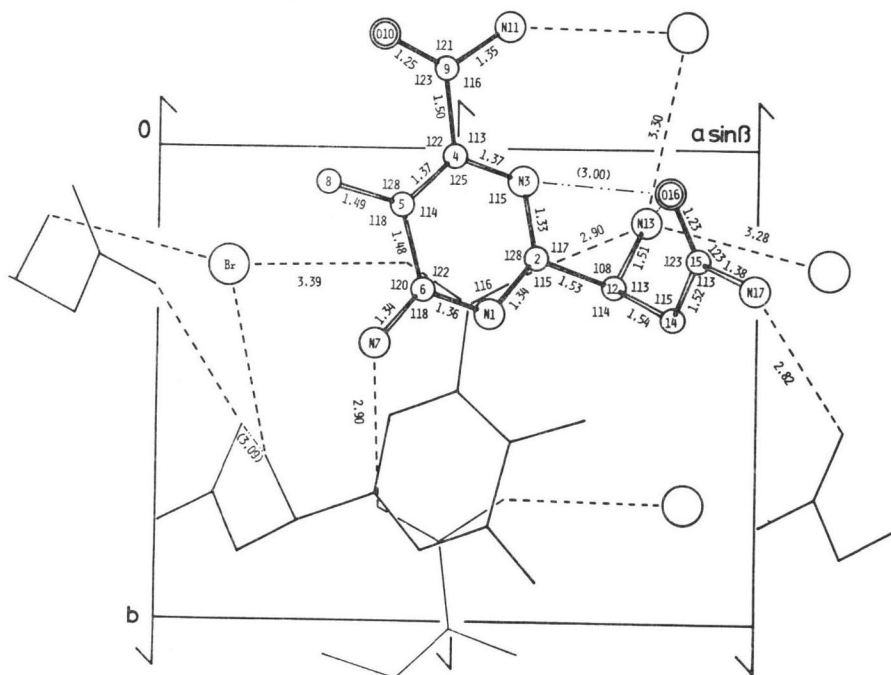
Thus, the absolute configuration of **II** was determined to be the *S*-configuration.

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