CHEMISTRY OF BLEOMYCIN. XIII*

THE X-RAY STRUCTURE DETERMINATION OF 4-AMINO-3-HYDROXY-2-METHYL-*n*-VALERIC ACID, AN AMINE COMPONENT OF BLEOMYCIN

Sir:

Previously, the structure of 4-amino-3-hydroxy-2-methyl-*n*-valeric acid (**I**), an acid hydrolysis product of bleomycin, was determined by chemical studies[1]. However, the stereochemistry remained unsolved. There are three consecutive asymmetric carbons in the molecule. Later, the stereochemistry of C-4 was assigned to the R-configuration by chemical interconversion method, that is, **I** was converted to D-alanine through dehydration followed by ozonolysis (Y. MURAOKA, T. TAKITA and H. UMEZAWA: unpublished data). In this communication we describe the result of the X-ray structure determination of **I**.

The crystals grown from aqueous butanol were transparent colorless prisms. A specimen of $0.36\times0.2\times0.16$ mm in size was selected for the X-ray diffraction work. The intensity

Fig. 1. Bond lengths and angles of molecules A and B (Fisher projection)

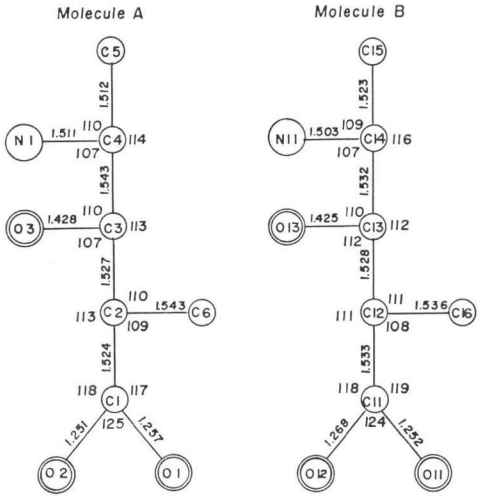

Fig. 2. Molecular packing and hydrogen bounding viewed along *c*-axis

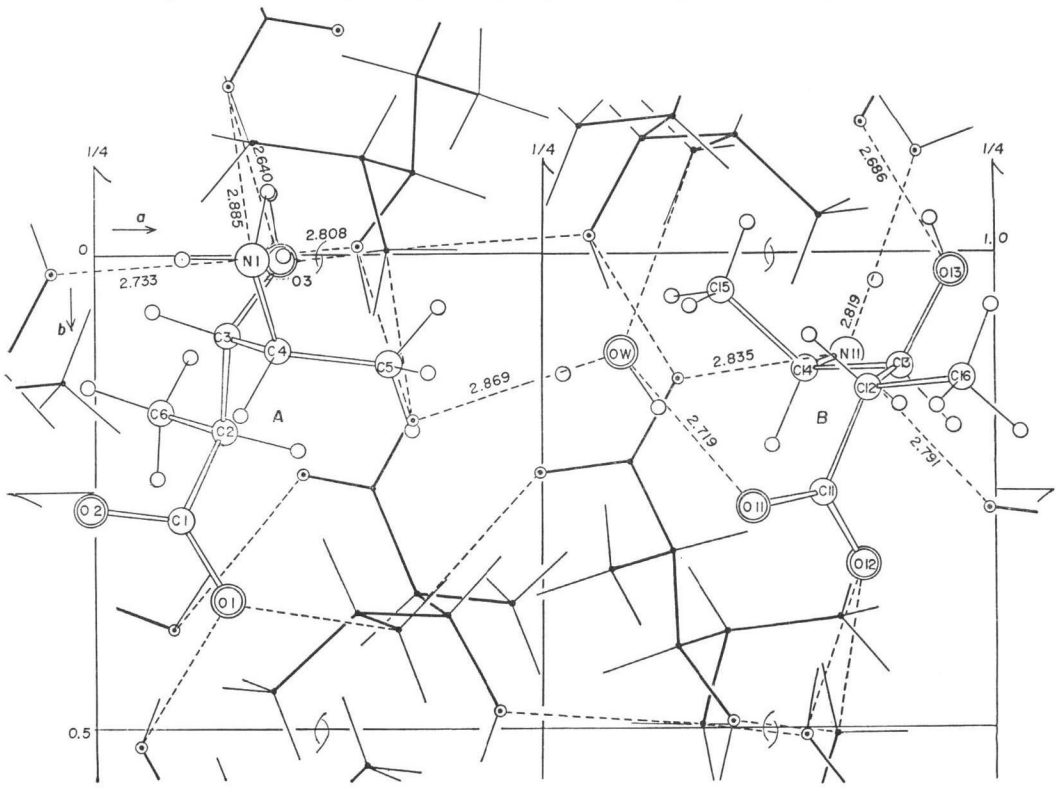

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Table 1. Final atomic parameters

Temperature factors are of the form $T=\exp[-\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{24}k^3l)]$

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C1	0.0904 (3)	0.2808 (3)	0.0425 (3)	0.0042 (3)	0.0028(2)	0.0034(3)	0.0001(2)	0.0001(3)	0.0007(2)
C2	0.1421 (3)	0.1867 (3)	−0.0269 (3)	0.0042 (3)	0.0021(2)	0.0042(3)	−0.0000(2)	0.0000(3)	0.0007(2)
C3	0.1464 (3)	0.0851 (3)	0.0513 (3)	0.0036 (3)	0.0025(2)	0.0039(3)	0.0003(2)	0.0003(3)	0.0001(2)
C4	0.2022 (3)	0.1021 (3)	0.1787 (3)	0.0041 (3)	0.0030(2)	0.0042(3)	−0.0001(2)	0.0004(3)	0.0002(3)
C5	0.3287 (4)	0.1180 (4)	0.1738 (4)	0.0039 (3)	0.0092(4)	0.0076(5)	−0.0022(3)	−0.0014(3)	0.0024(4)
C6	0.0730 (4)	0.1666 (3)	−0.1456 (4)	0.0071 (4)	0.0037(3)	0.0044(4)	0.0006(3)	−0.0009(3)	−0.0001(3)
N1	0.1750 (3)	0.0052 (2)	0.2565 (3)	0.0040 (2)	0.0039(2)	0.0039(3)	0.0008(2)	0.0002(2)	0.0008(2)
O1	0.1468 (2)	0.3658 (2)	0.0466 (3)	0.0047 (2)	0.0025(2)	0.0080(3)	−0.0009(2)	0.0025(2)	−0.0009(2)
O2	−0.0057 (2)	0.2690 (2)	0.0886 (3)	0.0041 (2)	0.0032(2)	0.0074(3)	−0.0003(2)	0.0013(2)	0.0001(2)
O3	0.2081 (2)	0.0075 (2)	−0.0181 (2)	0.0074 (2)	0.0022(1)	0.0048(2)	0.0005(2)	0.0017(2)	−0.0003(2)
C11	0.8110 (3)	0.2535 (3)	0.4737 (4)	0.0039 (3)	0.0032(2)	0.0052(4)	−0.0005(2)	−0.0008(3)	−0.0008(3)
C12	0.8614 (3)	0.1412 (3)	0.4612 (4)	0.0043 (3)	0.0028(2)	0.0051(4)	−0.0003(2)	0.0003(3)	0.0002(3)
C13	0.8943 (3)	0.1182 (3)	0.3271 (3)	0.0036 (3)	0.0027(2)	0.0043(3)	0.0003(2)	−0.0006(3)	0.0004(3)
C14	0.7924 (3)	0.1212 (3)	0.2399 (3)	0.0031 (3)	0.0035(2)	0.0041(3)	0.0001(2)	0.0001(3)	−0.0003(3)
C15	0.6996 (4)	0.0401 (4)	0.2661 (4)	0.0042 (3)	0.0070(3)	0.0067(4)	−0.0018(3)	−0.0009(3)	0.0006(3)
C16	0.9647 (3)	0.1337 (3)	0.5466 (4)	0.0054 (3)	0.0055(3)	0.0063(4)	−0.0008(3)	−0.0020(3)	0.0008(3)
N11	0.8358 (2)	0.1051 (2)	0.1107 (3)	0.0036 (2)	0.0029(2)	0.0042(3)	0.0002(2)	−0.0003(2)	−0.0003(2)
O11	0.7329 (3)	0.2681 (2)	0.5499 (3)	0.0067 (3)	0.0045(2)	0.0121(4)	−0.0001(2)	0.0038(3)	−0.0013(3)
O12	0.8552 (2)	0.3278 (2)	0.4105 (3)	0.0066 (2)	0.0025(2)	0.0082(3)	−0.0004(2)	0.0010(2)	0.0001(2)
O13	0.9510 (2)	0.0183 (2)	0.3154 (2)	0.0035 (2)	0.0029(2)	0.0060(2)	0.0007(2)	0.0008(2)	0.0006(2)
OW	0.0845 (2)	0.3973 (2)	0.4391 (3)	0.0059 (2)	0.0037(2)	0.0143(4)	−0.0009(2)	−0.0028(3)	0.0025(3)
HC2	0.2250(30)	0.2072(28)	−0.0550(35)	2.83 (0.88)					
HC3	0.0627(29)	0.0599(27)	0.0641(34)	2.28 (0.85)					
HC4	0.1604(28)	0.1680(26)	0.2221(30)	1.56 (0.75)					
HC5-1	0.3818(38)	0.0571(37)	0.1234(43)	6.75 (1.29)					
HC5-2	0.3700(34)	0.1269(33)	0.2418(38)	4.72 (1.05)					
HC5-3	0.3524(37)	0.1859(36)	0.1256(41)	5.68 (1.19)					
HC6-1	0.1050(37)	0.1079(36)	−0.1889(40)	5.58 (1.17)					
HC6-2	−0.0100(30)	0.1391(28)	−0.1266(34)	2.98 (0.87)					
HC6-3	0.0681(35)	0.2360(33)	−0.1950(38)	4.66 (1.07)					
HN1-1	0.0963(33)	0.0051(32)	0.2857(36)	4.65 (1.00)					
HN1-2	0.1911(38)	−0.0657(34)	0.2150(42)	5.42 (1.20)					
HN1-3	0.2095(33)	0.0017(36)	0.3217(38)	4.47 (1.05)					
HO3	0.1919(36)	−0.0618(34)	0.0106(40)	4.77 (1.13)					
HC12	0.7935(35)	0.0850(33)	0.4884(37)	4.35 (1.07)					
HC13	0.9560(31)	0.1819(31)	0.2903(35)	2.71 (0.94)					
HC14	0.7550(31)	0.2019(28)	0.2425(33)	2.50 (0.87)					
HC15-1	0.7280(33)	−0.0331(30)	0.2661(36)	4.12 (1.01)					
HC15-2	0.6430(33)	0.0452(32)	0.2044(37)	3.95 (1.05)					
HC15-3	0.6634(38)	0.0547(35)	0.3522(42)	5.63 (1.27)					
HC16-1	0.9983(34)	0.0547(30)	0.5465(38)	3.65 (0.99)					
HC16-2	1.0309(32)	0.1859(31)	0.5261(37)	3.73 (1.00)					
HC16-3	0.9381(37)	0.1591(36)	0.6498(41)	6.07 (1.20)					
HN11-1	0.7805(31)	0.1135(30)	0.0554(37)	3.66 (0.94)					
HN11-2	0.8689(31)	0.0290(30)	0.0983(36)	3.42 (0.95)					
HN11-3	0.8940(33)	0.1590(33)	0.0957(38)	4.64 (1.05)					
HO13	0.9298(36)	−0.0361(34)	0.3512(40)	5.22 (1.14)					
HOW-1	0.1265(34)	0.3381(34)	0.4348(40)	5.62 (1.10)					
HOW-2	0.0194(32)	0.3732(31)	0.4291(38)	4.05 (0.99)					

data were measured with Ni-filtered CuKα radiation on a Rigaku automatic four-circle X-ray diffractometer. The space group was determined by the WEISSENBERG photographs. Crystal data:

4-Amino-3-hydroxy-2-methyl-*n*-valeric acid hemihydrate, $C_6H_{13}O_3N \cdot 1/2\,H_2O$, Orthorhombic, $a=11.844\pm0.006$, $b=12.518\pm0.006$, $c=10.815\pm0.005$ Å

$Z=8$, $Dx=1.218\,g\,cm^{-3}$

Space group, $P2_12_12_1$.

Although the crystal density was not measured, it is clear that two crystallographically independent molecules are contained in an asymmetric unit. The intensities of 1281 symmetry independent reflexions were measured above 3σ level, out of 1595 theoretically possible ones within the 2θ value of 130°. The integrated intensity of each reflexion was measured by the ω-2θ scan method with a scan speed $4°2\theta$/min., and the background was measured by stationary counting at both sides of the diffraction peak for 10 seconds.

The normalized structure factors E were derived by use of the scale and temperature factors obtained by a WILSON plot. The structure was solved by the symbolic addition method. The specification of the origin and the assignment of unknown symbols were: $\varphi(1\ 11\ 0)=\pi/2$, $\varphi(0\ 11\ 4)=\pi/2$, $\varphi(2\ 0\ 1)=\pi/2$, $\varphi(3\ 8\ 0)=\pi/2$ (enantiomorph assignment), $\varphi(8\ 4\ 3)=a$, $\varphi(3\ 8\ 9)=b$. Several trial sets of phases in which a consecutive value of phases were assigned for a and b, were subjected to phase expansion and refinement. One of the sets in which were chosen $-3\pi/4$ for both a and b, gave an R value of 0.204 for 168 reflexions having E values greater than 1.5. The resulting E map showed the locations of 20 atoms out of 21 including the oxygen atom of the water of crystallization.

Refinement of the structure was carried out by the method of block-matrix least-squares coupled with three-dimensional difference FOURIER syntheses. The locations of the water oxygen atom and all of the 28 hydrogen atoms were determined on the difference maps. The final R value was 0.036 for 1281 observed reflexions. The final atomic parameters are given in Table 1.

The structures of the two crystallographically independent molecules, A and B are illustrated in Fig. 1, in which bond lengths and angles are also shown. The estimated standard deviations of these values do not exceed 0.006 Å and 0.35° respectively. In Fig. 2, the projection of the crystal structure along the c axis is shown. As seen in Figs. 1 and 2, both molecules have very similar dimensions and conformations. The differences in the corresponding bond lengths are all within the experimental error but the differences in angles are relatively larger, the largest being 4.7° in C2–C3–O3. The differences in internal rotation angles range from 9° to 18°, the largest one being observed in O1–C1–C2–C3 which define the orientation of the plane of the carboxyl group with respect to the C2–C3 bond. The conformation of the molecule may best be represented by defining the two trans planar groups, one being the N1, C4, C3, C2, C6 group and the other being C1, C2, C3, O3 group. These two groups intersect at the C2–C3 bond and the internal rotation angle C1–C2–C3–C4 is −52° in molecule A and −62° in B. The plane of the carboxyl group nearly bisects the angle C3–C2–C6 and the overall conformation of the molecules seems to be the most stable form. All the hydrogen atoms available for hydrogen bonding are involved in the hydrogen bonds listed in Table 2 forming a three dimensional network.

Table 2. Hydrogen bonds

Donor	Acceptor	Distance
N1	O13	2.733Å
N1	O12	2.885
N1	O3	2.808
N11	O1	2.835
N11	OW	2.819
N11	O2	2.791
O3	O12	2.640
O13	O1	2.686
OW	O12	2.869
OW	O11	2.719

The stereochemistry of C4 has been already deduced by the chemical method. Then, the absolute configuration of **I** was established as (2S, 3S, 4R)-4-amino-3-hydroxy-2-methyl-*n*-valeric acid.

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