ABSOLUTE STRUCTURE OF AMIDINOMYCIN

Sir:

Amidinomycin (**1**) was isolated from the culture filtrate of *Streptomyces flavochromogenes* and showed incomplete inhibition against spore-forming bacteria[1]. Later, amidinomycin[2] was proved to be identical with an antiviral antibiotic, myxoviromycin, produced by a *Streptomyces* similar to *Streptomyces albus*[3~5]. Amidinomycin gave 3-amino-1-carboxycyclopentane and 2-amidinoethylamine by acid hydrolysis and the structure was determined to be N-(2′-amidinoethyl)-3-aminocyclopentanecarboxamide[6,7]. 3-Amino-1-carboxycyclopentane was a new amino acid and its racemic form was synthesized by hydrogenation of 3-hydroxyimino-1-carboxycyclopentane over ADAMS catalyst in acetic acid[6,7]. Comparative paper chromatographies of the amino acid of natural origin and of the synthetic one obtained above showed the same Rf values. Further, both amino acids were respectively methylated after individual acetylation and the infrared absorption spectra in chloroform of these methyl esters of the acetates were compared and shown to be identical to each other. Nevertheless, the absolute configuration of the amino acid of natural origin has not yet been determined[6,7]. Thus, in order to establish the absolute configuration of amidinomycin, an X-ray crystallographic analysis of amidinomycin sulfate, $C_9H_{18}ON_4 \cdot H_2SO_4$, was carried out.

Single crystals of amidinomycin sulfate were obtained as colorless prisms by recrystallization from water and methanol.

Crystal data: $C_9H_{18}ON_4 \cdot H_2SO_4$, MW=296.3, orthorhombic, space group $P2_12_12_1$, a=10.046 (4) b=20.931 (8), c=6.506 (3) Å, Z=4, U=1368.0 Å^3, D_x=1.439 g·cm^{-3}.

The intensity data were measured on a Philips PW 1100 four-circle diffractometer with graphite-monochromated CuKα radiation using the θ-2θ scan method with a scan speed of 4° min^{-1} in θ. Out of the total of 1648 independent reflections within a 2θ value of 156°, 1418 reflections having intensities above 2σ(I) level were used for the structure determination and refinement. The intensities of 588 FRIEDEL pairs were also measured on the diffractometer. During the data collection, the intensities of three standard reflections, chosen in different regions of reciprocal space and measured every 120 minutes, remained essentially constant throughout. The intensities were corrected for LORENTZ-polarization factors and were placed on an absolute scale by WILSON's method. No absorption correction was made. The size of the crystal used for the data collection was about 0.3×0.25×0.2 mm.

The structure was determined by the heavy atom method. The atomic species were assigned on the difference electron-density map with the help of chemical considerations. Refinement of the positional and thermal parameters of the 19 atoms including those of the sulfate anion was carried out by the block-diagonal least-squares method with isotropic thermal parameters for all the atoms. When an R value was 0.098, anisotropic thermal parameters were introduced for all non-hydrogen atoms, and six cycles of calculations reduced R to 0.065.

The absolute configuration was determined by the anomalous dispersion method. The dispersion terms of the sulfur, the oxygen, and the nitrogen atoms for CuKα radiation were assumed to be f′=0.319, f″=0.557; f′=0.047, f″=0.032; f′=0.029, f″=0.018, respectively. Intensities were measured based on a right-handed axial system. The observed and calculated intensity ratios for the FRIEDEL pair reflections were compared. The ratios were only taken for the reflections with values of $[|Fobs(hkl)|-|Fobs(\bar{h}\bar{k}l)|] \geq 2\sigma[Fobs(hkl)]$. Out of 231 planes, 204 planes showed consistent values for $|Fobs(hkl)|/|Fobs(\bar{h}\bar{k}l)|$ and $|Fcal(hkl)|/|Fcal(\bar{h}\bar{k}l)|$.

Fig. 1. Chemical structure of amidinomycin.

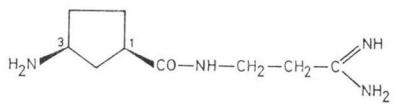

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Fig. 2. Stereoscopic drawing of the molecule of amidinomycin sulfate showing the correct absolute configuration.

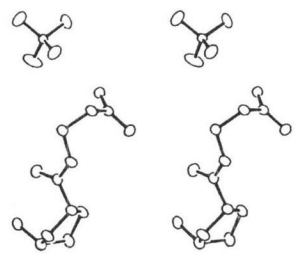
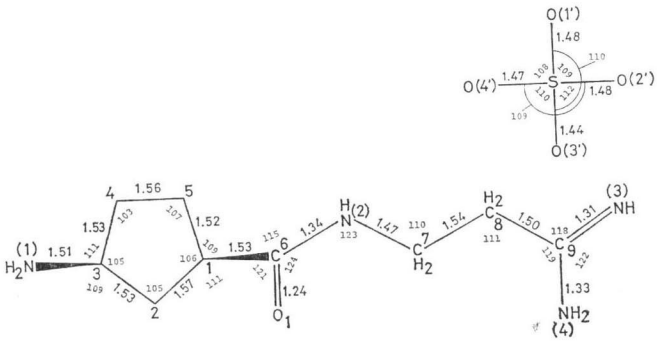


Table 1. Final atomic parameters of amidinomycin sulfate.

The fractional coordinates x, y and z are multiplied by 10^4. The temperature factor is of the form: $T=\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$, in which βij's are multiplied by 10^4.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	1848 (6)	4235 (3)	8096 (10)	73 (6)	16 (1)	207 (15)	5 (2)	−20 (9)	−8 (4)
C(2)	1280 (6)	4923 (3)	7733 (12)	75 (5)	13 (1)	294 (19)	3 (2)	5 (11)	−1 (4)
C(3)	−142 (7)	4894 (3)	8566 (10)	95 (7)	16 (1)	210 (16)	5 (3)	−4 (10)	2 (4)
C(4)	−79 (8)	4432 (3)	10382 (12)	117 (8)	25 (2)	201 (17)	19 (3)	22 (11)	9 (5)
C(5)	912 (8)	3913 (3)	9613 (10)	151 (9)	19 (1)	171 (14)	21 (3)	32 (12)	14 (4)
C(6)	1857 (6)	3853 (2)	6089 (9)	65 (5)	14 (1)	159 (12)	5 (2)	−4 (7)	3 (4)
C(7)	3229 (6)	3183 (3)	3802 (9)	76 (6)	16 (1)	155 (13)	9 (2)	19 (8)	7 (4)
C(8)	4138 (5)	2622 (3)	4411 (9)	56 (4)	14 (1)	175 (13)	4 (2)	16 (8)	1 (4)
C(9)	3494 (5)	2208 (2)	6018 (9)	57 (5)	13 (1)	155 (12)	2 (2)	17 (7)	1 (3)
O(1)	826 (4)	3774 (2)	5082 (7)	69 (4)	28 (1)	202 (10)	10 (2)	−29 (6)	−18 (3)
N(1)	−1054 (5)	4646 (2)	6901 (8)	63 (5)	18 (1)	216 (13)	3 (2)	−8 (7)	19 (3)
N(2)	3044 (5)	3606 (2)	5575 (8)	74 (4)	12 (1)	210 (12)	4 (2)	17 (8)	2 (3)
N(3)	2652 (5)	1769 (2)	5420 (8)	74 (5)	14 (1)	181 (12)	−5 (2)	−26 (7)	2 (3)
N(4)	3744 (6)	2322 (3)	7992 (7)	108 (6)	16 (1)	149 (11)	−6 (2)	−18 (8)	1 (3)
S	1217 (1)	1223 (1)	588 (2)	52 (1)	12 (0)	145 (3)	−1 (0)	7 (2)	0 (1)
O1(SO_4)	1401 (5)	568 (2)	1408 (8)	104 (5)	14 (1)	316 (15)	1 (2)	−36 (8)	27 (3)
O2(SO_4)	2401 (5)	1609 (2)	1120 (7)	108 (5)	22 (1)	184 (11)	−27 (2)	−7 (7)	−2 (3)
O3(SO_4)	13 (6)	1496 (2)	1406 (10)	104 (5)	20 (1)	557 (25)	10 (2)	129 (11)	−5 (5)
O4(SO_4)	1115 (6)	1178 (3)	−1662 (7)	145 (6)	30 (1)	150 (10)	−27 (3)	−27 (7)	5 (3)

Fig. 3. Bond lengths (Å) and bond angles (°).

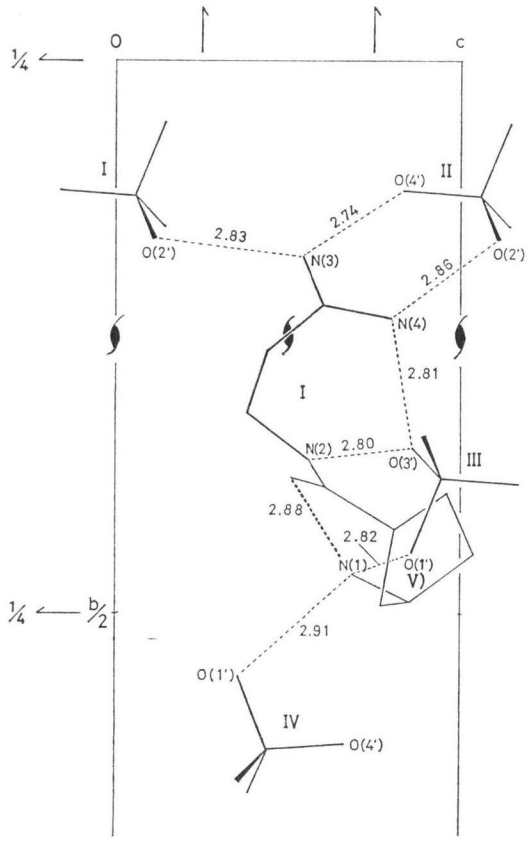


It was therefore concluded that the absolute configuration is described by taking a right-handed axial system for the atomic coordinates given in Table 1. At the final stage of the least-squares refinement, the anomalous dispersion effect was taken into account assuming the absolute configuration derived in this way. The final R factor was 0.062 for the 1,418 observed reflections. No hydrogen atoms were taken into account in the present structure determination. The final atomic parameters are listed in Table 1. A stereoscopic drawing of the molecule of amidinomycin sulfate produced by the ORTEP program[8] is shown in Fig. 2, which represents the correct absolute configuration. The bond lengths and angles lie in the normal range as shown in Fig. 3.

Thus, the chemical structure of amidinomycin already proposed[6,7] has been confirmed and the absolute configuration has been determined to be 1R, 3S as expressed by formula (**1**) in Fig. 1.

The cyclopentane ring is in an envelope conformation; C (1), C(2), C(4) and C(5) are almost coplanar within 0.05 Å and C(3), the flap, deviates by 0.55 Å from this plane in such a direction that

Fig. 4. Intra- and intermolecular hydrogen bonds. Symmetry operations are: I) x, y, z; II) x, y, 1+z; III) 1/2+x, 1/2−y, 1−z; IV) −x, 1/2+y, 1/2−z; V) −1/2+x, 1/2−y, 1−z.



the amino group at C(3) can form an intramolecular hydrogen bond to O(1) of the carbonyl group. The two bonds of the amidine group, C(9)-N(3) and C(9)-N(4), have almost the same bond length, and these three atoms and the adjacent carbon atom, C(9), N(3), N(4) and C(8) are also quite coplanar within 0.02 Å. This amidine group may possibly exist, after accepting H^+, as a charge-delocalized cation $-C(=NH_2)^+-NH_2$. As is seen in Fig. 4, all the nitrogen atoms of amidinomycin molecule are located within 3.0 Å from some oxygen atoms of the five surrounding sulfate anions, forming a net of hydrogen bonds.

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