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Structural Studies of Fortimicins. IV. The Crystal Structure of Fortamine Sulfate Trihydrate and the Anion Effect on Fortamine Conformation

Noriaki HIRAYAMA,* Kunikatsu SHIRAHATA, Yuji OHASHI,† and Yoshio SASADA†
Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.,
3-6-6 Asahimachi, Machida, Tokyo 194
† *Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology,*
Nagatsuta, Midori-ku, Yokohama 227

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The molecular and crystal structure of fortamine sulfate trihydrate, $C_8H_{18}N_2O_4 \cdot H_2SO_4 \cdot 3H_2O$, was determined by X-ray analysis. The space group is $P2_1$ with $a=7.2516(9)$, $b=11.193(1)$, $c=9.022(1)$ Å, $\beta=90.55(2)°$, and $Z=2$. The structure was solved by direct methods, and least-squares refinement using 1679 reflexions led to the final R value of 0.063. The ring conformation of fortamine sulfate is a chair form which corresponds to a chair obtained by inversion of that in the dihydrochloride. This is an extraordinary example of the cyclohexane ring inversion caused by the anion exchange in the crystal. The ring conformation of fortamines in solution and in crystal are compared and discussed.

Fortamine, 1,4-diaminocyclitol, is a component of potent aminocyclitol antibiotics fortimicins.[1)] Egan *et al.* have shown that fortamine moiety takes 'B' type conformation in fortimicin B, whereas it takes 'A' type conformation in fortimicin B sulfate and fortimicin A,[2,3)] as shown in Fig. 1. The conformation of fortamine moiety in fortimicins is probably related to their biological activity.[2)] Therefore, it is quite interesting to deduce the factors which control the conformation of fortamine. From such points of view we have determined the molecular structures of fortamine itself[4)] and its dihydrochloride salt[5)] by X-ray analysis. The results showed that the conformation of the former is 'B' type, but that of the latter is 'A' type. Although it has been believed that the 'A' type conformation is rather unstable because of the inherent 1,3-diaxial interactions, the latter analysis confirmed that 'A' type conformation could exist in the crystal structure and supported the assignment made by Egan *et al.*[2)]

The NMR results, however, revealed that in solution the fortamine rings of fortamine, fortamine dihydrochloride, and fortamine sulfate take essentially the same conformation, *i.e.*, the 'B' type conformation. It means that the ring conformation of fortamine dihydrochloride in the crystal is different from that in solution. This paradoxical observation has prompted us to compare the fortamine ring conformation of the sulfate in the crystal and in solution. In this connection we have undertaken the X-ray analysis of the sulfate. In this paper we will also report on the NMR results of fortamines.

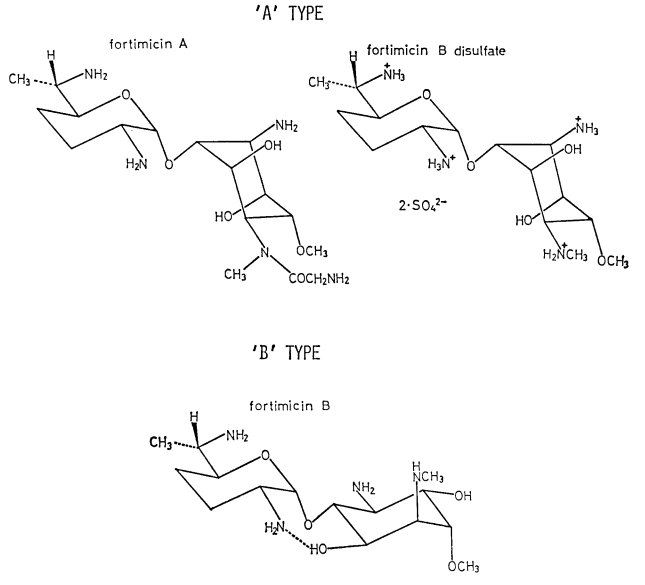

Fig. 1. Structures of fortimicins.

Experimental and Structure Determination

The prismatic crystals of the title compound were grown from a methanol solution. A crystal of $0.4 \times 0.4 \times 0.3$ mm^3 sealed in a glass capillary was used for data collection on a Rigaku four-circle automated diffractometer with graphite monochromated Mo $K\alpha$ radiation ($\lambda=0.71069$ Å). Preliminary unit-cell dimensions and space group were obtained from photographs. The space group was determined from the systematic absences ($0k0$ for odd k). Accurate cell dimensions were determined by least-squares calculation with 2θ values of 15 high-angle reflexions measured on the diffractometer. Crystal data are summarized in Table 1. All reflexions within the range of $2\theta \leqq 55°$ were collected by the use of the ω-2θ scan mode with a scanning rate of $4°(2\theta)$ min^{-1}. Stationary background counts were accumulated for 10 s before and after each scan. Periodic checks of the intensity values of three standard reflexions did not reveal any significant X-ray damage. Any corrections for absorption or extinction were not applied. A total of 1773 independent reflexions were obtained, of which 1679 ($|F_o| > 3.0\sigma(|F_o|)$) were considered as observed. The structure was solved by direct methods with MULTAN 78 program.[5)] The best set of phases was used to calculate an E map, which gave 13 chemically significant peaks. Other non-hydrogen

TABLE 1. CRYSTAL DATA

$C_8H_{18}N_2O_4 \cdot H_2SO_4 \cdot 3H_2O$	$b=11.193(1)$ Å
$F.W.=358.36$	$c=9.022(1)$ Å
$P2_1$	$\beta=90.55(2)°$
$Z=2$	$D_x=1.626$ g cm^{-3}
$a=7.2516(9)$ Å	μ(Mo $K\alpha$) $=2.869$ cm^{-1}

Table 2. Final atomic coordinates with their estimated standard deviations, multiplied by 10^4 for non-hydrogen and 10^3 for hydrogen atoms

Atom	x	y	z	B or B_{eq} / Å^2
C (1)	6432 (8)	3485 (7)	6092 (7)	1.8
C (2)	7703 (9)	4379 (6)	5338 (7)	1.7
C (3)	8081 (8)	4029 (6)	3751 (7)	1.6
C (4)	8786 (9)	2747 (6)	3585 (7)	1.7
C (5)	7534 (9)	1853 (6)	4388 (7)	1.7
C (6)	7172 (9)	2201 (6)	5984 (8)	1.9
C (7)	6486 (12)	4395 (9)	1453 (8)	3.4
C (8)	12106 (9)	3288 (9)	3235 (8)	3.0
N (1)	6257 (8)	3820 (6)	7691 (6)	2.2
N (4)	10743 (7)	2622 (5)	4120 (6)	1.9
O (1)	5829 (7)	1423 (5)	6578 (6)	2.9
O (2)	6985 (6)	5549 (4)	5440 (5)	2.2
O (3)	6346 (6)	4100 (5)	2990 (5)	2.0
O (5)	8390 (7)	711 (4)	4413 (5)	2.5
S	8474 (3)	−1780 (3)	1586 (2)	2.8
OS (1)	6904 (8)	−2010 (7)	679 (6)	4.8
OS (2)	8173 (9)	−1654 (12)	3103 (7)	7.6
OS (3)	9087 (18)	−3165 (12)	1757 (17)	2.0
OS (4)	9973 (25)	−1485 (21)	932 (22)	4.8
OS (5)	10210 (18)	−2063 (13)	917 (16)	2.1
OS (6)	8245 (25)	−334 (12)	1552 (16)	2.6
OS (7)	10038 (26)	−1075 (20)	1074 (24)	5.1
OS (8)	8813 (37)	−2563 (43)	2613 (53)	3.0
OW (1)	498 (1)	−380 (1)	869 (1)	6.4
OW (2)	205 (1)	−426 (1)	90 (1)	7.7
OW (3)	867 (2)	−398 (1)	857 (1)	11.3
HC (1)	495 (10)	351 (8)	569 (8)	3.3
HC (2)	881 (9)	437 (6)	607 (7)	1.8
HC (3)	878 (8)	461 (6)	332 (6)	0.7
HC (4)	875 (12)	256 (9)	249 (10)	4.6
HC (5)	640 (8)	183 (6)	398 (7)	0.9
HC (6)	835 (19)	215 (14)	654 (16)	10.9
HAN (1)	609 (10)	464 (8)	771 (7)	2.8
HBN (1)	706 (14)	391 (11)	821 (12)	7.1
HCN (1)	553 (13)	336 (12)	806 (11)	6.7
HAN (4)	1070 (10)	279 (7)	488 (9)	3.3
HBN (4)	1088 (12)	197 (9)	413 (10)	5.1
HO (1)	617 (8)	110 (6)	687 (6)	1.2
HO (2)	624 (9)	562 (6)	502 (7)	1.5
HO (5)	779 (12)	47 (10)	356 (10)	5.4
HAC (7)	752 (11)	396 (8)	91 (9)	4.0
HBC (7)	509 (17)	440 (14)	78 (14)	10.0
HCC (7)	704 (12)	506 (9)	132 (10)	5.3
HAC (8)	1346 (14)	316 (11)	367 (11)	7.1
HBC (8)	1175 (14)	311 (11)	247 (11)	7.1
HCC (8)	1191 (10)	398 (8)	352 (8)	2.9

atoms were located easily by the subsequent Fourier syntheses. The structural parameters were refined by block-diagonal least-squares methods with a modified HBLS program. The two of sulfate oxygen atoms were considerably disordered and when they were assumed to distribute over six positions with an occupancy of 0.3333, the refinement proceeded successfully. All hydrogen atoms except those of water molecules were located by difference syntheses. Refinement using anisotropic and isotropic thermal parameters for the non-hydrogen and hydrogen atoms, respectively, gave the final R value of 0.063 for 1679 reflexions. The weighting system used in the final stage was $w=(\sigma(|F_o|)^2+(0.015F_o)^2)^{-1}$. Atomic scattering factors were taken from "International Tables for X-Ray Crystallography."[6] The final positional and thermal parameters are given in Table 2. OS(3) to OS(8) denote the sulfate oxygen atoms refined with occupancy of 0.3333. OW denotes the water molecule. The table of observed and calculated structure factors and the list of the anisotropic thermal parameters are kept as Document No. 8144 at the Chemical Society of Japan.

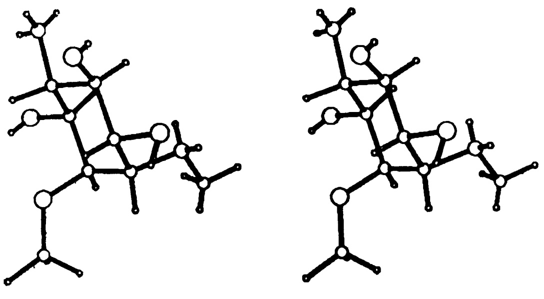

Fig. 2. Stereoscopic drawing of fortamine sulfate.

Table 3. Torsional angles (ϕ/°)

	Fortamine sulfate	Fortamine
C (3)-C (2)-C (1)-C (6)	54.1	53.9
C (4)-C (3)-C (2)-C (1)	−52.8	−56.1
C (5)-C (4)-C (3)-C (2)	51.0	56.3
C (6)-C (5)-C (4)-C (3)	−51.3	−56.4
C (1)-C (6)-C (5)-C (4)	53.4	54.6
C (2)-C (1)-C (6)-C (5)	−54.5	−52.6
O (2)-C (2)-C (1)-N (1)	−59.9	−62.2
O (3)-C (3)-C (2)-O (2)	−60.2	−59.8
N (4)-C (4)-C (3)-O (3)	170.4	173.9
O (5)-C (5)-C (4)-N (4)	−45.8	−60.1
O (1)-C (6)-C (5)-O (5)	−66.8	−57.9
N (1)-C (1)-C (6)-O (1)	65.2	65.2
C (4)-C (3)-O (3)-C (7)	−88.2	−110.1
C (3)-C (4)-N (4)-C (8)	−65.7	−70.9
C (5)-C (4)-N (4)-C (8)	169.7	166.5
C (2)-C (3)-O (3)-C (7)	150.3	129.2

The NMR spectra were measured in D_2O solution by means of an FX100 Model apparatus, JEOL Co., using DSS as the internal standard.

Results

Conformation of the Molecule. The ring conformation of fortamine dication, as shown in Fig. 2, is the 'B' type. The signs of torsional angles listed in Table 3 also represent the 'B' type conformation. The d values[3] of each ring-carbon atoms are as follows; C(1) 0.648, C(2) 0.632, C(3) 0.611, C(4) 0.619, C(5) 0.622, and C(6) 0.644 Å. The average C–C–C–C torsional angle is 52.9° and average d is 0.629 Å. They are quite similar to those of the dihydrochloride, 52.7° and 0.627 Å, respectively and smaller than those

Table 4. Bond lengths (l/Å) and angles (ϕ/°)

C (1) - C (2)	1.524 (6)	C (5) - C (4) - N (4)	110.4 (3)
C (1) - C (6)	1.538 (6)	C (4) - C (5) - O (5)	109.5 (3)
C (1) - N (1)	1.497 (6)	C (6) - C (5) - O (5)	107.2 (3)
C (2) - C (3)	1.512 (6)	C (5) - C (6) - O (1)	109.1 (4)
C (2) - O (2)	1.413 (5)	C (1) - C (6) - O (1)	108.0 (4)
C (3) - C (4)	1.532 (6)	C (3) - O (3) - C (7)	114.1 (4)
C (3) - O (3)	1.430 (5)	C (4) - N (4) - C (8)	114.5 (4)
O (3) - C (7)	1.430 (7)	OS (1) - S - OS (2)	116.8 (4)
C (4) - C (5)	1.537 (6)	OS (1) - S - OS (3)	95.7 (4)
C (4) - N (4)	1.501 (6)	OS (1) - S - OS (4)	117.3 (7)
N (4) - C (8)	1.478 (7)	OS (1) - S - OS (5)	114.8 (4)
C (5) - C (6)	1.516 (6)	OS (1) - S - OS (6)	95.0 (5)
C (5) - O (5)	1.421 (5)	OS (1) - S - OS (7)	122.5 (6)
C (6) - O (1)	1.416 (6)	OS (1) - S - OS (8)	116 (2)
S - OS (1)	1.419 (6)	OS (2) - S - OS (3)	92.8 (5)
S - OS (2)	1.395 (8)	OS (2) - S - OS (4)	124.5 (7)
S - OS (3)	1.619 (10)	OS (2) - S - OS (5)	125.5 (5)
S - OS (4)	1.285 (14)	OS (2) - S - OS (6)	84.3 (5)
S - OS (4)	1.437 (9)	OS (2) - S - OS (7)	112.7 (7)
S - OS (6)	1.628 (11)	OS (2) - S - OS (8)	53 (2)
S - OS (7)	1.460 (14)	OS (3) - S - OS (4)	93.3 (7)
S - OS (8)	1.297 (56)	OS (3) - S - OS (5)	65.7 (5)
C (1) - C (2) - C (3)	111.7 (4)	OS (3) - S - OS (6)	169.0 (5)
C (2) - C (3) - C (4)	113.5 (4)	OS (3) - S - OS (7)	109.5 (7)
C (3) - C (4) - C (5)	111.4 (4)	OS (3) - S - OS (8)	40 (2)
C (4) - C (5) - C (6)	112.9 (4)	OS (4) - S - OS (5)	27.8 (7)
C (5) - C (6) - C (1)	111.4 (4)	OS (4) - S - OS (6)	79.8 (7)
C (6) - C (1) - C (2)	111.8 (4)	OS (4) - S - OS (7)	18.7 (8)
C (6) - C (1) - N (1)	109.2 (4)	OS (4) - S - OS (8)	110 (3)
C (2) - C (1) - N (1)	108.9 (4)	OS (5) - S - OS (6)	107.5 (5)
C (1) - C (2) - O (2)	110.8 (4)	OS (5) - S - OS (7)	45.6 (7)
C (3) - C (2) - O (2)	111.8 (3)	OS (5) - S - OS (8)	89 (2)
C (2) - C (3) - O (3)	105.9 (3)	OS (6) - S - OS (7)	62.3 (7)
C (4) - C (3) - O (3)	107.3 (3)	OS (6) - S - OS (8)	135 (2)
C (3) - C (4) - N (4)	111.8 (3)	OS (7) - S - OS (8)	117 (3)

of the free base, 54.9° and 0.655 Å. These values show that the protonation at the nitrogen atoms causes the flattening of the fortamine ring as a whole. While C(1) and C(6) are puckered a little, C(3) and C(4) are flattened a little; the magnitude of puckering or flattening is smaller than that of the dihydrochloride and is almost the same as that of the free base. The smallest exocyclic O(5)–C(5)–C(4)–N(4) torsional angle of −45.8° is due to the intramolecular N(4)–H···O(5) hydrogen bond.

Bond Lengths and Angles. The bond lengths and angles are given in Table 4. The exocyclic C–C bond lengths are 1.512 to 1.538 Å, and the average value of 1.527 Å is essentially equal to those of the free base and the dihydrochloride. The C(2)–O(2) and C(6)–O(1) bond lengths, 1.413 and 1.416 Å, respectively, are significantly shorter than those of fortamine. This shortening is similar to that of the dihydrochloride. The C(1)–N(1), C(4)–N(4), and N(4)–C(8) bond lengths of the sulfate are as long as those of the dihydrochloride. Although the average C–C–C bond angle of 112.1° is the same as that of the dihydrochloride and larger than that of the free base, the scattering of the angles is small, *i.e.*, in the sulfate 111.4 to 113.5°, but in the dihydrochloride 109.7 to 114.3° and in the free base 109.9 to 112.6°. Most of the exocyclic bond angles are similar to those of the free base rather than those of the dihydrochloride. The bond lengths and angles show that the fortamine ring in the sulfate takes almost the same geometry as that of fortamine.

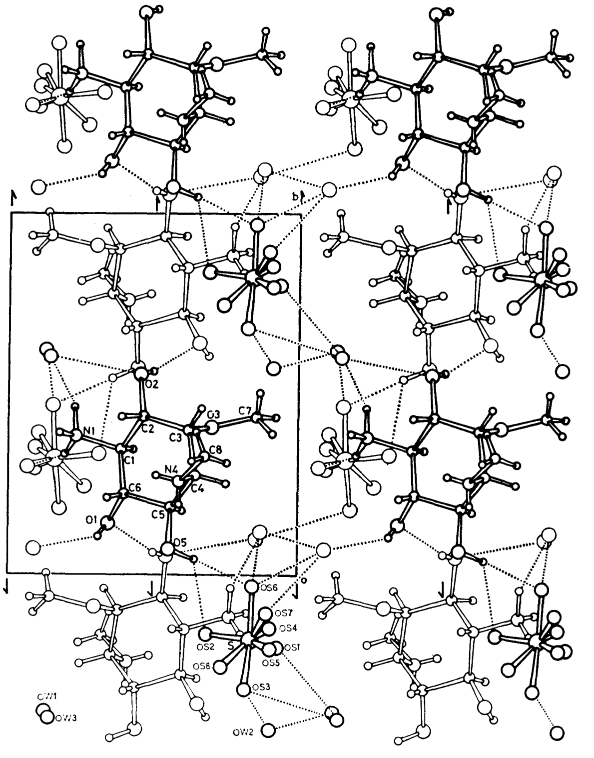

Fig. 3. Crystal structure of fortamine sulfate viewed along the a axis.

The sulfate oxygen atoms which are not involved in the hydrogen bonds are disordered; hence the relevant bond lengths and angles are distorted. The values are listed in Table 4.

Crystal Structure. The crystal structure viewed along the a axis is shown in Fig. 3. Hydrogen bond lengths and angles are listed in Table 5. Hydrogen bonds are shown by dotted lines in Fig. 3. The molecules linked by the O(2)–H···O(1) hydrogen bonds form helical chains along the b axis. There is no other intermolecular hydrogen bonds between the functional groups. The N(1) atom is hydrogen bonded to the OS(1) atom of the sulfate anion, but there is no sulfate anion sufficiently near the N(4) atom. The positive charge on N(4) may be partially transferred to O(5) through the N(4)–H···O(5) intramolecular hydrogen bond. The O(5) atom is hydrogen bonded to the OS(6) and OS(2) atoms. Therefore the positive charge may be neutralized by these hydrogen bonds. The O(1), O(2), and N(1) atoms are hydrogen bonded to water molecules. There is an extensive hydrogen bond networks between sulfate anion and water molecules.

Conformation in Solution. The NMR parameters of fortamine, its dihydrochloride, and its sulfate are shown in Table 6. The pD of the solution are 11.5, 3.5, and 1.5, respectively. The chemical shifts are

Table 5. Hydrogen bond distances (l/Å) and angles (ϕ/°)

A-H···B	A···B	∠A-H···B
N(4)-H$^{\mathrm{I}}$···O(5)$^{\mathrm{I}}$	2.751(6)	120(4)
O(5)-H$^{\mathrm{I}}$···OS(6)$^{\mathrm{I}}$	2.84(1)	142(10)
O(5)-H$^{\mathrm{I}}$···OS(2)$^{\mathrm{I}}$	2.903(9)	112(6)
O(2)-H$^{\mathrm{I}}$···O(1)$^{\mathrm{II}}$	2.892(5)	162(4)
N(1)-H$^{\mathrm{I}}$···OS(1)$^{\mathrm{II}}$	2.889(7)	152(5)
O(1)-H$^{\mathrm{I}}$···OW(2)$^{\mathrm{II}}$	2.841(7)	143(5)
N(1)-H$^{\mathrm{I}}$···OW(1)$^{\mathrm{III}}$	2.963(7)	151(5)
OS(3)$^{\mathrm{I}}$···OW(2)$^{\mathrm{IV}}$	2.60(1)	
OS(5)$^{\mathrm{I}}$···OW(2)$^{\mathrm{IV}}$	2.80(1)	
OS(6)$^{\mathrm{I}}$···OW(2)$^{\mathrm{V}}$	2.52(1)	
OS(7)$^{\mathrm{I}}$···OW(2)$^{\mathrm{V}}$	3.09(2)	
OW(1)$^{\mathrm{I}}$···OS(1)$^{\mathrm{VI}}$	3.024(8)	
OW(1)$^{\mathrm{I}}$···OW(2)$^{\mathrm{VI}}$	2.972(9)	
OW(3)$^{\mathrm{I}}$···OS(3)$^{\mathrm{VI}}$	3.03(1)	
OS(6)$^{\mathrm{I}}$···OW(1)$^{\mathrm{II}}$	2.91(1)	

Symmetry code

I:	(x	y	z)
II:	(1.0$-x$	0.5$+y$	1.0$-z$)
III:	(x	1.0$+y$	z)
IV:	($-x$	0.5$+y$	$-z$)
V:	(1.0$-x$	0.5$+y$	$-z$)
VI:	(x	y	1.0$+z$)

Table 6. 100 MHz ^{1}H-NMR parameters for fortamine free base, dihydrochloride, and sulfate

		Free base	Dihydro-chloride	Sulfate
Chemical shifts	H-1	2.80	3.47	3.43
	H-2	3.61	4.19	4.21
	H-3	3.67	3.98	3.97
	H-4	3.11	3.71	3.71
	H-5	3.78	4.19	4.16
	H-6	3.34	3.85	3.87
	OCH_3	3.45	3.48	3.49
	NCH_3	2.38	2.82	2.83
Coupling constants (Hz)	$J_{1,2}$	9.8	7.6	8.1
	$J_{2,3}$	4.4	3.3	3.3
	$J_{3,4}$	3.0	6.0	4.9
	$J_{4,5}$	4.6	4.5	4.9
	$J_{5,6}$	9.8	7.6	8.1
	$J_{6,1}$	9.8	7.6	8.1

expressed in ppm from the internal standard, DSS. The coupling constants are obtained by the first order analysis. The results apparently indicate that the ring conformations of the three are essentially same and they are in the 'B' type.

Discussion

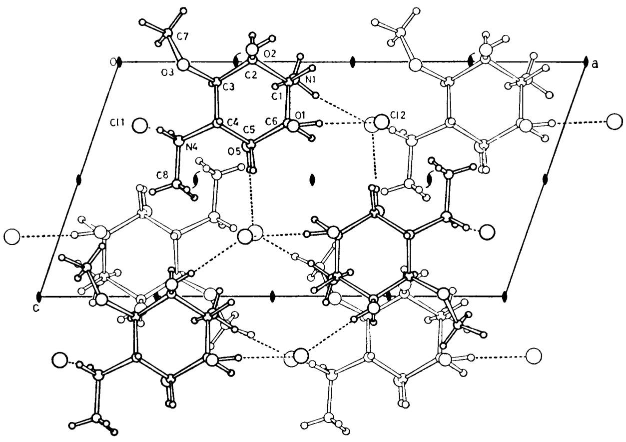

Fig. 4. Crystal structure of fortamine dihydrochloride viewed along the b axis.

Fortamine sulfate takes the 'B' type conformation in the crystal, whereas the chloride takes the 'A' type. It is interesting to examine the effect of different anions on the fortamine conformation in the crystal. The crystal structure of the dihydrochloride viewed along the b axis is shown in Fig. 4. In the crystal structure all functional groups are involved in intermolecular hydrogen bonds with the chloride anions, the anions locate near the two amino nitrogen atoms, and consequently the fortamine molecules are tightly packed. On the contrary, the fortamine molecules in the sulfate crystal as seen in Fig. 3 are loosely packed and anions are apparently solvated. The difference between the two crystal structures may come mainly from the different valence and the different size of the anions. In the crystal structure of the dihydrochloride, the Cl(1) atom is hydrogen bonded with the two protonated nitrogen atoms, *i.e.*, the N(1) and N(4) atoms. This makes the close packing possible. The sulfate anion, however, is divalent and bulkier than the chloride anion; hence it makes the close packing rather difficult and the structure which could accomodate the solvent molecules is built up.

The NMR results show that in aqueous solution the fortamine rings of the free base, the dihydrochloride, and the sulfate take essentially the same conformation, *i.e.*, the 'B' type conformation. In solution the anions might be solvated and they could not close to the cations easily; therefore it is conceivable that the environmental condition around the fortamine molecules in aqueous solution is close to that in the sulfate crystal. In the previous paper,[4] the electrostatic repulsion energy in the 'B' type conformation was calculated to be 0.50 kcal/mol higher than that in the 'A' type. Although further studies on the energy of molecular deformation would be required, these results suggest that intramolecular non-bonded interaction or intermolecular interaction must overcome the energy difference and the conformational change can easily occur in the stage of crystallization.

Figure 1 was drawn by TSD:XTAL, which is a computer-graphics interactive modeling programme for the NOVA 3 computer.[7]

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