THE X-RAY STRUCTURAL INVESTIGATION OF 6-(N,N-1,6-HEXYLENE-FORMAMIDINE)-PENICILLANIC ACID (MECILLINAM)

JANUSZ W. KRAJEWSKI, ZOFIA URBAŃCZYK-LIPKOWSKA,
PRZEMYSŁAW GLUZIŃSKI

Institute of Organic Chemistry, Polish Academy of Sciences,
01-224 Warszawa, Poland

ŁUKASZ LEBIODA

Institute of Chemistry, Jagiellonian University,
30-060 Kraków, Poland

IRENA BUŚKO-OSZCZAPOWICZ and JERZY CIEŚLAK

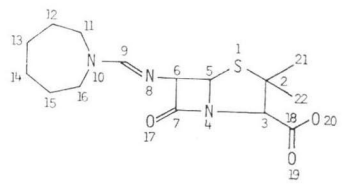
Institute of Pharmaceutical Industry, 02-516 Warszawa, Poland

(Received for publication July 21, 1980)

A new type of 6-formamidinepenicillanic acid in which the ω-nitrogen atom is involved in the azaheptane ring (mecillinam) having a strong selective activity against Gram-negative bacteria strains has been investigated by the X-ray single-crystal diffraction methods using crystals in the solvated state. The conformation of penam part as well as of the amidine group is discussed. Two independent molecules of mecillinam found in the asymmetric unit of the crystal cell differ from each other in their detailed conformations. The problem of the stability of the compound has been discussed also.

The 6-(N,N-1,6-hexyleneformamidine)-penicillanic acid (mecillinam) (Fig. 1) obtained independently in two different laboratories[1,2], represents a new type of N-substituted 6-aminopenicillanic acids (amidinopenicillins) having exceptional antibiotic properties. The most important feature of this compound is its selective and unusually strong activity against a number of Gram-negative bacteria strains, much stronger than the activity of previously known penicillins and cephalosporins. Mecillinam shows high activity in particular, against *Escherichia coli, Salmonella, Shigella* and also against *Proteus, Klebsiella, Enterobacter* and *Yersinia* species[1,3,4].

Fig. 1. Structural formula of the title compound giving the numbering of non-hydrogen atoms.



However, distinct stability differences are found between various mecillinam derivatives[5,6]. Since this is one of the most essential problems in drug chemistry, the exact knowledge of the molecular and crystal structure of the title compound seems to be necessary. Special attention should be given to the configuration and conformation of the amidine group, one of the most important parts of amidinopenicillins. Besides, the compound can be obtained in different solvation states (including the solvent free form) which may also give rise to the stability differences.

Preliminary results of these investigations were presented previously[7].

Materials and Methods

Crystal properties

The crystals of mecillinam for X-ray diffraction measurements were obtained from a purified sample by recrystallization from a water - ethanol - acetone mixture. They are colourless, stable in air at room temperature during the period of the experiment. A well shaped crystal of the dimensions of $0.4 \times 0.35 \times 0.3$ mm was selected for the measurements.

X-ray measurements

All reflection intensities were collected on a four-circle automatic diffractometer CAD-4 (Enraf-Nonius) (SLAF & BS, Jagiellonian University, Kraków, Poland) using MoKα radiation. The scanning mode was $\omega/2\theta$ up to $2\theta_{max}=150°$. The stability of the compound was monitored after every 20 measurements on two independent control reflections. From a total of 2432 collected reflections 2295 with $I>2\sigma_I$ were considered as observed. These were used for the structure refinement.

Computations

All computations were done on CDC-CYBER-73 Computer. The main program systems used were: for structure solution—program SHELX[8], for structure refinement and geometry calculations—the X-RAY System-70[9]. The measured intensities were converted into the structure amplitudes by LORENTZ and polarization corrections. No absorption correction was applied.

Results

The antibiotic crystallizes in the monoclinic system, space group $P2_1$. Table 1 gives the crystal data. The unit cell contains 4 molecules of the compound ($Z=4$), thus, there are two symmetrically independent molecules in the asymmetric unit. The number of observed reflections was rather small.

A separate problem relates to the unknown solvation mode of the crystal since the analytical results gave no clear answer. The existence of several water molecules in the crystal unit cell was certain, but their number as well as the possibility of the presence of an ethanol molecule (solvation by acetone is less probable) is still questionable. The calculated crystal density values for various solvation possibilities gave the best agreement with the experimental value for the presence of either $8H_2O$ or $5H_2O+C_2H_5OH$ with two molecules of mecillinam in a unit cell.

Table 1. Crystal data.

$C_{15}H_{23}N_3O_3S+(nH_2O)$	$M_r=325.47$ (solvent free)
$a=10.384(2)$ Å	$D_m=1.23$ Mg m^{-3}
$b=22.278(4)$ Å	Space group $P2_1$
$c=10.322(2)$ Å	$Z=4$
$\beta=116.86(2)°$	$V=2,130.2$Å^3

Several runs of the SHELX program (computation of absolute structure factors, tangent refinement of phases, electron density maps) with all non-zero structure factors as input data revealed the crude positional parameters of the penam parts for both independent molecules. The remaining atoms are found by a series of FOURIER syntheses. These revealed also six possible positions for non-hydrogen atoms of solvent molecules, assumed for further refinement process to be O-atoms. The refinement runs varied the positional and anisotropic thermal parameters for all non-hydrogen atoms keeping invariant the calculated hydrogen parameters in mecillinam molecules (the carboxyl and solvent H-atoms were still unknown). The weighting scheme was $w=1/\sigma_{F_o}{}^2$. The final reliability factors were then $R=0.11$, $R_w=0.12$. During the refinement process one of the solvent O-atoms was discarded. Table 2 gives the refined fractional coordinates for non-hydrogen atoms in mecillinam molecules as well as for five solvent O-atoms.

It should be mentioned that the number of observed reflections may be regarded as inadequate

Table 2. Fractional coordinates ($\times 10^4$) with esd's in parentheses.

Atom	Molecule I			Molecule II		
	x/a	*y/b*	*z/c*	*x/a*	*y/b*	*z/c*
S (1)	9,037(7)	362(3)	24(8)	2,443(6)	−1,068(3)	−5(6)
C (2)	7,174(29)	579(9)	−1,360(31)	1,792(21)	−532(10)	−1,527(23)
C (3)	6,406(25)	776(8)	−353(28)	2,700(23)	−690(9)	−2,376(23)
N (4)	7,187(19)	505(6)	1,030(18)	3,077(19)	−1,299(7)	−2,117(18)
C (5)	8,725(22)	433(9)	1,598(28)	2,606(22)	−1,656(8)	−1,190(22)
C (6)	8,610(22)	−153(8)	2,396(21)	4,033(23)	−2,011(8)	−634(21)
C (7)	7,038(21)	−46(7)	1,498(22)	4,539(24)	−1,517(7)	−1,322(20)
N (8)	9,328(17)	−720(6)	2,401(17)	4,908(19)	−2,115(6)	926(18)
C (9)	8,812(21)	−1,056(8)	1,151(20)	5,601(25)	−1,714(8)	1,857(23)
N (10)	9,377(19)	−1,554(7)	1,102(20)	6,344(22)	−1,797(7)	3,266(19)
C (11)	10,599(27)	−1,802(9)	2,334(27)	7,076(26)	−1,304(10)	4,214(25)
C (12)	10,807(33)	−2,449(13)	2,252(39)	6,679(35)	−1,243(12)	5,392(32)
C (13)	9,574(41)	−2,826(12)	2,167(49)	5,051(38)	−1,365(13)	4,989(36)
C (14)	8,617(39)	−2,960(14)	460(34)	4,912(36)	−2,000(15)	5,192(36)
C (15)	7,857(29)	−2,430(12)	−402(27)	5,089(30)	−2,440(12)	4,075(25)
C (16)	8,768(28)	−1,899(10)	−339(23)	6,332(30)	−2,350(10)	3,874(26)
O (17)	5,931(16)	−345(6)	1,140(16)	5,592(14)	−1,329(5)	−1,309(14)
C (18)	6,247(29)	1,462(10)	−238(31)	1,842(25)	−554(9)	−4,006(23)
O (19)	5,187(19)	1,675(6)	−1,183(18)	1,177(17)	−956(7)	−4,846(16)
O (20)	7,241(21)	1,750(7)	783(21)	1,846(22)	−8(8)	−4,353(18)
C (21)	7,292(34)	1,127(11)	−2,213(27)	2,239(27)	95(9)	−788(27)
C (22)	6,274(32)	68(9)	−2,418(26)	178(20)	−591(11)	−2,448(23)

Solvent:	Atom	*x/a*	*y/b*	*z/c*
	W(1)	9,874(20)	2,271(7)	2,290(20)
	W(2)	12,072(29)	1,450(11)	3,552(29)
	W(3)	12,161(27)	180(11)	3,013(28)
	W(4)	2,820(24)	1,102(9)	−3,806(24)
	W(5)	9,464(31)	2,839(12)	4,605(34)

when compared with the number of parameters to be refined in the structure. Furthermore, the considerable mosaic character of the crystal had lowered the overall accuracy of the measurements. These problems probably contributed to the failure to locate the water and carboxyl H-atoms. The relatively small number of observed reflections resulted in large standard deviations of all refined parameters.

A view of the mecillinam molecules in the asymmetric unit is presented in a parallel projection (Fig. 2). The most important bond lengths and valence angles for both molecules are listed in Tables 3 and 4, respectively.

Discussion

It was found that the thiazolidine rings in the two mecillinam molecules possess different conformations (Fig. 3). Recently[10], various conformations of thiazolidine rings in several penicillin derivatives investigated by X-ray methods have been systematized based on their relative atomic positions. There are three types of envelope conformations called S, C5 and C3, depending on which ring atom deviates mostly from the best plane defined by the four remaining atoms. In the present

Fig. 2. Parallel projection of both symmetrically independent molecules **I** and **II** in an asymmetric unit.

W-solvent atoms (assumed to be oxygen). Close acceptor-donor contacts (potential hydrogen bonds) shown by dashed lines.

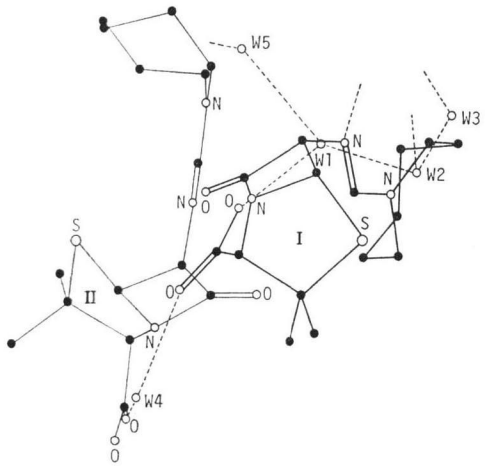


Fig. 3. Views of molecules **I** and **II** oriented along their amidine planes.

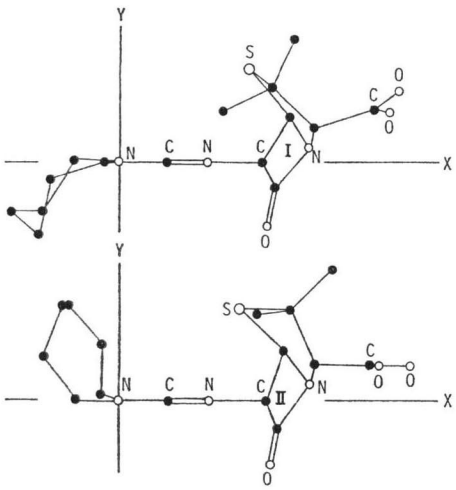


Table 3. Some bond lengths (Å) with esd's in parentheses.

	Molecule **I**	Molecule **II**
S (1)-C (2)	1.88(2)	1.84(2)
S (1)-C (5)	1.80(3)	1.85(2)
C (2)-C (3)	1.63(5)	1.59(4)
C (3)-C (18)	1.55(3)	1.54(3)
C (18)-O (19)	1.19(3)	1.22(2)
C (18)-O (20)	1.27(3)	1.27(3)
C (3)-N (4)	1.42(3)	1.40(3)
N (4)-C (5)	1.44(3)	1.49(3)
N (4)-C (7)	1.35(2)	1.45(3)
C (7)-O (17)	1.23(2)	1.17(3)
C (5)-C (6)	1.58(3)	1.54(3)
C (6)-C (7)	1.49(3)	1.53(3)
C (6)-N (8)	1.47(3)	1.47(2)
N (8)-C (9)	1.37(2)	1.27(2)
C (9)-N (10)	1.27(3)	1.32(3)

Table 4. Some valence angles (°) with esd's in parentheses.

	Molecule **I**	Molecule **II**
C (2)-S (1)-C (5)	97(1)	90(1)
S (1)-C (2)-C (3)	103(2)	104(1)
N (4)-C (5)-S (1)	105(2)	102(1)
C (3)-N (4)-C (5)	119(2)	120(2)
C (2)-C (3)-N (4)	108(2)	107(2)
C (3)-N (4)-C (7)	130(2)	124(2)
C (6)-C (5)-S (1)	119(2)	118(1)
N (4)-C (5)-C (6)	88(2)	88(3)
N (4)-C (7)-C (6)	96(2)	91(2)
C (7)-C (6)-C (5)	82(1)	86(2)
C (5)-N (4)-C (7)	92(1)	91(1)
C (5)-C (6)-N (8)	124(1)	117(2)
C (7)-C (6)-N (8)	124(2)	120(2)
C (6)-N (8)-C (9)	119(1)	125(2)
N (8)-C (9)-N (10)	122(2)	126(2)
C (9)-N (10)-C (11)	123(2)	121(2)
C (9)-N (10)-C (16)	119(2)	120(2)
C (11)-N (10)-C (16)	119(2)	119(2)

case (Table 5) the thiazolidine ring in molecule **II** may be assigned to the S-type (the S atom deviates by 0.82(5) Å from the least-squares plane through the other four atoms). In contrast, the thiazolidine ring in molecule **I** cannot be assigned to any of the above types, although it also has an envelope conformation. This conformation may be described as the N4-type, the N(4) atom deviating by −0.37(1) Å from the least-squares plane through the remaining atoms (Table 5). Similarly, the corresponding bond lengths and angles (Tables 3 and 4) in the thiazolidine rings of the two mecillinam molecules are different: these of molecule **II** conform to averaged values of those of the S-type, while these of mole-

Table 5. Least-squares planes in thiazolidine rings of mecillinam.

	Molecule I	Molecule II
Plane defined by:	S (1), C (2), C (3), C (5)	C (2), C (3), N (4), C (5)
Deviations (in Å) of ring atoms from planes (esd's in parentheses)		
S (1)	0.040 (6)	0.818 (5)
C (2)	−0.04 (2)	0.00 (2)
C (3)	0.03 (2)	0.00 (2)
N (4)	−0.37 (1)	0.00 (1)
C (5)	−0.03 (2)	0.00 (2)

cule **I** do not conform to any of the earlier specified types.

The tertiary amino N-atoms [N(10)] in both molecules are almost planar (rather of hybridization sp^2 than sp^3) as it may be shown by the deviations of N(10) from the planes through C(9), C(11) and C(16): 0.01(1) and 0.03(1) Å, for molecule **I** and **II**, respectively. The conformations of the hexamethyleneimine rings and their orientation relative to the rest of the molecules are different and do not correspond to any explicitly defined conformation. The X-ray investigations recently done by PALM and CSÖREGH[11] on bacmecillinam hydrochloride salt lead to very similar conclusions regarding the hexamethyleneimine ring structure. Conversely, in N-hexylene-N′-*p*-nitrophenylformamidine[12] the seven-membered aza-ring was found to possess the chair conformation with the N-atom also having a planar arrangement. The amidine chains $>N_A-CH=N_I-$penam in both molecules are coplanar with the amino N-atom planes. The penam parts and hexamethyleneimine rings are attached in a trans configuration with respect to the $-CH=N_I-$ double bond. The β-lactam rings are twisted with respect to the amidine plane in very similar manner by about 116°.

Considering the bond lengths in amidine groups it was found that in the molecule **II** of mecillinam the $-CH=N_I-$ distance exhibits the value of 1.27(2) Å, which is nearly normal for an isolated $-CH=N_I-$ double bond. It is spectacularly shorter than the bonding distance of the imino nitrogen and penam carbon atoms. Conversely, in the molecule **I** the corresponding $-C=N_I-$ distance is elongated by 0.1 Å (over 4 esd.) with simultaneous shortening of the bond to the amino nitrogen atom. The anomalous bond lengths in the amidine group for molecule **I** indicate the strong perturbation of bond multiplicity. As is discussed below, this unusual feature may be due to the hydrogen bridging or even protonating effect. The $-CH=N_I-$ bond length in molecule **II** of mecillinam may be compared to that found in N-hexylene-N′-*p*-nitrophenylformamidine[12] (within the double esd. value).

Table 6 gives the shortest intermolecular contacts involving non-hydrogen atoms in the crystal. In the absence of information concerning the hydrogen atomic positions at potentially hydrogen-donoring atoms (carboxyl and water O-atoms) this is the only basis for identifying hydrogen bonds. The short contact (2.67 Å) between one of the carboxyl O-atoms [O(19)] in molecule **II** at $1+x, y, 1+z$ and the amidine nitrogen N(8) in molecule **I** implies the existence of a rather strong intermolecular hydrogen bridge between the carboxyl group and amidine nitrogen atom (Fig. 2).

A significant number of close contacts of potential hydrogen donors and acceptors indicate the possibility of the existence of a rather complex hydrogen bonding system in the crystal lattice. None of these contact values, however,

Table 6. Interatomic distances (Å) with esd's in parentheses, offering the possibility of hydrogen bonding.

W (1) −O (20)	2.72 (3)
O (20)*−W (4)	2.63 (3)
W (1) −W (5)	2.90 (4)
O (19) −W (4)	3.00 (2)
W (3) −O (20)*[i]	2.91 (4)
N (8) −O (19)*[i]	2.67 (2)
W (1) −W (2)	2.74 (3)
W (3) −W (2)	2.86 (3)
W (2) −W (4)[i]	2.59 (4)
W (5) −O (19)*[ii]	2.80 (3)

Symmetry codes: [i] $1+x, y, 1+z$,
[ii] $1-x, 1/2+y, -z$.
* atom from molecule **II**.

correspond to a relatively strong hydrogen bond (Table 6). Thus, it seems that the solvent molecules may easily dissociate from crystal lattice and thus make the mecillinam susceptible to destruction. This may be regarded as one of the reasons for the relatively low stability of this drug form.

References

1) LUND, F. & L. TYBRING: 6-β-Amidinopenicillanic acids, a new group of antibiotics. Nature, New Biology 236: 135~137, 1972
2) BUŚKO-OSZCZAPOWICZ, I. & J. CIEŚLAK (in Polish): Semisynthetic penicillins. X. Synthesis of 6-formamidinopenicillanic acid derivatives of cyclic secondary amines. Acta Polon. Pharm. 30: 43~48, 1973
3) DZIERŻYŃSKA, I.; D. JASTALSKA, I. BUŚKO-OSZCZAPOWICZ & J. CIEŚLAK (in Polish): Semisynthetic penicillins. XII. Bacteriostatic and bactericidal activity of 6-(N,N-1,6-hexyleneformamidine)-penicillanic acid against Gram-negative bacteria *in vitro*. Acta Polon. Pharm. 30: 443~446, 1973
4) BOROWSKI, J.; M. ZAREMBA, J. CIEŚLAK & I. BUŚKO-OSZCZAPOWICZ: Sensitivity of *Yersinia pseudotuberculosis* and enterocolitica to a 6-β-amidinopenicillanic acid derivative. J. Antimicr. Chemoth. 4: 387~388, 1978
5) CIEŚLAK, J.; I. BUŚKO-OSZCZAPOWICZ & J. KAZIMIERCZAK (in Polish): Method of preparation of anhydrous 6-(N,N-1,6-hexyleneformamidine)-penicillanic acid. Polish Patent 93,674, Dec. 18, 1974
6) BUŚKO-OSZCZAPOWICZ, I.; J. CIEŚLAK, J. KULAK, H. ZAKRZEWSKA & P. LENKOWSKI (in Polish): Method of preparation of crystalline sodium or potassium salts of 6-(N,N-1,6-hexyleneformamidine)-penicillanic acid for injection purposes. Polish Patent 100,273, June 24, 1974
7) KRAJEWSKI, J. W.; Z. URBAŃCZYK-LIPKOWSKA, P. GLUZIŃSKI, L. LEBIODA, I. BUSKO-OSZCZAPOWICZ & J. CIEŚLAK: The molecular structure of 6-(N,N-1,6-hexyleneformamidine)-penicillanic acid. International Symposium on Antibiotics, Weimar, May 14, 1979
8) SHELDRICK, G. M.: SHELX. Program for crystal structure determination. Univ. of Cambridge, England, 1976
9) STEWART, J. M.; F. A. KUNDELL & J. C. BALDWIN: The XRAY-70 System. Computer Science Center, Univ. of Maryland, College Park, Maryland, U.S.A., 1970
10) DOMIANO, P.; M. NARDELLI, A. BALSAMO, B. MACCHIA & F. MACCHIA: Crystal and molecular structure of *p*-methoxybenzyl 2α-methyl-2β-[(*R*)-acetoxy(methoxy)methyl]-6β-phenoxyacetamidopenam-3α-carboxylate. Acta Cryst. B35: 1363~1372, 1979
11) PALM, T. B. & I. CSÖREGH: The crystal and molecular structure of (*S*)-1′-ethoxycarbonylethyl 6β-[(hexahydro-1H-azepin-1-yl)methyleneamino] penicillanate hydrochloride (Bacmecillinam hydrochloride). Acta Cryst. B34: 138~143, 1978
12) KRAJEWSKI, J. W.; Z. URBAŃCZYK-LIPKOWSKA, P. GLUZIŃSKI, I. BUŚKO-OSZCZAPOWICZ, J. OSZCZAPOWICZ, J. BLEIDELIS & A. KEMME: Amidines. VIII. X-Ray investigation of amidine group in N^1-(hexylene-1,6)-N^2-*p*-nitrophenylformamidine. Polish J. Chem. (in press)