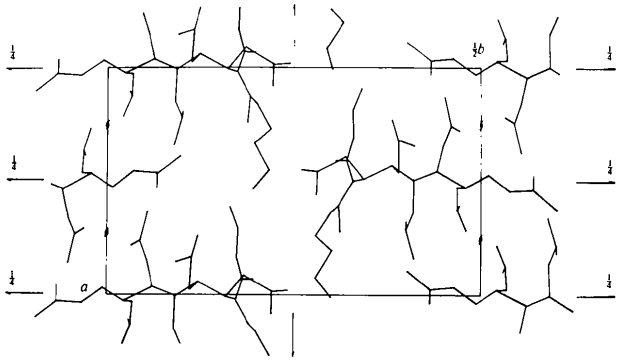

Fig. 3. The crystal structure.

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The Structure of the Antibiotic Amicetin Consisting of Nucleobase, Disaccharide and Amino Acid Moieties

BY JANET L. SMITH† AND M. SUNDARALINGAM‡

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin – Madison, Madison, Wisconsin 53706, *USA*

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Abstract

Amicetin ($C_{29}H_{42}N_6O_9$), 1-[4-*O*-(4,6-dideoxy-4-dimethylamino-α-D-glucopyranosyl)-2,3,6-trideoxy-β-D-*erythro*-hexopyranosyl]-N^4-[4-(2-methyl-L-serylamino)-benzoyl]cytosine, is one of a group of cytosine nucleoside antibiotics which inhibit peptide bond formation during protein biosynthesis. It contains a nucleobase, a disaccharide and an amino acid moiety. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 25{\cdot}793\ (5)$, $b = 11{\cdot}897\ (2)$, $c = 10{\cdot}185\ (2)$ Å, $Z = 4$; $D_m = 1{\cdot}293$, $D_c = 1{\cdot}315$ Mg m^{-3} and $V_c = 3125{\cdot}4\ (1{\cdot}7)$ Å^3. The crystal structure was solved by direct methods and refined to an R value of 0·06. The molecule is in a highly extended conformation with an end-to-end distance of 25 Å. The terminal hydroxyl group O(17)—H of the α-methyl-L-serine moiety exhibits a twofold conformational disorder (occupancy factors of 0·62 and 0·38) and both hydroxyl positions participate in intermolecular hydrogen bonds. Concomitant with this disorder, the C(17) atom attached to the hydroxyl group also appears to be disordered. The cytosine base is in the *anti* conformation [$\chi = 25{\cdot}7\ (5)°$] with respect to the trideoxyglucose ring. The amide group C(4)—N(4)—C(7)—O(7) is markedly distorted from planarity [$\omega = -159{\cdot}2\ (7)°$]. The disaccharide component possesses a C(1) axial–C(4) equatorial bridge, with the *trans* conformation for the bridge torsions C(2*)—C(1*)—O(1*)—C(4′) [−163·8 (5)°] and C(1*)—O(1*)—C(4′)—C(5′) [−162·8 (5)°], and a +*gauche* conformation for the bridge torsions O(5*)—C(1*)—O(1*)—C(4′) [72·6 (5)°] and C(1*)—O(1*)—C(4′)—C(3′) [76·4 (5)°]. An unusual feature of the crystal structure is that only four of the eight available protons take part

† Present address: Laboratory for the Structure of Matter, Code 6030, Naval Research Laboratory, Washington, DC 20375, USA.

‡ To whom correspondence should be addressed.

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in intermolecular hydrogen-bond interactions, three of these involving the Watson–Crick base pairing sites of the cytosine base. In addition, there is suggestion of a C–H···O and an N–H···N intramolecular hydrogen bond.

Introduction

Peptidyl transfer from the peptidyl tRNA in the *P*-site to the aminoacyl tRNA in the *A*-site of the ribosome, catalyzed by the enzyme peptidyl transferase, can be inhibited by several classes of antibiotics of diverse chemical structure (Vazquez, 1974). Among these are the cytosine nucleoside antibiotics whose best characterized members are amicetin (Fig. 1), gougerotin, and blasticidin S. These compounds have been used extensively in *in vitro* studies of protein biosynthesis (Vazquez, Staehelin, Celma, Battaner, Fernandez-Munoz & Munro, 1969), and appear to have nearly identical inhibitory activity (Harris & Pestka, 1973; Barbacid & Vazquez, 1974). They have in common a cytosine base, at least one pyranose sugar and a peptide tail which may be either substituted at the amino group of the base (amicetin) or attached to the sugar (gougerotin and blasticidin S). The relative inhibitory activity of several analogs of the cytosine antibiotics has also been assayed and shows that there is an absolute requirement for an intact cytosine base (Coutsogeorgopoulos, Bloch, Watanabe & Fox, 1975; Krayevsky, Kukhanova & Gottikh, 1975). This is in contrast to the wide diversity of saccharide and peptide structures which can be accommodated without loss of activity. These facts implicate the cytosine base as the major determinant of the mode of action of the cytosine antibiotics and suggests that the cytosine residue in these compounds competes with the 3′-CCA terminus of aminoacyl tRNA's for a specific binding site within the ribosomal acceptor *A*-site, thus inhibiting peptidyl transfer (Coutsogeorgopoulos *et al.*, 1975).

We have chosen to study these antibiotics crystallographically to look for common structural features which may confer common biological activity. To date no crystal structures for an intact cytosine antibiotic have been reported, although the structures of two hydrolytic products are known, *viz.*, the crystal structures of cytosamine triacetate (Sygusch, Brisse & Hanessian, 1974), a derivative of the nucleoside portion of amicetin, and the *C*-substance of gougerotin (Swaminathan, McAlister & Sundaralingam, 1980). Here we report the crystal structure of amicetin, the first three-dimensional structure of a complete cytosine nucleoside antibiotic. Its full chemical structure was determined by Hanessian & Haskell (1964). This molecule is unique among the cytosine antibiotics in that it contains a disaccharide.

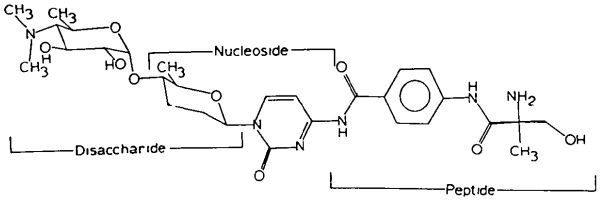

Fig. 1. Chemical structure of amicetin.

Experimental

A sample of amicetin was kindly supplied by Dr George B. Whitfield Jr of the Upjohn Company, Kalamazoo, Michigan. Crystals were grown by evaporation from a mixture of *n*-propanol, *n*-butanol and water (7:7:1) and the unit-cell dimensions were determined by least-squares refinement of diffractometer-measured setting angles of thirty general reflections. A crystal of approximate dimensions 0·3 × 0·3 × 0·1 mm was chosen for analysis. Data were collected on an Enraf–Nonius CAD-4 diffractometer using Ni-filtered Cu $K\alpha$ (λ = 1·5418 Å) radiation to a resolution of 0·79 Å using an $\omega/2\theta$ scan at 293 K. The scan width was [0·75 + 0·213 tan (θ)]° with a varying scan speed of 0·38–2·0° min.$^{-1}$ depending upon the intensity of each reflection. A helium-filled flow tube was placed between the crystal and the detector in order to decrease both the background counts and the loss of intensity due to air scatter. Lorentz, polarization and decay corrections were applied to the reduced data. Of 3728 independent reflections scanned, 2718 reflections whose intensities were greater than or equal to 1·5 times their standard deviations, were used in the structure analysis.

Structure solution and refinement

The structure was solved by multisolution methods (Germain, Main & Woolfson, 1971) with normalized structure amplitudes ($|E|$) calculated using molecular scattering factors (Swaminathan *et al.*, 1980; Debye, 1915). The coordinates of the cytosamine triacetate (Sygusch *et al.*, 1974) crystal structure were used for computing molecular scattering factors. Of the 44 non-H atoms in amicetin, 36 were found in the initial *E* map and the remaining eight in a subsequent electron density map.

The amicetin structure was refined by full-matrix least-squares techniques minimizing the quantity $\sum w\Delta F^2$ where $w = 1/[\sigma^2 + (0{\cdot}02|F_o|)^2]$, $\Delta F = |F_o| - |F_c|$, σ = standard deviation of $|F_o|$ based on counting statistics. 36 of the 42 H atoms were located by difference Fourier synthesis. The difference electron density map also revealed an alternative position for the hydroxyl O(17), indicating that this atom is dis-

ordered, and some residual density around C(17), indicating a slightly shifted alternative site for the C(17) atom as well. The refinement of the two O(17) sites yielded values of 0·62 [O(17)*A*] and 0·38 [O(17)*B*] for their relative occupancies. The C(17)–O(17) distances corresponding to these sites were 1·394 (6) and 1·640 (6) Å respectively. The residual density lobe on C(17) mentioned above was pointing in the direction of O(17)*B* and this, taken in conjunction with the inordinately long C(17)–O(17)*B* distance, suggested a possible disorder of the C(17) atom as well. Therefore, C(17) was partitioned into C(17)*A* (major site) and C(18)*B* (minor site located on the density lobe) with occupancies corresponding to the O(17)*A* and O(17)*B* atoms respectively. This disordering gave considerably better values for the C(17)*A*–O(17)*A* and C(17)*B*–O(17)*B* distances (Fig. 2). Several cycles of refinement of the heavy atoms [with C(17)*B* fixed] and three cycles on the H atom positions gave a final *R* value of 0·06 for the observed reflections, where $R = \sum||F_o| - |F_c||/\sum|F_o|$. The final shifts were less than 0·15 times their estimated errors. Of the six H atoms not found in difference electron density maps, four are in the disordered region. These six H atoms were fixed geometrically during refinement. Both of the disordered O(17) positions are involved in hydrogen bonds as donors (see below), and their H's were fixed geometrically to lie between the donor and acceptor atoms and were assigned the same occupancies as their covalently linked O atoms.

All the calculations were carried out on a minicomputer PDP 11/35 using the programs developed in these laboratories (Rao, McAlister & Merritt, 1979, unpublished results). The scattering factors used for C, N, and O atoms are from Cromer & Waber (1965) and for H atoms from Stewart, Davidson & Simpson (1965).

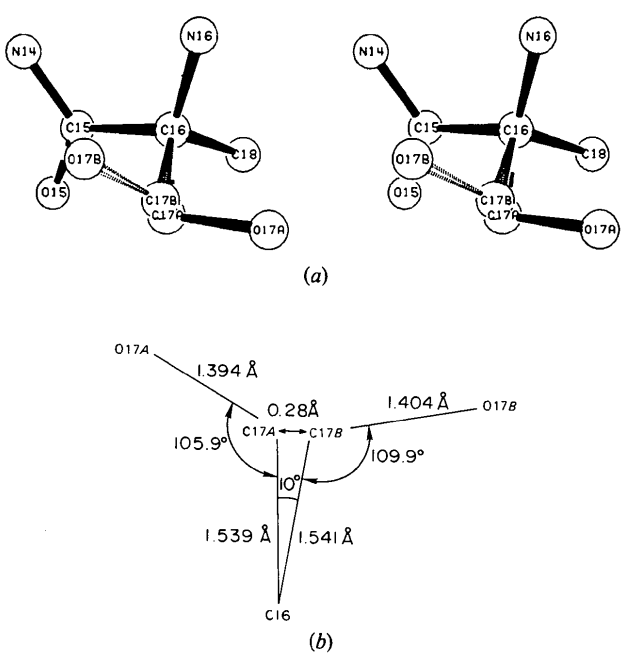

Fig. 2. (*a*) A stereoscopic diagram showing the disorder of the α-methylserine moiety. (*b*) The geometry involving the disordered atoms.

Results and discussion

The final positional parameters for the non-H atoms are listed in Table 1.* An *ORTEP* (Johnson, 1965) drawing of the molecule showing the overall conformation of amicetin and the atom numbering are given in Fig. 3. The bond lengths and angles of amicetin are shown in Table 2. Some pertinent torsion angles are listed in Table 3. The molecule exhibits a highly extended conformation. The conformation and geometry of amicetin are generally in good agreement with those observed for cytosamine triacetate (Sygusch *et al.*, 1974).

The cytosine base

The cytosine-base geometry is very similar to the average geometry of well determined crystal structures containing a nonprotonated cytosine base (Smith, 1978). The only significant differences between the geometry of the cytosine base of amicetin and the average cytosine is at the amino end due to the substitution at N(4): N(3)–C(4) is shortened, C(4)–N(4) and C(5)–C(6) are lengthened, and the N(3)–C(4)–C(5) angle is increased with an accompanying decrease in C(4)–C(5)–C(6). The C(5)–C(4)–N(4) bond angle [121·6 (5)°], is widened with a concomitant reduction in the N(3)–C(4)–N(4) angle [114·4 (5)°], probably resulting from the intramolecular C(5)–H···O(7) [H···O(7) 2·12 (5) Å] interaction (see also below).

The glycosyl conformation

The glycosyl torsion angle χ [O(5′)–C(1′)–N(1)–C(6)] describing the disposition of the base relative to the sugar is 25·7 (5)° and thus falls in the *anti* domain. In cytosamine triacetate, this value is 42·5 (5)°.

* Lists of structure factors, anisotropic thermal parameters for non-H atoms and positional parameters for the H atoms have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35906 (16 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Fractional positional and equivalent isotropic temperature factors B_{eq} of the non-H atoms of amicetin*

$B_{eq} = \frac{4}{3}\sum_i \sum_j \mathbf{a}_i \mathbf{a}_j \beta_{ij}$ where $\mathbf{a}_i$'s are the cell constants of the unit cell. The numbers alongside the disordered atoms C(17) and O(17) are the occupancy factors.

	x	y	z	B_{eq} (Å²)
N(1)	0·7065 (1)	0·3732 (3)	0·1192 (4)	4·8 (1)
C(2)	0·6734 (2)	0·2874 (4)	0·0803 (5)	4·7 (1)
O(2)	0·6914 (1)	0·1960 (3)	0·0492 (4)	6·5 (1)
N(3)	0·6211 (1)	0·3076 (3)	0·0832 (4)	4·6 (1)
C(4)	0·6038 (2)	0·4066 (4)	0·1204 (5)	4·6 (1)
N(4)	0·5507 (1)	0·4161 (3)	0·1211 (4)	4·7 (1)
C(5)	0·6364 (2)	0·4983 (4)	0·1557 (5)	4·7 (1)
C(6)	0·6880 (2)	0·4770 (4)	0·1511 (5)	5·2 (1)
C(7)	0·5204 (2)	0·5046 (4)	0·1619 (5)	4·4 (1)
O(7)	0·5379 (1)	0·6006 (3)	0·1744 (4)	5·7 (1)
C(8)	0·4668 (2)	0·4795 (4)	0·1946 (4)	4·2 (1)
C(9)	0·4510 (2)	0·3737 (4)	0·2374 (5)	4·4 (1)
C(10)	0·4011 (2)	0·3553 (4)	0·2831 (5)	4·7 (1)
C(11)	0·3658 (2)	0·4414 (4)	0·2866 (5)	4·6 (1)
C(12)	0·3802 (2)	0·5453 (4)	0·2432 (6)	5·4 (1)
C(13)	0·4297 (2)	0·5648 (4)	0·1981 (5)	5·0 (1)
N(14)	0·3154 (1)	0·4308 (3)	0·3376 (5)	5·8 (1)
C(15)	0·2892 (2)	0·3358 (4)	0·3648 (6)	6·1 (1)
O(15)	0·3072 (1)	0·2421 (3)	0·3458 (5)	8·0 (1)
C(16)	0·2341 (2)	0·3481 (6)	0·4176 (6)	7·2 (2)
N(16)	0·2223 (2)	0·4627 (4)	0·4446 (6)	9·4 (2)
C(17)*A* 0·62	0·1996 (2)	0·2989 (7)	0·3085 (7)	10·0 (2)
C(17)*B* 0·38	0·1992	0·3207	0·2991	5·4
O(17)*A* 0·62	0·1494 (2)	0·2980 (7)	0·3591 (8)	10·4 (3)
O(17)*B* 0·38	0·2097 (4)	0·3955 (11)	0·1959 (13)	10·6 (4)
C(18)	0·2284 (2)	0·2789 (7)	0·5421 (7)	10·6 (2)
C(1′)	0·7629 (2)	0·3510 (4)	0·1120 (5)	5·0 (1)
C(2′)	0·7836 (2)	0·3601 (4)	−0·0229 (5)	5·2 (1)
C(3′)	0·8429 (2)	0·3438 (5)	−0·0174 (5)	5·6 (1)
C(4′)	0·8672 (1)	0·4240 (4)	0·0801 (5)	4·3 (1)
C(5′)	0·8416 (2)	0·4162 (5)	0·2117 (5)	5·5 (1)
O(5′)	0·7860 (1)	0·4323 (3)	0·1976 (3)	5·7 (1)
C(6′)	0·8594 (2)	0·4976 (8)	0·3080 (7)	12·0 (3)
O(1*)	0·9210 (1)	0·3960 (3)	0·1025 (3)	4·6 (1)
C(1*)	0·9541 (2)	0·4300 (4)	−0·0018 (4)	4·7 (1)
C(2*)	1·0048 (1)	0·3670 (4)	0·0151 (5)	4·7 (1)
O(2*)	0·9944 (1)	0·2495 (3)	0·0254 (4)	6·4 (1)
C(3*)	1·0365 (2)	0·4094 (4)	0·1272 (5)	4·7 (1)
O(3*)	1·0856 (1)	0·3552 (3)	0·1368 (4)	6·3 (1)
C(4*)	1·0425 (2)	0·5363 (4)	0·1186 (5)	5·0 (1)
N(4*)	1·0726 (2)	0·5874 (3)	0·2262 (5)	6·6 (1)
C(5*)	0·9895 (2)	0·5921 (4)	0·1058 (5)	5·2 (1)
O(5*)	0·9617 (1)	0·5464 (3)	−0·0065 (3)	5·6 (1)
C(6*)	0·9928 (3)	0·7162 (5)	0·0819 (6)	8·5 (2)
C(7*)	1·1277 (3)	0·5997 (6)	0·1946 (9)	11·6 (2)
C(8*)	1·0662 (2)	0·5333 (5)	0·3527 (6)	7·2 (2)

Note: In this and the following tables e.s.d.'s are not given for atom C(17)*B* because it was not refined.

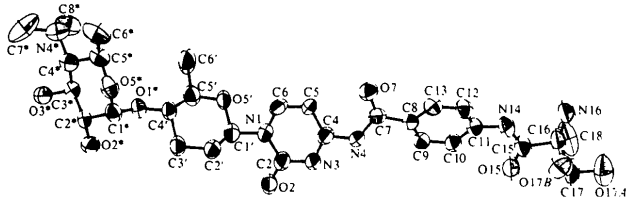

Fig. 3. An *ORTEP* (Johnson, 1965) drawing of the amicetin molecule showing 50% probability surfaces for the thermal ellipsoids of the non-H atoms and the atom numbering.

Table 2. *Bond lengths* (Å) *and angles* (°) *involving non-H atoms of amicetin with e.s.d.'s in parentheses*

N(4*)–C(4*)	1·474 (7)	C(2)–O(2)	1·224 (6)
N(4*)–C(7*)	1·465 (9)	N(3)–C(4)	1·315 (6)
N(4*)–C(8*)	1·450 (8)	C(4)–C(5)	1·424 (7)
C(4*)–C(3*)	1·520 (7)	C(4)–N(4)	1·374 (6)
C(4*)–C(5*)	1·525 (7)	N(4)–C(7)	1·376 (6)
C(5*)–O(5*)	1·455 (6)	C(5)–C(6)	1·356 (7)
C(5*)–C(6*)	1·499 (8)	C(7)–O(7)	1·235 (6)
C(3*)–C(2*)	1·492 (7)	C(7)–C(8)	1·453 (7)
C(3*)–O(3*)	1·425 (6)	C(8)–C(9)	1·393 (7)
C(2*)–C(1*)	1·517 (6)	C(8)–C(13)	1·395 (7)
C(2*)–O(2*)	1·427 (6)	C(9)–C(10)	1·386 (7)
C(1*)–O(1*)	1·422 (5)	C(10)–C(11)	1·371 (7)
C(1*)–O(5*)	1·399 (6)	C(11)–C(12)	1·364 (7)
O(1*)–C(4′)	1·445 (4)	C(11)–N(14)	1·406 (6)
C(4′)–C(5′)	1·497 (7)	C(12)–C(13)	1·377 (7)
C(4′)–C(3′)	1·513 (7)	N(14)–C(15)	1·346 (6)
C(3′)–C(2′)	1·543 (7)	C(15)–O(15)	1·223 (6)
C(2′)–C(1′)	1·478 (7)	C(15)–C(16)	1·527 (7)
C(5′)–O(5′)	1·454 (6)	C(16)–N(16)	1·424 (9)
C(5′)–C(6′)	1·453 (10)	C(16)–C(17)*A*	1·539 (9)
O(5′)–C(1′)	1·432 (6)	C(16)–C(17)*B*	1·541
C(1′)–N(1)	1·480 (6)	C(16)–C(18)	1·519 (10)
N(1)–C(2)	1·388 (6)	C(17)*A*–O(17)*B*	1·394 (8)
N(1)–C(6)	1·363 (6)	C(17)*B*–O(17)*B*	1·404
C(2)–N(3)	1·371 (6)		

C(6′)–C(5′)–O(5′)	106·9 (6)	C(6*)–C(5*)–O(5*)	105·6 (5)
C(5′)–O(5′)–C(1′)	112·4 (5)	C(2*)–C(3*)–O(3*)	112·8 (5)
N(1)–C(1′)–C(2′)	112·8 (5)	C(2*)–C(3*)–C(4*)	110·3 (5)
N(1)–C(1′)–O(5′)	105·0 (4)	O(3*)–C(3*)–C(4*)	111·3 (5)
C(2′)–C(1′)–O(5′)	111·5 (5)	C(1*)–C(2*)–O(2*)	109·3 (4)
C(2)–N(1)–C(6)	121·3 (5)	C(1*)–C(2*)–C(3*)	113·1 (5)
C(2)–N(1)–C(1′)	117·3 (5)	C(3*)–C(2*)–O(2*)	112·2 (5)
C(1′)–N(1)–C(6)	121·2 (5)	O(1*)–C(1*)–C(2*)	107·0 (4)
N(1)–C(2)–O(2)	119·6 (5)	O(1*)–C(1*)–O(5*)	113·0 (4)
N(1)–C(2)–N(3)	118·0 (5)	C(2*)–C(1*)–O(5*)	111·8 (5)
O(2)–C(2)–N(3)	122·3 (5)	C(1*)–O(5*)–C(5*)	114·3 (4)
C(2)–N(3)–C(4)	119·8 (5)	C(1*)–O(1*)–C(4′)	113·2 (4)
N(3)–C(4)–C(5)	124·0 (5)	C(3′)–C(4′)–O(1′)	110·9 (4)
C(4)–N(4)–C(7)	129·1 (5)	O(1*)–C(4′)–C(5′)	105·5 (4)
N(1)–C(6)–C(5)	121·4 (5)	C(2′)–C(3′)–C(4′)	110·8 (5)
N(4)–C(7)–O(7)	122·1 (5)	C(1′)–C(2′)–C(3′)	108·4 (6)
N(4)–C(7)–C(8)	116·9 (5)	C(4′)–C(5′)–O(5′)	109·8 (5)
O(7)–C(7)–C(8)	121·0 (5)	C(4′)–C(5′)–C(6′)	115·1 (6)
C(7)–C(8)–C(9)	122·4 (5)	C(11)–C(12)–C(13)	120·9 (6)
C(7)–C(8)–C(13)	120·6 (6)	C(8)–C(13)–C(12)	121·5 (6)
C(9)–C(8)–C(13)	116·7 (6)	C(11)–N(14)–C(15)	128·0 (5)
C(8)–C(9)–C(10)	121·3 (6)	N(14)–C(15)–O(15)	122·8 (5)
C(9)–C(10)–C(11)	120·5 (6)	N(14)–C(15)–C(16)	117·4 (5)
C(10)–C(11)–C(12)	119·2 (6)	O(15)–C(15)–C(16)	119·8 (6)
C(10)–C(11)–N(14)	123·8 (5)	C(15)–C(16)–N(16)	111·0 (6)
C(12)–C(11)–N(14)	116·9 (5)	C(15)–C(16)–C(17)*A*	104·3 (6)
C(4*)–N(4*)–C(7*)	112·9 (6)	C(15)–C(16)–C(17)*B*	104·3
C(4*)–N(4*)–C(8*)	114·7 (5)	N(16)–C(16)–C(17)*A*	112·3 (7)
C(8*)–N(4*)–C(7*)	110·5 (7)	N(16)–C(16)–C(17)*B*	103·2
C(3*)–C(4*)–N(4*)	114·9 (5)	C(16)–C(17)*A*–O(17)*A*	105·9 (7)
C(3*)–C(4*)–C(5*)	110·2 (5)	C(16)–C(17)*B*–O(17)*B*	109·9
N(4*)–C(4*)–C(5*)	110·9 (5)	C(18)–C(16)–C(17)*A*	109·9 (7)
C(4*)–C(5*)–C(6*)	113·1 (6)	C(18)–C(16)–C(17)*B*	118·9
C(4*)–C(5*)–O(5*)	110·3 (5)	C(18)–C(16)–N(16)	109·7 (7)
N(4)–C(4)–N(3)	114·4 (5)	C(18)–C(16)–C(15)	109·4 (6)
N(4)–C(4)–C(5)	121·6 (5)	C(3′)–C(4′)–C(5′)	111·5 (5)
C(4)–C(5)–C(6)	115·3 (6)		

The disaccharide

The bond distances and bond angles in the saccharide moieties are generally similar to the values found in cytosamine triacetate. C(1′)–C(2′), C(4′)–C(5′) and C(5′)–C(6′) are considerably shortened. Both pyranose rings are in the preferred $^4C_1$ confor-

mation. The puckering parameters (Cremer & Pople, 1975) for the sugar rings are as follows:

	Q	θ	φ
Trideoxyglucose	0·567 Å	3·2°	48·4°
Aminomethylated glucose	0·537	4·5	275·0

The distortion from a perfect chair ($\theta = 0°$) in both cases is very small. The trideoxyglucose puckering is least at the C(3′) and C(4′) and most at the C(1′) and O(5′) atoms, while the aminomethylated sugar ring is flattened at C(1*) and C(2*) and puckered most at O(5*) and C(5*). Thus, there is a preferred flattening of the sugar rings at the C–C bonds adjacent to the bridge glycosidic linkages of the disaccharide moiety.

The glycosidic conformations

The torsion angles about the glycosidic linkages reveal the disaccharide to be in a less than fully extended conformation. The torsion angles φ_1 [O(5*)–C(1*)–O(1*)–C(4′)], φ_1' [C(2*)–C(1*)–O(1*)–C(4′)], φ_2 [C(1*)–O(1*)–C(4′)–C(3′)] and φ_2' [C(1*)–O(1*)–C(4′)–C(5′)] are 72·6 (5), −163·8 (3), 76·4 (5) and −162·8 (4)° respectively. They deviate from the fully extended values of ±120° (Sundaralingam, 1968) by approximately equal rotations around both the glycosidic bonds.

Table 3. *Torsion angles in the amicetin structure* (°)

Glycosyl C–N	O(5′)–C(1′)–N(1)–C(6)	25·7 (5)
2,3,6-Trideoxyglucose	C(1′)–C(2′)–C(3′)–C(4′)	−53·6 (5)
	C(2′)–C(3′)–C(4′)–C(5′)	52·7 (5)
	C(3′)–C(4′)–C(5′)–O(5′)	−54·1 (5)
	C(4′)–C(5′)–O(5′)–C(1′)	58·9 (5)
	C(5′)–O(5′)–C(1′)–C(2′)	−62·7 (5)
	O(5′)–C(1′)–C(2′)–C(3′)	58·2 (5)
Glycosidic bridge C–O bonds	C(5′)–C(4′)–O(1*)–C(1*)	−162·8 (4)
	C(3′)–C(4′)–O(1*)–C(1*)	76·4 (5)
	C(4′)–O(1*)–C(1*)–C(2*)	−163·8 (3)
	C(4′)–O(1*)–C(1*)–O(5*)	72·6 (5)
Endocyclic torsions in aminomethyl glucose	C(1*)–C(2*)–C(3*)–C(4*)	−50·8 (5)
	C(2*)–C(3*)–C(4*)–C(5*)	53·0 (5)
	C(3*)–C(4*)–C(5*)–O(5*)	−55·7 (5)
	C(4*)–C(5*)–O(5*)–C(1*)	58·2 (5)
	C(5*)–O(5*)–C(1*)–C(2*)	−55·2 (5)
	O(5*)–C(1*)–C(2*)–C(3*)	51·6 (5)
Torsion angles of amide group linking cytosine base and phenyl group	N(3)–C(4)–N(4)–C(7)	175·7 (4)
	C(5)–C(4)–N(4)–C(7)	−5·2 (8)
	C(4)–N(4)–C(7)–O(7)	18·6 (8)
	C(4)–N(4)–C(7)–C(8)	−159·2 (5)
	N(4)–C(7)–C(8)–C(9)	26·6 (7)
	N(4)–C(7)–C(8)–C(13)	−160·6 (4)
Torsion angles in the disordered methylserine moiety	C(12)–C(11)–N(14)–C(15)	167·4 (5)
	C(10)–C(11)–N(14)–C(15)	−15·2 (8)
	C(11)–N(14)–C(15)–O(15)	−0·8 (8)
	C(11)–N(14)–C(15)–C(16)	−178·9 (5)
	O(15)–C(15)–C(16)–C(18)	53·2 (7)
	O(15)–C(15)–C(16)–C(17)*A*	−64·3 (9)
	O(15)–C(15)–C(16)–C(17)*B*	−75·0
	O(15)–C(15)–C(16)–N(16)	174·4 (5)
	N(14)–C(15)–C(16)–N(16)	−7·4 (7)
	N(14)–C(15)–C(16)–C(18)	−128·6 (5)
	N(14)–C(15)–C(16)–C(17)*A*	113·8 (6)
	N(14)–C(15)–C(16)–C(17)*B*	103·2
	O(17)*A*–C(17)*A*–C(16)–C(15)	174·0 (9)
	O(17)*A*–C(17)*A*–C(16)–N(16)	−65·7 (8)
	O(17)*A*–C(17)*A*–C(16)–C(18)	56·8 (8)
	O(17)*B*–C(17)*B*–C(16)–C(15)	−59·2
	O(17)*B*–C(17)*B*–C(16)–N(16)	57·0
	O(17)*B*–C(17)*B*–C(16)–C(18)	178·6

Conformational disorder of the amino acid α-methylserine

The twofold conformational disorder observed for the α-methylserine hydroxyl group is quite common, particularly in cases such as this one where two hydrogen bonds can be formed. Any positional disorder in the C(17) atom would ordinarily be absorbed by the anisotropic thermal parameters, but in this case the time/lattice averaged electron density for C(17) cannot be modelled as an ellipsoid and residual difference density adjacent to the major site appears. Using geometric arguments, a plausible disordered model can be proposed. However, the separate C(17) sites cannot be refined owing to their short separation (Fig. 2). There is little change in the R factor when the two C(17) positions are included in the model at the occupancies of their respective hydroxyl oxygens.

The torsions around N(16)–C(17) for O(17)*A* are *trans*, *gauche*$^-$ and *gauche*$^+$ respectively, for C(15), N(16) and C(18) (Table 3), while those for O(17)*B* are *gauche*$^-$, *gauche*$^+$ and *trans*. Thus, the two O(17) positions are related by a +120° rotation about C(16)–C(17). We believe that such a conformational change can effect the valency angle changes at C(16) which our model necessitates due to the 0·28 Å C(17)*A*–C(17)*B* separation.

The C(16)–N(16) bond length of 1·424 Å is significantly shortened compared to the normal C–N single-bond value of about 1·48 Å (Pauling, 1960). There are no intermolecular contacts shorter than 3·5 Å to the amino N(16); however, N(16) is involved in an intramolecular hydrogen bond [2·07 (5) Å] with N(14).

The non-planar amide

The central portion of the amicetin molecule comprising the various sections of the 'conjugated system' from C(1′) to C(16) is nonplanar. The twist angles between the least-squares planes through the base [C(1′) to N(4)], the amide group [C(4) to C(8)], the phenyl ring [C(7) to N(14)] and the serine peptide [C(11) to C(16)] are 14·9 (8), 35·9 (8) and 28·9 (8)° respectively. The largest deviation from planarity is localized in the N(4)–C(7) amide bond which displays an ω torsion angle of −159·2 (5)°. Winkler & Dunitz (1971) defined the angles χ_N, χ_C and τ to describe the out-of-plane bending at N and C′ and twisting of the amide bond, respectively, as components of amide

nonplanarity. Using their equations, it is seen that the nonplanarity of the amide group is due mainly to out-of-plane bending at N(4) ($\chi_N = -15{\cdot}8°$) and somewhat to twist of the N(4)–C(7) bond ($\tau = -11{\cdot}8$), while there is very little out-of-plane bending at C(7) ($\chi_C = 2{\cdot}2°$) as expected. Due to the greater tetrahedral character of N(4), the amide bond N(4)–C(7) [1·376 (6) Å] exhibits greater single-bond character, and is significantly longer than the average peptide bond (1·32 Å) (Ramachandran, Lakshminarayanan & Kolaskar, 1973). The peptide bond N(14)–C(15) [1·346 (6) Å] linking the α-methylserine moiety is closer in length to the average peptide bond.

The non-planarity of the amide group (ω = 159·2°) and the opening of the angle C(5)–C(4)–N(4) occur to minimize the van der Waals contact between H(C5) and O(7).

Hydrogen bonding and crystal packing

The most unusual feature of the amicetin crystal structure is the participation of only half the available protons in intermolecular hydrogen bonds. The eight potential donor protons are localized in three functional groups: the cytosine, the serine and the aminomethylated glucose. These functional groups are at the ends and middle of the amicetin molecule, separated by the phenyl ring and trideoxyglucose sugar. As a result, the termini of two adjacent molecules join at the cytosine of a third, saturating its three Watson–Crick base-pairing sites and creating a tight cluster of hydrogen bonds (Table 4 and Fig. 4). The fourth hydrogen bond occurs between the serine and glucose termini. Thus, hydrogen bonds are formed between all combinations of functional groups: cytosine–serine, cytosine–glucose and glucose–serine. The spatial separation of hydrogen-bonding functional groups by the phenyl ring and trideoxyglucose sugar places severe constraints on the hydrogen-bonding and crystal-packing possibilities for the elongated amicetin molecule. This may be partially responsible for the paucity of hydrogen bonds as well as for the formation of two intramolecular interactions.

Table 4. *Inter- and intramolecular hydrogen-bond parameters and the shorter contacts in amicetin*

D–H···*A*	*D*–H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*–H···*A* (°)
N(4)–HN(4)···O(2*)(i)	0·83 (5)	2·15 (5)	2·867 (6)	144 (5)
O(17)*A*–HO(17)*A*···O(3*)(ii)	0·92	1·97	2·881 (8)	178
O(17)*B*–HO(17)*B*···O(2)(i)	0·91	1·85	2·76 (1)	179
O(3*)–HO(3*)···N(3)(iii)	1·02 (5)	2·11 (5)	3·100 (5)	164 (4)
N(14)–HN(14)···N(16)(v)	1·02 (5)	2·07 (5)	2·664 (6)	115 (4)
C(5)–HC(5)···O(7)(v)	1·02 (5)	2·12 (5)	2·824 (6)	125 (4)

The shorter contacts (Å)

C(6)···O(15)	3·156 (6)	C(9)···O(2*)(iii)	3·251 (6)
O(17)*B*···C(7*)(ii)	3·221 (14)	O(2*)···C(8*)(vi)	3·256 (7)
C(5)···O(15)(iv)	3·245 (6)	O(17)*A*···O(3*)(ii)	3·293 (11)

Symmetry code: (i) = $x-\frac{1}{2}, \frac{1}{2}-y, \bar{z}$; (ii) = $x-1, y, z$; (iii) = $\frac{1}{2}+x, \frac{1}{2}-y, \bar{z}$; (iv) = $1-x, \frac{1}{2}+y, \frac{1}{2}-z$; (v) = x,y,z; (vi) = $2-x, y-\frac{1}{2}, \frac{1}{2}-z$.

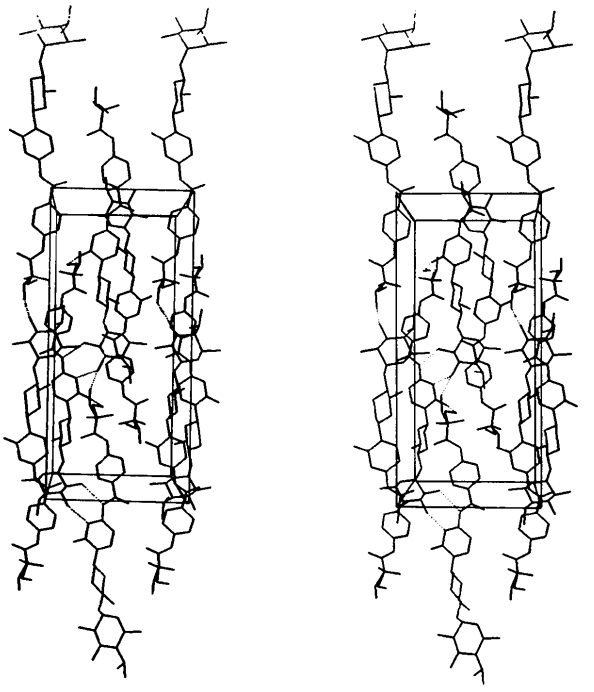

Fig. 4. Stereoscopic packing diagram of amicetin viewed along the *c* axis. Hydrogen bonds are indicated by broken lines.

The cytosine base is the focus of intermolecular interactions in the amicetin crystal as evidenced by the concentration of hydrogen bonds at its Watson–Crick base-pairing face. This need not be the case; the C(4)–N(4) bond could be rotated so as to place N(4)H away from the Watson–Crick face and O(7) towards it. Three factors favor the observed orientation: an attractive interaction is formed between O(7) and C(5)H, a hydrogen bond exists between N(4)H and O(2*), and there is not the repulsion between the electron rich O(7) and N(3) atoms as would be the case in the 'flipped' conformation. The hydrogen bond involving O(2) is not oriented as in a Watson–Crick base pair, but is 48° above the plane of the cytosine and equally displaced between N(1) and N(3), illustrating the large solid angle subtended by the O(2) lone pairs.

Conclusion

The crucial role of the cytosine base in the intermolecular interactions of crystalline amicetin serves to highlight its importance in the activity of the antibiotic. The crystal structure, taken together with the analog studies, suggest that specific cytosine hydrogen-bonding interactions likely determine the site and orientation of amicetin binding near the peptidyl transferase center of the ribosome. These same interactions probably also play a role in the ribosomal

acceptor site binding of the –CCA terminus of aminoacyl tRNA's. The nature of the specific interactions of the cytosine residue with the ribosome is not clear, although base pairing to ribosomal RNA or specific hydrogen bonding to RNA and/or protein components would be involved. It is noteworthy that the glycosyl conformations are all *anti* for amicetin, the *C*-substance of gougerotin and the 3′-cytosine of the –CCA terminal trinucleotide of yeast phenylalanyl tRNA (Stout *et al.*, 1978). Such a conformation could also be required for ribosomal acceptor site binding of both the substrates and inhibitors. Further interactions of the amino acid and saccharide portions of the cytosine antibiotics with the ribosome are likely, although as mentioned above, the analog studies would suggest a less specific role for such interactions. Crystal structures of other intact cytosine antibiotics should aid in identifying the structural components of these secondary interactions.

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