Structure of 1-[2-(Adenin-9-yl)ethyl]-3-carbamoylpyridinium (AC2N+) Chloride Monohydrate, a Pyridine–Coenzyme Model

By Toshimasa Ishida, Yoko Miyamoto, Ayakazu Nakamura and Masatoshi Inoue

Osaka College of Pharmacy, 2-10-65 Kawai, Matsubara-City, Osaka 580, Japan

Abstract

$C_{13}H_{14}N_7O^+.Cl^-.H_2O$, $M_r = 337.8$, crystallizes in space group $P2_1/a$ with $a = 12.073$ (5), $b = 8.280$ (2), $c = 15.086$ (8) Å, $\beta = 94.16$ (4)°, $U = 1504$ (1) Å³, $Z = 4$, $D_m = 1.488$ (1), $D_x = 1.485$ Mg m⁻³. The final R is 0.078 for 2622 independent reflections. Although no prominent overlap between the adenine and pyridinium rings has been observed, the molecule takes a folded conformation. In the crystal, the layers formed by the hydrogen bonds either between the polar groups of the adjacent adenine moieties or between the carbamoyl groups of the neighboring nicotinamide moieties are alternately arranged perpendicular to the c axis, and form a network of hydrogen bonds parallel to the ab plane. The crystal waters and chloride ions filling the space among these layers stabilize the molecular packing in the crystal by hydrogen bonds and van der Waals contacts.

Introduction

Nicotinamide adenine dinucleotide (NAD⁺), with its reduced form (NADH), is an important coenzyme in oxidation–reduction reactions in many living cells.

Various spectroscopic studies (for example, Oppenheimer, Arnold & Kaplan, 1978; Ross, Rousslang, Motten & Kwiram, 1979; Sovago & Martin, 1979) suggested that in aqueous solution, NAD⁺ occurs in a folded conformation, in which the adenine and pyridinium rings form an intramolecular stack. In the crystal structure of the NAD lithium complex (Saenger, Reddy, Mühlegger & Weimann, 1977), however, the NAD⁺ molecule takes an extended conformation.

As model compounds of NAD⁺, the 1-[2-(adenin-9-yl)ethyl]-3-carbamoylpyridinium (AC2N⁺) and 1-[2-(adenin-9-yl)propyl]-3-carbamoylpyridinium (AC3N⁺) cations were examined: they displayed similar hypochromism to NAD⁺ (Craig, Huang, Scott & Leonard, 1972) and the former compound exhibits the same behavior as NAD⁺ on the addition of cyanide ions (Okubo & Ise, 1973*a*). The crystal structures of

the latter compound (AC3N⁺) and its dicationic form (AH⁺C3N⁺), further protonated at N(1) of the adenine ring, were determined by X-ray analysis (Johnson, Frank & Paul, 1973; Johnson, Maier & Paul, 1973), in which the conformation of AC3N⁺ was shown to be an extended form, while AH⁺C3N⁺ had a folded conformation without intramolecular stacking between the two aromatic rings.

The present paper describes the crystal structure of AC2N⁺ chloride monohydrate.

Experimental

AC2N⁺ chloride was synthesized according to the previously described method (Okubo & Ise, 1973*b*). Light-yellow prismatic crystals were obtained from 80% aqueous ethanol solution by slow evaporation at room temperature. A crystal of approximately 0.4 × 0.2 × 0.4 mm was mounted on a Rigaku automated four-circle diffractometer equipped with a graphite monochromator. The cell parameters were obtained by least-squares methods from the 2θ values of 42 independent reflections measured with Cu $K\alpha$ radiation. 2622 independent reflections were measured by the ω–2θ scan mode ($2\theta_{max} = 130°$) at a speed of 4° min⁻¹ with background counts for 5s. Corrections were applied for Lorentz and polarization effects but not for absorption.

Structure determination and refinement

The structure was solved by Patterson–Fourier methods and refined by block-diagonal least squares with anisotropic thermal parameters for all non-hydrogen atoms. All H atoms, except those of the crystal water, found on a difference Fourier map were introduced in the refinement. The final least-squares refinement was computed with the following weighting scheme: $w = 0.5$ for $F_o = 0.0$, $w = 1.0$ for $0 < F_o \leq 16.0$ and $w = 1.0/[1.0 + 0.166(F_o - 16.0)]$ for $F_o > 16.0$. In the last cycle of refinement, none of the positional parameters shifted more than one quarter of

their estimated standard deviations. Residual fluctuations in the difference map were within the range $\pm 0 \cdot 3$ e Å^{-3}, but H atoms of the water were not revealed. The final R value was 0·078.* The published atomic scattering factors were used (*International Tables for X-ray Crystallography*, 1974). All numerical calculations were made on an ACOS-700 computer at the Computation Center of Osaka University, using *The Universal Crystallographic Computing System* (1979). The final atomic parameters are listed in Table 1.

---

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36272 (14 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Atomic coordinates for nonhydrogen atoms* $(\times 10^4)$ *and hydrogen atoms* $(\times 10^3)$ *with their standard deviations in parentheses*

	x	y	z	B_{eq} (Å^2)*
N(1)	1472 (3)	3451 (4)	4267 (2)	3·6 (2)
C(2)	540 (4)	3069 (5)	3767 (3)	4·3 (3)
N(3)	−279 (3)	4036 (5)	3471 (2)	3·8 (2)
C(4)	−78 (3)	5560 (5)	3743 (3)	3·0 (2)
C(5)	831 (3)	6125 (5)	4256 (3)	3·0 (2)
C(6)	1641 (3)	4980 (5)	4534 (3)	3·1 (2)
N(6)	2561 (3)	5344 (4)	5047 (3)	4·0 (2)
N(7)	755 (3)	7784 (4)	4388 (2)	3·3 (2)
C(8)	−192 (3)	8172 (5)	3956 (3)	3·5 (3)
N(9)	−743 (3)	6884 (4)	3559 (2)	3·1 (2)
C(10)	−1784 (3)	6927 (6)	3016 (3)	3·3 (2)
C(11)	−1611 (3)	7290 (5)	2049 (3)	3·5 (3)
N(12)	−978 (3)	5978 (4)	1633 (2)	2·9 (2)
C(13)	−1542 (3)	4731 (5)	1264 (2)	2·9 (2)
C(14)	−983 (3)	3474 (5)	895 (2)	3·1 (2)
C(15)	173 (3)	3514 (5)	934 (3)	3·5 (3)
C(16)	724 (4)	4814 (6)	1333 (3)	3·7 (3)
C(17)	131 (3)	6061 (5)	1671 (3)	3·7 (3)
C(18)	−1673 (3)	2162 (5)	447 (3)	3·2 (2)
O(19)	−2564 (2)	2501 (4)	51 (2)	3·8 (2)
N(20)	−1245 (3)	684 (5)	493 (3)	3·6 (2)
Cl	794 (1)	10049 (1)	1989 (1)	3·2 (1)
O(W)	−1857 (5)	1222 (8)	2697 (4)	5·6 (4)
H(2)	49 (5)	190 (7)	350 (4)	4 (1)
H(6a)	303 (5)	455 (7)	522 (4)	4 (1)
H(6b)	272 (4)	647 (6)	527 (3)	2 (1)
H(8)	−53 (4)	934 (6)	393 (3)	1·8 (9)
H(10a)	−225 (4)	778 (7)	326 (4)	3 (1)
H(10b)	−222 (3)	574 (5)	307 (3)	1·2 (8)
H(11a)	−116 (4)	836 (6)	203 (3)	2 (1)
H(11b)	−228 (3)	735 (5)	164 (3)	1·3 (8)
H(13)	−240 (4)	473 (5)	124 (3)	1·4 (9)
H(15)	61 (4)	260 (6)	68 (3)	2 (1)
H(16)	156 (5)	490 (7)	138 (4)	4 (1)
H(17)	52 (3)	713 (5)	197 (3)	1·4 (9)
H(20a)	−171 (5)	−21 (7)	22 (4)	4 (1)
H(20b)	−69 (5)	39 (7)	88 (4)	3 (1)

* The equivalent isotropic temperature factors for nonhydrogen atoms, B_{eq}, have been calculated by $B_{eq} = \frac{4}{3}(a^2 B_{11} + 2ab \cos \gamma \times B_{12} + \cdots)$.

Results and discussion

Molecular structure

The bond lengths and angles are shown in Fig. 1, with the atomic numbering used in the present work. The least-squares planes of the purine, pyridinium and carbamoyl moieties are given in Table 2, together with displacements of atoms from the planes.
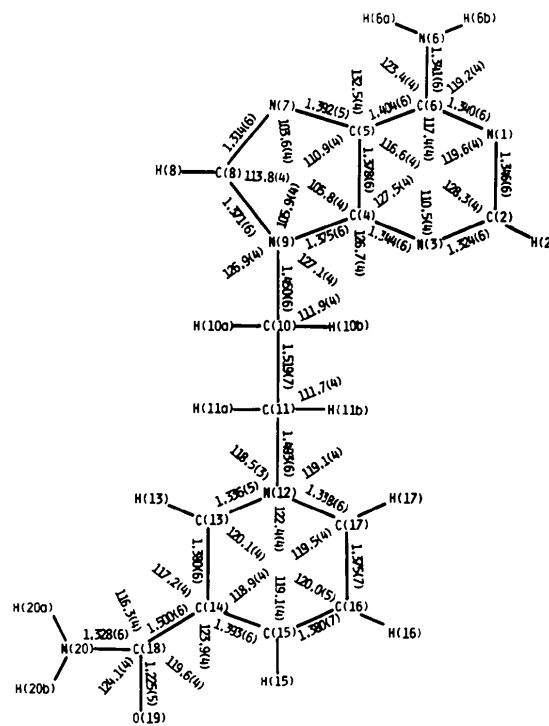

Fig. 1. Bond lengths (Å) and angles (°) between nonhydrogen atoms with their estimated standard deviations.

Table 2. *The least-squares planes and deviations of atoms* (Å) *from the planes*

Equations of the best planes are expressed by $m_1X + m_2Y + m_3Z = d$ in orthogonal space.

Plane	m_1	m_2	m_3	d
Adenine ring	0·53377	0·17184	−0·82798	−4·13429
Pyridinium ring	0·02040	0·46976	−0·88256	0·12894
Carbamoyl group	0·51753	0·15900	−0·84076	−1·34206

Deviations from the best planes (atoms with asterisks define the plane)

Adenine ring		Pyridinium ring		Carbamoyl group	
N(1)*	−0·009 (4)	N(12)*	0·000 (4)	C(14)*	−0·002 (6)
C(2)*	−0·006 (5)	C(13)*	0·008 (5)	C(18)*	0·009 (5)
N(3)*	−0·002 (4)	C(14)*	−0·008 (5)	O(19)*	−0·002 (5)
C(4)*	0·007 (5)	C(15)*	0·000 (6)	N(20)*	−0·003 (6)
C(5)*	0·010 (4)	C(16)*	0·012 (6)	C(13)	0·669 (7)
C(6)*	0·013 (5)	C(17)*	−0·009 (5)	C(15)	−0·678 (7)
N(7)*	−0·006 (4)	C(11)	0·059 (7)	H(20a)	0·04 (6)
C(8)*	−0·014 (5)	C(18)	−0·076 (7)	H(20b)	0·20 (6)
N(9)*	0·007 (4)	H(13)	−0·00 (5)		
N(6)	0·037 (6)	H(15)	0·01 (5)		
C(10)	−0·037 (6)	H(16)	0·02 (6)		
H(2)	−0·16 (6)	H(17)	−0·04 (4)		
H(8)	0·00 (5)				

The bond lengths and angles of the adenine moiety agree well with those of the neutral adenine derivatives summarized by Voet & Rich (1970). Nine atoms in the purine ring are almost on a plane with a maximum deviation of 0·014 (5) Å for C(8), but the N(6) and C(10) atoms attached to the ring deviate slightly from the plane.

In the pyridinium ring, the mean C—C and C—N distances are 1·382 and 1·337 Å, respectively. The corresponding values are 1·382 and 1·342 Å in AC3N⁺, 1·384 and 1·351 Å in AH⁺C3N⁺, 1·367 and 1·347 Å in 3-carbamoyl-1-[2-(indol-3-yl)-ethyl]pyridinium chloride (Herriott, Camerman & Deranleau, 1974), 1·382 and 1·348 Å in the 3-carbamoyl-1-methylpyridinium−N-acetyl-L-tryptophan complex (Ash, Herriott & Deranleau, 1977), 1·375 and 1·358 Å in 1,1'-trimethylenebis(3-carbamoyl-pyridinium) dichloride (Frank, Thayer & Paul, 1973) and 1·376 and 1·350 Å in the 3-carbamoyl-1-methyl-pyridinium−3-indoleacetic acid complex (Ishida, Tomita & Inoue, 1980). Therefore the C—N value in this crystal is slightly shorter than those observed in the other pyridinium compounds. On the other hand, the bond angles are reasonable compared with those of the other pyridinium derivatives. The atoms in the pyridinium ring lie almost on the best plane (the deviations ranging from −0·009 to 0·012 Å). The dimensions of the carbamoyl group are also normal.

The selected torsion angles are listed in Table 3. The conformation of AC2N⁺, projected onto the adenine
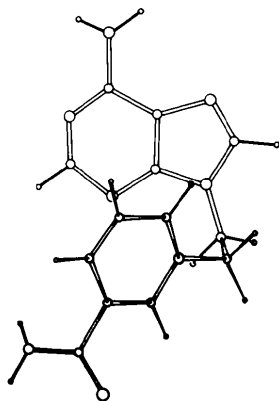
ring, is shown in Fig. 2. The AC2N⁺ molecule adopts a folded conformation, in which the relevant torsion angles, χ and θ, lie near 90°, and ψ near 60°; in the related AC3N⁺, the conformation is in an extended form ($\chi = 95\cdot6$, $\theta = 97\cdot4°$, and two torsion angles, ψ_1 and ψ_2, connecting both the aromatic rings are −171·5 and 178·1°), while AH⁺C3N⁺ takes a folded conformation ($\chi = -63\cdot3$, $\theta = 123\cdot9$, $\psi_1 = -51\cdot5$ and $\psi_2 = -52\cdot8°$). There is, however, no prominent overlap between the adenine and pyridinium rings [dihedral angle 34·7 (2)°], probably due to the shortness of the chain connecting both aromatic rings. The folded conformation of the AC2N⁺ molecule is more energetically stable than the extended one (Miyamoto, Ishida & Inoue, unpublished data); the conformations having χ, θ and ψ of either 60 or 300° are energetically stable folded forms compared with the extended conformation having ψ of near 180°. The observed conformation may therefore be possible also in solution. The plane of the adenine ring is almost parallel to, but does not overlap, the carbamoyl group [dihedral angle 1·4 (2)°]. The dihedral angle between the pyridinium ring and carbamoyl group is 34·2 (3)°, and the torsion angle τ is 34·3 (6)°. This conformation has also been observed in AC3N⁺ ($\tau = 38\cdot7°$), although the most frequently observed value of τ is near 0° in the other 3-carbamoylpyridinium derivatives.

Crystal structure

Fig. 3 shows the packing diagram viewed down the *a* axis; the dotted lines represent the hydrogen bonding between the carbamoyl groups and between the adenine rings. The distances and angles for hydrogen bonds and

Fig. 2. The conformation of the AC2N⁺ molecule observed in the crystal structure, projected onto the adenine ring.

Table 3. *Selected torsion angles* (°)

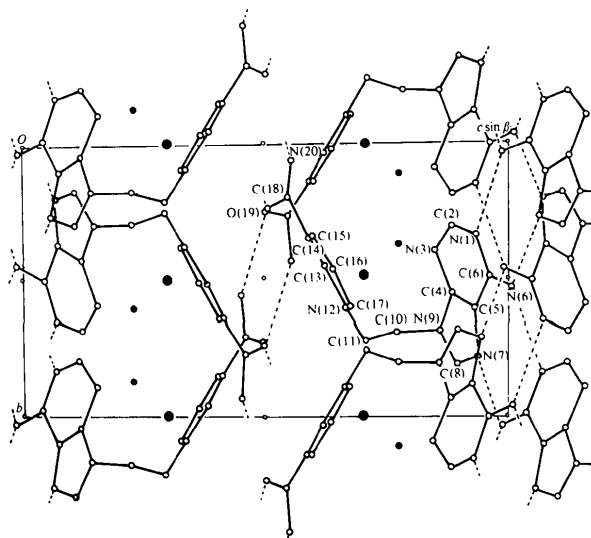|		
C(8)—N(9)—C(10)—C(11)	χ	85·7 (5)
C(4)—N(9)—C(10)—C(11)		−90·6 (5)
N(9)—C(10)—C(11)—N(12)	ψ	64·7 (5)
C(10)—C(11)—N(12)—C(13)	θ	87·9 (5)
C(10)—C(11)—N(12)—C(17)		−89·7 (5)
C(13)—C(14)—C(18)—O(19)	τ	34·3 (6)
C(15)—C(14)—C(18)—O(19)		−143·7 (4)

Fig. 3. Crystal-packing diagram viewed down the *a* axis. The dotted lines represent the hydrogen bonds. The large and small filled circles represent the Cl⁻ ions and water molecules, respectively.

Table 4. *Hydrogen bonds and short contacts*

D–H$\cdots A$ hydrogen bonds

D	A	$D\cdots A$	$H\cdots A$	D–H$\cdots A$
O(W)$\cdots$N(3)		3·178 (8) Å		
N(20)$\cdots$O(19^i)		3·085 (5)	2·12 (6) Å	163 (5)°
N(20)$\cdots$Cl^{ii}		3·259 (4)	2·38 (6)	171 (5)
N(6)$\cdots$N(1^{iii})		2·979 (6)	2·01 (5)	162 (4)
N(6)$\cdots$N(7^{iv})		3·017 (5)	2·13 (6)	176 (5)
O(W)$\cdots$N(1^v)		3·232 (8)		

D–H$\cdots$Cl short contacts

D	$D\cdots$Cl	$H\cdots$Cl	D–H$\cdots$Cl
C(2)$\cdots$Cl^{ii}	3·696 (5) Å	2·79 (6) Å	144 (4)°
C(15)$\cdots$Cl^{ii}	3·340 (5)	2·89 (5)	108 (3)
C(10)$\cdots$Cl^{vi}	3·601 (5)	2·88 (4)	122 (3)
C(13)$\cdots$Cl^{vi}	3·476 (4)	2·53 (5)	151 (3)

Short contacts

N(3)$\cdots$N(12)	3·263 (5) Å	N(3)$\cdots$C(17)	3·260 (6) Å	
C(4)$\cdots$N(12)	3·306 (5)	C(4)$\cdots$C(17)	3·181 (6)	
C(8)$\cdots$C(11)	3·321 (6)	N(9)$\cdots$N(12)	2·994 (5)	
N(9)$\cdots$C(17)	3·182 (6)	C(10)$\cdots$C(13)	3·239 (6)	
C(10)$\cdots$C(17)	3·265 (7)	C(13)$\cdots$O(19)	2·820 (5)	
C(15)$\cdots$N(20)	2·949 (6)	C(17)$\cdots$Cl	3·423 (5)	
N(20)$\cdots$O(W)	3·487 (8)	O(19)$\cdots$C(11^i)	3·256 (5)	
O(19)$\cdots$C(13^i)	3·170 (5)	O(19)$\cdots$N(12^i)	3·239 (5)	
O(W)$\cdots$Cl^{ii}	3·581 (7)	O(W)$\cdots$C(11^{ii})	3·418 (8)	
O(19)$\cdots$C(15^v)	3·236 (6)	C(8)$\cdots$N(6^{vi})	3·497 (6)	
C(11)$\cdots$Cl^{vi}	3·679 (5)	N(1)$\cdots$C(8^{vii})	3·461 (6)	
N(1)$\cdots$N(9^{vii})	3·468 (5)	C(2)$\cdots$N(7^{vii})	3·365 (6)	
C(4)$\cdots$C(6^{vii})	3·351 (6)			

Symmetry code: (i) $-0\cdot5 - x$, $-0\cdot5 + y$, $-z$; (ii) x, $-1 + y$, z; (iii) $0\cdot5 - x$, $0\cdot5 + y$, $1 - z$; (iv) $0\cdot5 - x$, $-0\cdot5 + y$, $1 - z$; (v) $-0\cdot5 + x$, $0\cdot5 - y$, z; (vi) $-0\cdot5 + x$, $1\cdot5 - y$, z; (vii) $-x$, $1 - y$, $1 - z$.
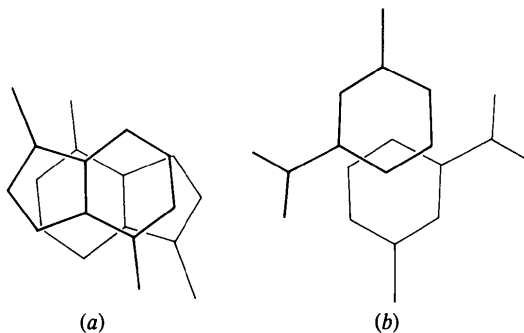

(a)   *(b)*

Fig. 4. Overlap (*a*) between the adenine rings and (*b*) between the pyridinium rings.

short contacts less than 3·5 Å (3·7 Å for the Cl$^-$ ion) are listed in Table 4.

N(6) is hydrogen bonded to N(1) and N(7) of the adjacent adenine moiety related by twofold screw symmetry. This kind of hydrogen bond is also observed in deoxyadenosine (Watson, Sutor & Tollin, 1965). The carbamoyl groups around a twofold screw axis are linked by a hydrogen bond between N(20) and O(19). The Cl$^-$ ions stabilize the crystal packing of the AC2N$^+$ molecule by a hydrogen bond [Cl$\cdots$N(20)] and van der Waals contacts. The crystal water is weakly hydrogen bonded to N(3) and N(1) of the adenine ring.

Fig. 4 shows the overlap between adenine rings and between pyridinium rings related by a center of symmetry. The adenine rings are extensively stacked and their average interplanar spacing is 3·356 Å, which is slightly shorter than the normal van der Waals separation (3·4 Å). A centrosymmetric arrangement would be favorable for dipole–dipole interactions, and the short contacts caused by electrostatic interactions occur between electron-rich–electron-deficient pairs: $-0\cdot282e$[N(1)] $-$ $0\cdot072e$[C(8)], $-0\cdot305e$[N(7)] $-$ $0\cdot102e$[C(2)] (Pullman & Pullman, 1963). A similar overlap has been observed in adenine hydrochloride (Broomhead, 1948) and the 9-ethyladenine–5-fluoro-1-methyluracil complex (Tomita, Katz & Rich, 1967).

On the other hand, no prominent stacking is observed between the pyridinium rings, because the overlap area is small and the interplanar spacing is 3·630 Å.

References

Ash, R. P., Herriott, J. R. & Deranleau, D. A. (1977). *J. Am. Chem. Soc.* **99**, 4471–4475.

Broomhead, J. M. (1948). *Acta Cryst.* **1**, 324–329.

Craig, J. H., Huang, P. C., Scott, T. G. & Leonard, N. J. (1972). *J. Am. Chem. Soc.* **94**, 5872–5879.

Frank, J. K., Thayer, N. N. & Paul, I. C. (1973). *J. Am. Chem. Soc.* **95**, 5386–5390.

Herriott, J. R., Camerman, A. & Deranleau, D. A. (1974). *J. Am. Chem. Soc.* **96**, 1585–1589.

International Tables for X-ray Crystallography (1974). Vol IV. Birmingham: Kynoch Press.

Ishida, T., Tomita, K. & Inoue, M. (1980). *Arch. Biochem. Biophys.* **200**, 492–502.

Johnson, P. L., Frank, J. K. & Paul, I. C. (1973). *J. Am. Chem. Soc.* **95**, 5377–5385.

Johnson, P. L., Maier, C. A. & Paul, I. C. (1973). *J. Am. Chem. Soc.* **95**, 5370–5377.

Okubo, T. & Ise, N. (1973a). *J. Am. Chem. Soc.* **95**, 4031–4036.

Okubo, T. & Ise, N. (1973b). *Tetrahedron Lett.* pp. 321–322.

Oppenheimer, N. J., Arnold, L. J. & Kaplan, N. O. (1978). *Biochemistry*, **17**, 2613–2619.

Pullman, B. & Pullman, A. (1963). *Quantum Biochemistry*, pp. 185–291. New York: Interscience.

Ross, J. B. A., Rousslang, K. W., Motten, A. G. & Kwiram, A. L. (1979). *Biochemistry*, **18**, 1808–1813.

Saenger, W., Reddy, B. S., Mühlegger, K. & Weimann, G. (1977). *Nature (London)*, **267**, 225–229.

Sovago, I. & Martin, R. B. (1979). *FEBS Lett.* **106**, 132–134.

The Universal Crystallographic Computing System (1979). Library of Programs, Computing Center of Osaka Univ.

Tomita, K., Katz, L. & Rich, A. (1967). *J. Mol. Biol.* **30**, 545–549.

Voet, D. & Rich, A. (1970). *Prog. Nucleic Acid Res. Mol. Biol.* **10**, 183–265.

Watson, D. G., Sutor, D. J. & Tollin, P. (1965). *Acta Cryst.* **19**, 111–124.