chain becomes saturated with cations, and the ratio crown to cation should approach 1:1 even for difluorenylbarium. In systems favoring 2:1 crown–cation complexes the increased distance between crown units should affect the complex formation constant, and at a low crown to styrene ratio intermolecular complex formation may occur.

The Crystal Structure of the Antitumor Agent 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide Monohydrate Hydrochloride (NSC-45388)[1]

Steven L. Edwards, James S. Sherfinski, and Richard E. Marsh*

Contribution from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91109. Received September 20, 1973

Abstract: The structure of the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) has been investigated by single-crystal X-ray diffraction techniques. The drug crystallizes as the hydrochloride monohydrate in the monoclinic space group $P2_1/n$ with cell dimensions $a = 7.893$ (3) Å, $b = 13.961$ (2) Å, $c = 10.224$ (3) Å, $\beta = 93.20$ (4)°, $Z = 4$. Full-matrix least-squares refinement converged at a final R index of 0.058 based on 2291 counter-collected data. The molecule is nearly planar, in part held in that conformation by an internal N–H$\cdots$N hydrogen bond. This intramolecular hydrogen bond may be important regarding the biological activity of this and related drugs.

Aryltriazene compounds are interesting cancer-related drugs now under investigation. The exact mechanism of the actions of these drugs is not known, but it is hypothesized that more than one mechanism may be involved. Several reports suggest that the compound 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388, DTIC, Figure 1a) is active as an alkylating agent.[2] The related drug 5-(3,3-bis(2-chloroethyl)-1-triazeno)imidazole-4-carboxamide (NSC-82196, DCTIC, Figure 1b) displays a different specificity to various cancers, and it has been proposed that DCTIC operates in a fashion other than that of DTIC.[3] Shealy and his coworkers[4,5] have suggested that DTIC and DCTIC both decompose to diazeno derivatives, allowing, for instance, DCTIC to liberate $NH(C_2H_4Cl)_2$.[6] This amine, nornitrogen mustard, is a known alkylating agent in itself.

Results of activity–structure studies on drugs related to aryltriazenes indicate that the terminal substituents on the triazeno group are important in determining potency.[3] At least one of these substituents must be a

methyl group or the compound is inactive. The exception to this rule is the case of DCTIC which, as evidenced above, acts by a different mechanism. Substituting a benzene ring for the imidazole ring has little effect on activity.[7]

Although the crystallization of DCTIC has not yet been successful, the crystal structures of its transformation product[8] (Figure 1c) and of a pyrazole derivative[9] (Figure 1d) have been reported. This paper presents the results of a crystallographic investigation of DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, as the hydrochloride monohydrate.

Experimental Section

A sample of DTIC was supplied by Dr. Corwin Hansch of Pomona College. A small amount was dissolved in a toluene-glacial acetic acid solution and placed in a desiccator charged with $CaCl_2$. Clear, well-shaped, prismatic crystals formed within a few days. Precession photographs indicated a monoclinic unit cell and space group $P2_1/n$ (systematic absences: $0k0$, k odd; $h0l$, $h + l$ odd). Accurate cell dimensions were obtained by a least-squares fit to the observed $\sin^2 \theta/\lambda^2$ values for 16 reflections as measured on a diffractometer; the density was measured by flotation in a bromobenzenechlorobenzene solution. Crystal data are given in Table I.

The measured density is in fair agreement with the value 1.43 g/cm⁻³ calculated for four molecules of DTIC and four molecules of acetic acid per unit cell, and we presumed the crystals to have that composition. It was only during the course of the structure solution that it became apparent that the compound was actually the hydrochloride monohydrate salt, rather than the acetate. We

(1) Contribution No. 4734 from the Arthur Amos Noyes Laboratory of Chemical Physics. This investigation was supported in part by Public Health Service Grant No. GM 16966 from the National Institutes of Medical Sciences, National Institutes of Health. One of us (J. S. S.) would like to acknowledge the support of an NIH Traineeship.

(2) J. Luce, W. Thurman, B. Isaacs, and R. Talley, *Cancer Chemother. Rep.*, 54, 122 (1970).

(3) C. Hansch, N. Smith, R. Engle, and H. Wood, *Cancer Chemother. Rep.*, 56, 443 (1972).

(4) Y. F. Shealy, J. A. Montgomery, and W. R. Laster, Jr., *Biochem. Pharmacol.*, 11, 674 (1962).

(5) Y. F. Shealy, R. P. Struck, L. B. Holum, and J. A. Montgomery, *J. Org. Chem.*, 26, 2396 (1961).

(6) C. L. Vogel, C. Denham, T. P. Waalkes, and T. De Vita, *Cancer Res.*, 30, 1651 (1970).

(7) Y. T. Lin, T. L. Loo, S. Vadlomudi, and A. Goldin, *J. Med. Chem.*, 15, 201 (1972).

(8) D. Abraham, J. Rutherford, and R. Rosenstein, *J. Med. Chem.*, 12, 189 (1969).

(9) J. E. Whinnery and W. H. Watson, *Acta Crystallogr., Sect. B*, 28, 3635 (1972).
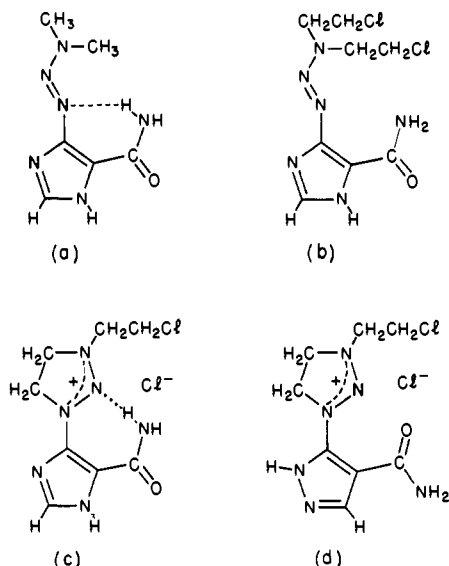
Figure 1. (a) The structure of DTIC, (b) DCTIC, (c) a transformation product of DCTIC, (d) a transformation product of the pyrazole derivative of DCTIC.

Figure 2. A view normal to the $\overline{2}02$ plane showing the intermolecular hydrogen bonding.

Table I. Crystal Data

$C_6H_{11}ON_6{}^+\cdot Cl^-\cdot H_2O$	Monoclinic space group $P2_1/n$
	FW = 236.7
a = 7.893 (3) Å	$F(000)$ = 496
b = 13.961 (2) Å	D_m = 1.40 (1) g/cm^{-3}
c = 10.224 (3) Å	D_x = 1.397 (1)
β = 93.20 (4)°	
V = 1125 Å	
λ(Cu Kα) = 1.5418 Å	
μ = 29.4 cm^{-1}	

being 2° at 2θ = 20° and 3.2° at 2θ = 147°. Four check reflections were monitored regularly during data collection and exhibited a gradual decrease in intensity to approximately 92% of the original values. Each reflection was assigned a variance $\sigma^2(I)$ based on counting statistics plus a term $(0.02\ S)^2$, where S is the scan count. Intensities and their variances were then corrected for the observed decay as well as for Lorentz and polarization effects and placed on an approximately absolute scale by means of a Wilson plot[10] (assuming an acetate of crystallization). Of the 2291 reflections measured, 129 had net intensities less than zero. Absorption was neglected; we estimate that it could have caused errors in F(obsd) amounting to no more than 4%, and that the only systematic effect

Table II. Final Nonhydrogen Atom Parameters ($\times 10^4$)[a,b]

Atom	x	y	z	$U_{11}{}^c$	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	6670 (2)	4469 (1)	4194 (2)	563 (10)	447 (9)	445 (9)	−10 (8)	−40 (8)	−13 (8)
C(2)	6624 (3)	3516 (2)	4149 (2)	609 (13)	462 (11)	503 (12)	−49 (10)	2 (10)	−45 (9)
N(3)	7548 (2)	3170 (1)	5155 (2)	607 (11)	389 (9)	540 (10)	15 (8)	38 (9)	−6 (8)
C(4)	8240 (2)	3923 (1)	5893 (2)	507 (11)	434 (10)	456 (10)	3 (8)	31 (9)	0 (8)
C(5)	7670 (2)	4743 (1)	5284 (2)	495 (11)	434 (10)	400 (9)	−2 (8)	28 (8)	−2 (8)
N(6)	8041 (2)	5661 (1)	5701 (2)	547 (10)	415 (8)	432 (8)	−6 (7)	−18 (8)	−6 (7)
N(7)	7364 (2)	6295 (1)	4920 (2)	571 (10)	414 (8)	480 (9)	1 (7)	2 (8)	1 (7)
N(8)	7690 (2)	7172 (1)	5296 (2)	702 (12)	400 (9)	578 (11)	−12 (8)	73 (9)	−5 (8)
C(9)	7035 (5)	7930 (2)	4440 (4)	1094 (25)	517 (15)	862 (21)	178 (17)	126 (19)	166 (14)
C(10)	8708 (4)	7381 (2)	6481 (3)	721 (17)	576 (14)	723 (16)	−85 (13)	42 (14)	−176 (12)
C(11)	9401 (3)	3734 (2)	7029 (2)	541 (12)	514 (11)	480 (11)	44 (9)	22 (9)	62 (9)
O(12)	9869 (2)	2911 (1)	7260 (2)	759 (11)	522 (9)	680 (10)	104 (8)	−112 (8)	103 (8)
N(13)	9899 (3)	4488 (2)	7742 (2)	796 (14)	572 (13)	603 (12)	79 (11)	−208 (11)	−24 (10)
Cl	7580 (1)	9605 (4)[d]	5275 (1)	789 (4)	444 (3)	603 (3)	90 (3)	−127 (3)	18 (2)
O(14)	9868 (3)	284 (1)	2948 (2)	798 (13)	683 (11)	562 (10)	110 (9)	−155 (10)	−58 (9)

[a] The final value of the extinction parameter g^{17} is 4.4 (6) $\times$ 10^{-6} e^{-2}. [b] Numbers in parentheses here and in following tables are estimated standard deviations of the least significant figures. [c] The form of the anisotropic temperature factor is $T = \exp[-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2klb^*c^*U_{23})]$. [d] The y coordinate for Cl has been multiplied by 10^5.

presume that the HCl was present as a minor contaminant in the acetic acid; similar crystals can be grown reproducibly from a reagent grade toluene–acetic acid solution to which a trace of concentrated HCl has been added.

For intensity measurements a crystal was cut to a roughly rectangular prism with dimensions 0.35 $\times$ 0.25 $\times$ 0.25 mm and mounted with the b axis slightly skew to the ϕ axis of a locally modified G.E. XRD-5 quarter-circle diffractometer automated by Datex. Using Ni-filtered copper radiation (λ 1.5418 Å), one quadrant of data was measured up to 150° in 2θ employing the θ–2θ scan technique. The following conditions were selected for the data collection: (1) a scan rate of 1°/min in 2θ; (2) 50-sec backgrounds counted before and after each scan; (3) a scan range that varied linearly with 2θ,
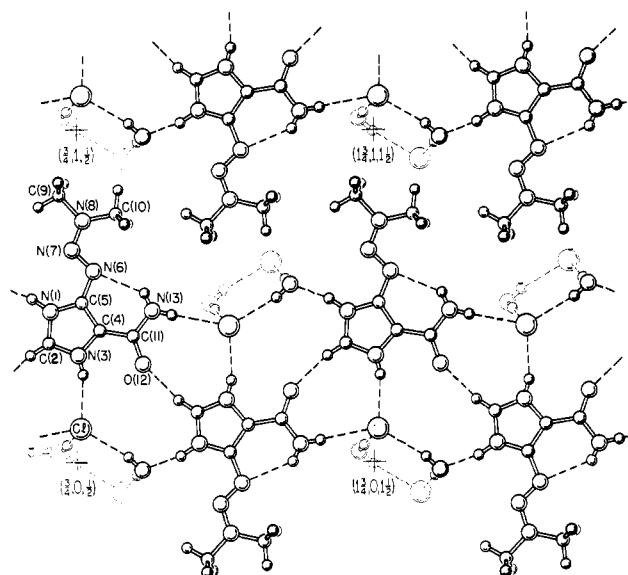
would be a decrease of about 0.0010 Å^2 in the isotropic component of the temperature coefficients U_{ij} (Table II).

Solution and Refinement. From a list of normalized structure factors (E's) and a Σ_2 interaction list,[11,12] three reflections and their signs were chosen to specify the origin[13] ($\overline{1}$.13.3, E = +3.78; $\overline{2}$89, E = +3.49; 438, E = −3.35). The $\overline{2}02$ reflection and its higher

(10) A. J. C. Wilson, *Nature (London)*, **150**, 151 (1942).
(11) R. Destro, "Program ASIG-2," private communication, 1970.
(12) W. H. Zachariasen, *Acta Crystallogr.*, **5**, 68 (1952).
(13) H. Hauptman and J. Karle, "Solution of the Phase Problem. I. The Centrosymmetric Crystal," A. C. A. Monograph No. 3, Polycrystal Bookservice, Pittsburgh, Pa., 1953.
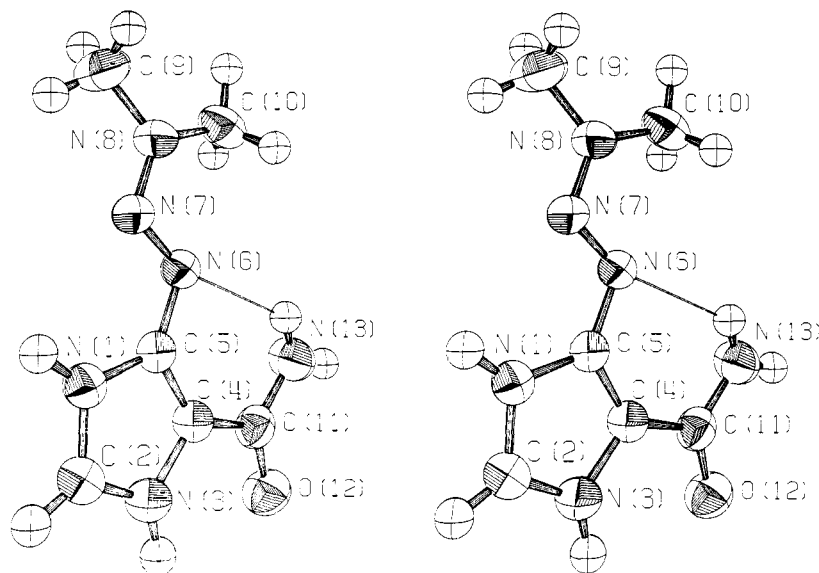
Figure 3. A stereoview of the DTIC cation: C. K. Johnson, "ORTEP, A Fortran Thermal Ellipsoid Program for Crystal Structure Illustrations," U. S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965. The C and N thermal ellipsoids and hydrogen atom thermal spheroids (B = 3.0 $Å^2$) are drawn at the 50% probability level.

orders are very intense, which indicated that the DTIC molecule lies approximately parallel to those planes. From packing considerations a phase of π was assigned to the $\overline{2}02$ reflection, and the phase of the $\overline{4}04$ was set equal to zero. Hand manipulation of the Σ_2 relations led to phases for 100 reflections.[11] The resulting E map indicated a reasonable structure for the DTIC molecule but had many additional peaks. An acetate grouping was fitted to four of these peaks, and structure factors were calculated for 826 low-angle data. The R index ($= (\Sigma||F_o| - |F_c||/\Sigma|F_o|)$) was a disappointing 0.68; nevertheless, refinement was initiated. It was only after several structure factor difference map cycles that we became convinced of the presence of a chloride ion and a water molecule, rather than an acetate group. The standard $AgNO_3$ test confirmed the presence of halide.

Refinement was by least-squares minimization of the quantity $\Sigma w|F_o{}^2 - s^2F_c{}^2|^2$, where $1/s$ is the scale factor for F_o. Scattering factors for C, N, and O were from the "International Tables for X-ray Crystallography,"[14] for Cl⁻ from Cromer and Waber,[15] and for H from Stewart, Davidson, and Simpson.[16] Weights w were set equal to $\sigma^{-2}(F_o{}^2)$; the $\overline{2}02$ reflection, which had exceeded the counter capacity, was given a weight of 0. All calculations were done under the CRYM computing system using an IBM 370/155 computer. When refinement reached an R index of 0.072, hydrogen peaks were predominant on a difference map. Hydrogen positions were assigned accordingly. The final least-squares cycle included, in a single matrix, coordinates for all 28 atoms, isotropic temperature factors for the 13 hydrogen atoms, anisotropic temperature factors for the 15 heavy atoms, a secondary extinction parameter,[17] and the scale factor. No parameter shifted by as much as 0.4σ. The goodness of fit, $[\Sigma w(F_o{}^2 - s^2F_o{}^2)/(N - P)]^{1/2}$, is 1.99 for N = 2290 reflections of nonzero weight (including those with negative net intensity) and P = 189 parameters. The R index for 2162 reflections with intensities greater than zero is 0.058.[18] The final parameters are listed in Tables II and III.

Discussion

The crystal structure is comprised of planar networks of protonated DTIC cations and chloride ions, the sheets being held together in pairs by water molecules of crystallization. These planar arrays are parallel to and midway between the $\overline{2}02$ planes; a view of

(14) "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham, 1962, pp 202–203.

(15) D. T. Cromer and J. T. Waber, *Acta Crystallogr.*, **18**, 104 (1965).

(16) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).

(17) A. C. Larson, *Acta Crystallogr.*, **23**, 664 (1967).

(18) See paragraph at end of paper regarding supplementary material.

Table III. Final Hydrogen Atom Parameters[a]

Atom	x	y	z	B, Å
H(1)	611 (3)	484 (2)	354 (2)	5.9 (6)
H(2)	607 (2)	316 (1)	348 (2)	3.9 (4)
H(3)	774 (3)	256 (2)	531 (2)	6.9 (7)
H(4)	653 (4)	760 (3)	366 (3)	9.6 (1.0)
H(5)	638 (4)	833 (3)	480 (3)	11.2 (1.2)
H(6)	798 (4)	829 (2)	415 (3)	9.2 (9)
H(7)	876 (3)	811 (2)	658 (2)	6.8 (6)
H(8)	818 (3)	709 (2)	726 (2)	6.8 (7)
H(9)	985 (4)	710 (2)	644 (3)	7.9 (8)
H(10)	944 (3)	503 (2)	758 (3)	8.0 (8)
H(11)	1056 (4)	438 (2)	842 (3)	8.2 (8)
H(12)	916 (3)	44 (2)	359 (3)	8.1 (8)
H(13)	1051 (3)	−10 (2)	329 (3)	6.7 (8)

[a] Coordinates have been multiplied by 10^3.

one such array is shown in Figure 2. Details of the hydrogen bonds are given in Table IV. Included is a

Table IV. Distances Involving Hydrogen Bonds (D–H···A)

Atom D	Atom H	Atom A	H···A, Å	D···A, Å
N(13)	H(10)	N(6)	2.34	2.974
N(13)	H(11)	Cl(a)	2.46	3.311
N(1)	H(1)	O(14)(b)	1.78	2.696
C(2)	H(2)	O(12)(c)	2.14	3.052
N(3)	H(3)	Cl	2.24	3.088
O(14)	H(12)	Cl	2.30	3.208
O(14)	H(13)	Cl(d)	2.37	3.158

(a) at $x + 0.5, -y + 0.5, z + 0.5$
(b) at $-x + 1.5, y + 0.5, -z + 0.5$
(c) at $x - 0.5, -y + 0.5, z - 0.5$
(d) at $-x + 2, -y, -z + 1$

C(2)–H···O contact which, by essentially any criterion,[19] must be considered a hydrogen bond, albeit a rather weak one.

A stereoscopic view of the DTIC cation is shown in Figure 3; bond distances and angles are given in Figure

(19) W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968, pp 14–16.
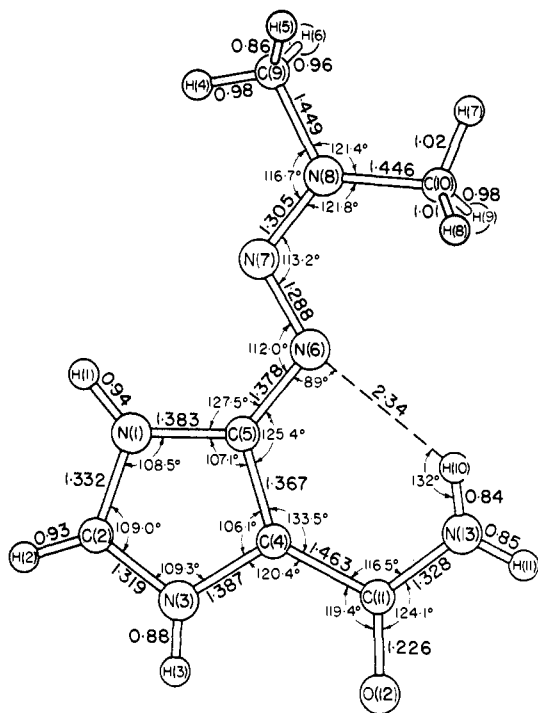
Figure 4. Bond distances and angles for the DTIC cation. The estimated standard deviation of a C–C bond is 0.003 Å, for a C–H bond 0.03 Å, and for a C–C–C angle about 0.4°.
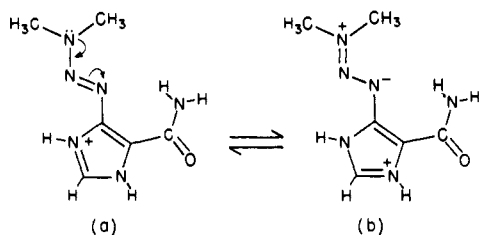

Figure 5. Resonance contributors to the molecular structure of the DTIC cation.

Table V. Angles (deg) Involving Hydrogen Atoms[a]

H(1)–N(1)–C(2)	121	H(7)–C(10)–N(8)	107
H(1)–N(1)–C(5)	130	H(8)–C(10)–N(8)	110
H(2)–C(2)–N(1)	124	H(9)–C(10)–N(8)	111
H(2)–C(2)–N(3)	127	H(7)–C(10)–H(8)	110
H(3)–N(3)–C(2)	125	H(8)–C(10)–H(9)	107
H(3)–N(3)–C(4)	125	H(9)–C(10)–H(7)	112
H(4)–C(9)–N(8)	105	H(10)–N(6)–C(5)	89
H(5)–C(9)–N(8)	115	N(6)–H(10)–N(13)	132
H(6)–C(9)–N(8)	108	H(10)–N(13)–C(11)	119
H(4)–C(9)–H(5)	115	H(10)–N(13)–H(11)	123
H(5)–C(9)–H(6)	107	H(11)–N(13)–C(11)	117
H(6)–C(9)–H(4)	106	H(12)–O(14)–H(13)	104

[a] Standard deviations are approximately 1 to 1.5°.

4 and Table V. Considerable double-bond character in the N(7)–N(8) bond is evidenced by the short bond distance of 1.305 Å and by the planarity of the bonding about N(8), indicating sp^2 hybridization. In valence-bond terms, this double-bond character can be explained by a relatively large contribution from the resonance form shown in Figure 5b. The resulting build-up of negative charge density at N(6) would tend to make the N(13)–H···N(6) internal hydrogen-bond conformation particularly stable.
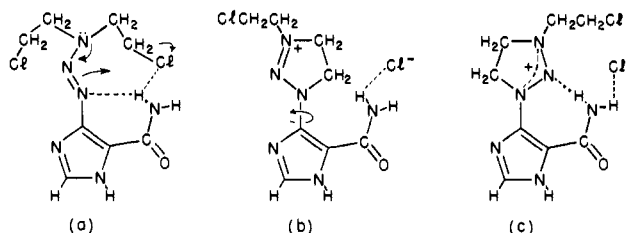

Figure 6. A possible cyclization mechanism for DCTIC.

The dimensions of the imidazole ring agree well with values found for the protonated ring in histidine compounds,[20-23] despite the π character in the exocyclic bonds C(4)–C(11) and C(5)–N(6); the largest difference is a lengthening of the N(3)–C(4) bond by about 0.02 Å. The atoms of the imidazole ring are coplanar within

Table VI. The Least-Squares Plane of the Imidazole Ring[a]

Atom	Deviation, Å	Atom	Deviation, Å
N(1)*	0.002	Cl⁻	−0.050
C(2)*	0.000	H(1)	0.03
N(3)*	−0.002	H(2)	0.05
C(4)*	0.003	H(3)	0.03
C(5)*	−0.003	H(4)	0.23
N(6)	−0.014	H(5)	−0.57
N(7)	0.013	H(6)	0.88
N(8)	−0.002	H(7)	−0.08
C(9)	0.086	H(8)	−0.88
C(10)	−0.053	H(9)	0.72
C(11)	0.077	H(10)	−0.23
O(12)	0.246	H(11)	−0.01
N(13)	−0.031		

[a] The least-squares plane was passed through the five atoms marked with an asterisk, all weighted equally. Direction cosines of the plane normal are 0.835, −0.001, and −0.596 relative to a, b, and c; the origin-to-plane distance is 1.834 Å.

experimental error (Table VI). Out-of-plane deviations of the side-chain atoms result primarily from a twist of about 1.5° about the N(7)–N(8) bond, an out-of-plane bending of the C(4)–C(11) bond by about 3°, and a twist of about 9.5° about the C(4)–C(11) bond. The latter two distortions result in a slight lengthening of the internal hydrogen bond.

This internal hydrogen bond suggests a possible mechanism for the deactivation of the bis(chloroethyl) compound DCTIC, Figure 1b, which cyclizes rapidly under mild conditions[6] to form the inactive product shown in Figure 1c. The structure of this latter compound has already been reported;[8] it also has an internal hydrogen bond, but the acceptor atom is the second nitrogen atom of the triazoline ring rather than the first. A possible mechanism for the ring closure is outlined in Figure 6. An internal hydrogen bond in the parent compound DCTIC, as found in the structure of DTIC, would hold the nitrogen atom of the amide group in a position such that the chlorine atom of a terminal chloroethyl group could approach (Figure

(20) J. Donohue, L. R. Lavine, and J. S. Rollett, Acta Crystallogr., 9, 655 (1956).
(21) J. Donohue and A. Caron, Acta Crystallogr., 17, 1178 (1964).
(22) I. Bennett, A. Davidson, M. Harding, and I. Morelle, Acta Crystallogr., Sect. B, 26, 1722 (1970).
(23) T. Kistenmacher, D. Hunt, and R. Marsh, Acta Crystallogr., Sect. B, 28, 3352 (1972).

6a). Electron transfer could then result in ring formation with the concomitant release of a chloride ion (Figure 6b). A subsequent rotation about the C–N bond would then bring the product into the conformation found by Abraham, et al.[8] We note that the related inactive compound shown in Figure 4d, which differs by having a pyrazole ring in place of the imidazole ring, adopts a nonplanar conformation with no internal hydrogen bond and the amide group rotated by 180°;[9] nevertheless, a similar mechanism can be proposed for the cyclization of the active parent compound.

Conformation of Cyclic Peptides. VIII. Cyclic Hexapeptides Containing the L-Pro-D-Phe Sequence[1]

Kenneth D. Kopple,* Thomas J. Schamper and Anita Go

Contribution from the Department of Chemistry, Illinois Institute of Technology, Chicago, Illinois 60616. Received October 24, 1973

Abstract: The cyclic peptides *cyclo*(L-xxx-L-Pro-D-Phe)$_2$, where xxx is Ala, Orn, or His, were prepared and their conformations were studied by proton magnetic resonance and model building. The data show that these peptides exist in two conformations with average C_2 symmetry. One of these (conformation A) has trans xxx-Pro peptide bonds and is composed of two β turns, with the amide protons of the xxx residues solvent shielded *and* transannularly hydrogen bonded. The other conformation (B) probably has cis xxx-Pro peptide bonds; a likely conformation is proposed. Only conformation A occurs in chloroform or hexafluoro-2-propanol. Conformation B is favored, but not exclusively, by solvents of high dielectric constant that can serve as good hydrogen bond proton acceptors (water, dimethyl sulfoxide). Conformation B is somewhat more favored in the histidine case than in the ornithine or alanine analogs, and potassium ion slightly increases its stability (alanine peptide in dimethyl sulfoxide). From the data collected on the internal peptide protons of conformation A, it is argued that the magnetic anisotropy of the 2–3 peptide bond of a β turn has no important influence on the chemical shift of the internal proton, rather, that the principal factor is hydrogen bonding.

In continuation of studies of conformation determining sequences in oligopeptides we have prepared the cyclic hexapeptides *cyclo*(L-xxx-L-Pro-D-Phe)$_2$, where the variable residue is alanine, histidine, or ornithine, and carried out a conformational study based on proton magnetic resonance and model building techniques. This work, described below, complements a previous study of the retro isomers of these peptides, *cyclo*(L-xxx-D-Phe-L-Pro)$_2$.[2]

Experimental Section

Spectra. Proton magnetic resonance spectra were obtained using the Bruker HX 270 spectrometer of the University of Chicago and the 250-MHz spectrometer at the NMR Facility for Biomedical Research, Carnegie-Mellon University. Most of the spectra were obtained as single scans in the continuous wave mode, although in some cases signal enhancement with the Carnegie-Mellon spectrometer was obtained using the multiple rapid scan correlation technique developed by Bothner-by, Dadok, and Sprecher.[3] We are grateful to Drs. Joseph Dadok and Richard F. Sprecher for instruction in the use of the method.

Resonances were assigned to amino acid residues of the peptides using the usual spin-decoupling technique and, in some instances, homonuclear indor.[4]

Peptide Synthesis. The linear precursors of the cyclic peptides were prepared by stepwise synthesis of tripeptide fragments, which were coupled to form the open-chain hexapeptides. Intermediates were not thoroughly purified, but thin-layer chromatography and proton magnetic resonance were used to ensure that each intermediate had the required composition before the next step was undertaken. The cyclization steps, purifications, and characterizations of final products are described below. For use in the procedures below the N,N-dimethylformamide was freed of amines and water by storage over and distillation from an ethylene–maleic anhydride copolymer (Monsanto EMA 11), and the N-methylmorpholine was freed of secondary amine by distillation from phenyl isocyanate.

cyclo(D-**Phe**-L-**Orn**-L-Pro)$_2$. Crude Boc-(D-Phe-L-Orn(Z)-L-Pro)$_2$-NHNH$_2$, 2.65 g, was dissolved in 25 ml of anhydrous trifluoroacetic acid and stored at room temperature for 15 min. The trifluoroacetic acid was evaporated under vacuum and the residue was triturated with a large excess of anhydrous ether, washed with ether, and dried under vacuum over phosphorus pentoxide. An almost homogeneous, ninhydrin-positive product (2.8 g) was obtained.

The above product (2.25 mmol) was dissolved in 8 ml of di-

(1) This work was supported by U. S. Public Health Service Grant GM 14069 for the Institute of General Medical Sciences, National Institutes of Health, and by a PHS Career Development Award to K. D. K., GM 47357, from NIGMS. The proton magnetic resonance work reported was performed in large part at the NMR Facility for Biomedical Studies, Carnegie-Mellon University, which is supported by Grant RR-00292 from the National Institutes of Health. The 270-MHz nmr spectrometer at the University of Chicago, also used in this work, is supported by a grant (GP 33116) from the National Science Foundation.

(2) K. D. Kopple, A. Go, T. J. Schamper, and C. S. Wilcox, *J. Amer. Chem. Soc.*, **95**, 6090 (1973).

(3) (a) J. Dadok, R. F. Sprecher, and A. A. Bothner-by, 13th Experimental NMR Conference, Asilomar, Calif., 1972, paper 15-4; (b) J. Dadok and R. F. Sprecher, 14th Experimental NME Conference, Boulder, Colorado, 1973, Section 2C.

(4) W. A. Gibbons, H. Alms, R. S. Bockman, and H. R. Wyssbrod, *Biochemistry*, **11**, 1721 (1972).