PACK, G. R. & LOEW, G. H. (1978). *Biochim. Biophys. Acta*, **519**, 163–172.
PATEL, D. J. (1979). *Biopolymers*, **16**, 2739–2754.
PATEL, D. J. & CANUEL, L. L. (1977). *Proc. Natl Acad. Sci USA*, **74**, 2624–2628.
PEARLSTEIN, R. A., DRENO, P. L., PENSAK, M. & HOPFINGER, A. J. (1981). *Biochim. Biophys. Acta*, **655**, 432–445.
QUIGLEY, G. J., WANG, A. H. J., UGHETTO, G., VAN DER MAREL, G., VAN BOOM, J. H. & RICH, A. (1980). *Proc. Natl Acad. Sci. USA*, **77**, 7204–7208.
REDDY, B. S. SESHADRI, T. P., SAKORE, T. D. & SOBELL, H. M. (1979). *J. Mol. Biol.* **135**, 787–812.
SHIEH, H.-S., BERMAN, H. M., DABROW, M. & NEIDLE, S. (1980). *Nucleic Acids Res.* **8**, 85–97.

Acta Cryst. (1984). B**40**, 429–434

The Structure of Pilocarpine Hydrochloride, $C_{11}H_{17}N_2O_2^+.Cl^-$: A Muscarinic Alkaloid

BY PENELOPE W. CODDING*

Departments of Chemistry and Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada T2N 1N4

AND MICHAEL N. G. JAMES

Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

(*Received* 24 *October* 1983; *accepted* 5 *April* 1984)

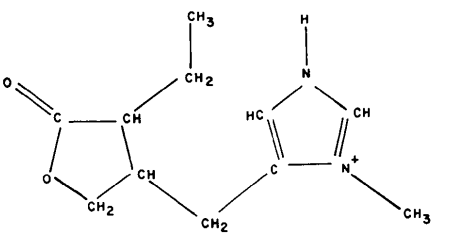
Abstract

$M_r = 244{\cdot}72$, at ambient room temperature (~295 K) and at 77 K, monoclinic, $P2_1$, $Z = 2$, $a = 11{\cdot}057\ (1)$ [10·965 (5)], $b = 9{\cdot}212\ (1)$ [9·177 (3)], $c = 6{\cdot}697\ (1)$ [6·507 (3)] Å and $\beta = 110{\cdot}05\ (2)$ [109·19 (4)]°, $V = 637{\cdot}8\ (2)$ [618·4 (5)] Å^3 (the values in brackets for the 77 K determination). $D_x = 1{\cdot}276$ g cm^{-3}, $F(000) = 260$, $\lambda(\text{Mo } K\alpha) = 0{\cdot}71069$ Å, $\mu(\text{Mo } K\alpha) = 2{\cdot}92$ cm^{-1}, $R = 0{\cdot}043$ [0·031] for 2751 [3864] contributing reflections. The *N*-methylimidazole ring is protonated at the position of the secondary N atom and the ring is planar. The NMR spectrum of the compound was studied over the pH range 2·6–10·6; these spectra show that there is a delocalization of π electrons over the bonds between the two N atoms of the protonated imidazole ring. The Cl^- ion in the crystal structure is hydrogen-bonded to the protonated N atom at a distance of 3·035 (2) [3·030 (2)] Å. The conformation of the molecule differs significantly from that found in pilocarpine trichlorogermanate and from models of muscarinic agents.

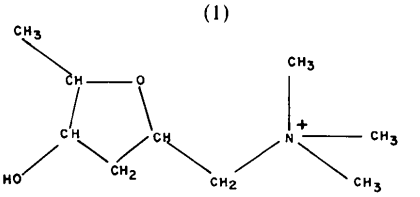
Introduction

Pilocarpine (1) is one of several alkaloids that mimic the effect of acetylcholine on autonomic effector cells that control smooth muscle contraction. The compound binds to the muscarinic class of acetylcholine receptors (Birdsall, Burgen & Hulme, 1978) and exhibits partial agonist activity.

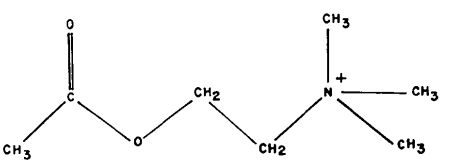
Muscarine (2), pilocarpine (1), and acetylcholine (3) have considerable differences in their chemical structures yet all bind to the muscarinic receptor while only acetylcholine has activity at the nicotinic receptor. An explanation for the affinity of muscarinic agents for their receptor has been sought in terms of a specific binding conformation. Three distinct models appear in the literature: in the first, Kier (1967, 1968) predicted on the basis of extended Hückel theory (EHT) that there were two preferred low-energy conformations of acetylcholine. One, the folded conformation with a hydrogen-bond acceptor



(1)



(2)



(3)

* Author to whom correspondence should be addressed.

0108-7681/84/040429-06$01.50

3·0 Å from the quaternary N atom, was assumed to be the muscarinic binding conformation. In the second model, Chothia (1970) and Baker, Chothia, Pauling & Petcher (1971) predicted that the acetylcholine molecule had a muscarinic face containing a methyl group approximately 5·4 Å from the positive N atom. The pilocarpine structure found in the trichlorogermanate hemihydrate determined by Fregerslev & Rasmussen (1968) fits this muscarinic face of acetylcholine. The third model for the muscarinic receptor (Schulman, Sabio & Disch, 1983) defined two essential receptor sites, an anionic site (*P*) 3·0 Å from the N atom of the trimethylammonium group and an electrostatic site (*Q*) 1·2 Å from the ether O atom. This model required these sites to be 6·6–6·8 Å apart and the *Q*–O–N–*P* dihedral angle to be in the range 100–117°; these two requirements placed the terminal methyl C atom that is typical of muscarinic agents approximately 8·5 Å from *P*, the anionic site.

Attempts to define the conformational stereochemistry required for molecules to bind to the muscarinic receptor site have been inconclusive because of the flexibility of acetylcholine. In six crystal structure analyses of acetylcholine a *gauche* N–C–C–O conformation was found; however, there are variations from this average position and both *gauche* conformers have been observed. (A composite diagram of all six structures has been deposited, see deposition footnote.) Both NMR studies (Makriyannis, Theard & Mautner, 1979) and theoretical calculations (Reed, Murray, Roche & Domelsmith, 1981) have confirmed the flexibility of acetylcholine and have led to a proposal that flexibility confers activity on acetylcholine analogs. Since structural models for muscarinic agents have assumed restricted geometry while spectroscopic and theoretical studies find some conformational flexibility, the crystal structure of pilocarpine with a different anion, the Cl^- ion, was undertaken to probe these issues. In addition, solution NMR spectra were studied as a function of pH to characterize further the resonance form of the imidazole ring and the site of protonation.

Experimental section

The sample of pilocarpine hydrochloride was obtained from Sigma Chemical Co., St Louis, MO, and crystallized by the vapor diffusion of ether into an ethanolic solution of the compound (Codding & James, 1973). Eeles (1953) previously reported the space group, $P2_1$, and cell dimensions $a = 6·7$, $b = 9·2$, $c = 10·8$ Å and $\beta = 105·5°$ for pilocarpine hydrochloride. If the values of a and c are interchanged, the Eeles cell dimensions are quite similar to those in this work except for a ~4·5° difference in the β angle in the two determinations.

Colorless crystal, 0·6×0·5×0·3 mm, data collection at room temperature, 295 K, and at liquid N_2 temperature, 77 K, automated Picker FACS-1 diffractometer. The general details common to both data collections are as follows: 2θ values for reflections used in lattice determination 26·7–50·7°, 2θ scan of 2° min^{-1}, range 1·5° with two 10 s fixed-position background counts, 6% average variation in three standard reflections (600, 060, 006), graphite monochromator, Mo radiation, $2\theta_{max} = 80°$, no absorption correction, 4032 reflections measured, reflections classified as observed if $I > 3\sigma(I)$ where $\sigma(I) = (B + T + kI^2)^{1/2}$ (B the total background count, T the total peak count, I the net intensity and k taken as 0·04). For the 77 K data, $+h$, $+k$, $\pm l$ reflections collected, max (h, k, l) 20, 18, 12; for the 295 K data, $\pm h$, $+k$, $+l$ reflections collected, max (h, k, l) 20, 18, 12.

The low-temperature apparatus used was the Cryotip Refrigerator System, Air Products and Chemical Inc., Allentown, PA, with modifications to the compressed gas (N_2) inlet plumbing to allow three tanks to be connected in parallel and the addition of two vertical drying columns for the compressed gas. The crystal was mounted with silicone grease and cooled to 77 K. This system suffered from occasional warm-ups which caused the crystal to move in the grease; an automatic crystal-alignment routine allowed the data collection to proceed.

The NMR spectra were obtained on a Varian A-60-D spectrometer at a probe temperature of 298 K. Spectra were recorded at sweep rates of 1 Hz s^{-1} overall and 0·2 Hz s^{-1} for the CH_3 doublet. Chemical shifts were measured relative to the central resonance of the tetramethylammonium (TMA) ion triplet; this resonance is 3·17 p.p.m. downfield from the methyl resonance of sodium 3-(trimethylsilyl)-1-propanesulfonic acid (TMPSA).

Structure solution by tangent refinement of phases calculated from the coordinates of two atomic positions identified in an $|E|^2 - 1$ Patterson map and subsequent identification of most atomic positions in an E synthesis. Completion of model by an observed Fourier synthesis. Unit-weight full-matrix refinement on F with anisotropic thermal parameters, positions of all 17 H atoms from a difference Fourier synthesis. H atoms assigned the isotropic thermal parameter of the bonded atom and included in the model but not refined. Final $R_w = 0·043$, $S = 3·7$, $(\Delta/\sigma)_{max} = 0·76$, peaks in final ΔF map +0·17 to −0·23 e Å^{-3} (in bonds, especially double bonds).

The low-temperature data were refined using the final coordinates of the C, N, O and Cl^- atoms of the room-temperature structure, isotropic thermal parameters of 2·0 Å^2, and unit weights. After full-matrix, anisotropic refinement the H atoms were identified in a difference Fourier synthesis and their parameters refined [$\sum w(\Delta F)^2$ minimized], with isotropic thermal parameters, along with the rest of the

Table 1. *Fractional atomic coordinates* ($\times 10^5$) *and average root-mean-square vibrations* (Å, $\times 10^2$) *for the non-hydrogen atoms of pilocarpine hydrochloride at* 77 K

The y coordinate of Cl was fixed at 0 to define the origin. The root-mean-square vibration values were calculated with *ORTEP* (Johnson, 1965).

	x	y	z	$\langle u^2\rangle^{1/2}$
N(1)	34036 (10)	−9917 (12)	45972 (18)	11
C(2)	40932 (12)	−12865 (14)	66486 (23)	12
N(3)	43240 (10)	−471 (16)	77609 (15)	12
C(4)	37613 (12)	10865 (14)	63737 (21)	12
C(5)	31801 (11)	4928 (14)	43708 (20)	11
C(6)	24889 (12)	11679 (15)	22233 (20)	12
C(7)	23425 (11)	28138 (14)	23362 (20)	11
C(8)	18799 (14)	34586 (16)	398 (22)	13
O(9)	12760 (11)	48264 (13)	2478 (17)	14
C(10)	9455 (12)	48264 (14)	20774 (21)	12
C(11)	13385 (11)	34135 (13)	33168 (19)	11
O(12)	3955 (11)	58587 (13)	25143 (20)	11
C(13)	1432 (12)	24419 (15)	29481 (22)	12
C(14)	−9374 (15)	31291 (19)	36156 (28)	14
C(15)	29912 (14)	−20726 (16)	28613 (23)	13
Cl	57965 (3)	0	126003 (4)	11

structure. The average shift in parameter was less than the accuracy of the IBM 360 computer word [$(\Delta/\sigma)_{max} = 5{\cdot}8$ (errors were very small)] so the refinement was terminated. Final $R_w = 0{\cdot}036$, $S = 3{\cdot}9$, peaks in final ΔF map $\pm 0{\cdot}5$–$0{\cdot}6$ e Å^{-3}. Programs used: *XRAY*70 system (Stewart, Kundell & Baldwin, 1970). Scattering factors for C, N, O, Cl from Cromer (1968), for H from Mason & Robertson (1966).

The final atomic coordinates for the non-hydrogen atoms of the low-temperature model are in Table 1. The atomic labeling scheme and the interatomic distances for the two structures are given in Fig. 1 and the interbond angles are in Table 2.*

* The room-temperature atomic coordinates, the least-squares planes, the anisotropic thermal parameters, the hydrogen-atom parameters and interatomic distances involving hydrogen atoms as well as the structure factors for both data sets and two figures have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 39366 (76 pages). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

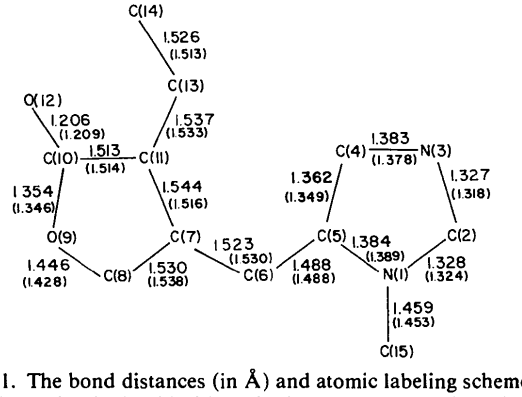


Fig. 1. The bond distances (in Å) and atomic labeling scheme for pilocarpine hydrochloride at both temperatures. The values at 295 K are in parentheses. The estimated standard deviations are 0·001–0·002 Å at 77 K and 0·003–0·005 Å at 295 K.

Table 2. *Bond angles* (°) *for pilocarpine hydrochloride*

	77 K	295 K
C(15)–N(1)–C(2)	124·7 (1)	125·0 (2)
C(5)–N(1)–C(15)	126·0 (1)	126·3 (2)
C(5)–N(1)–C(2)	109·3 (1)	108·7 (2)
N(1)–C(2)–N(3)	108·5 (1)	108·9 (2)
C(2)–N(3)–C(4)	108·9 (1)	108·8 (2)
N(3)–C(4)–C(5)	107·0 (1)	107·3 (2)
C(4)–C(5)–N(1)	106·3 (1)	106·3 (2)
C(4)–C(5)–C(6)	131·7 (1)	132·0 (2)
C(6)–C(5)–N(1)	121·8 (1)	121·7 (2)
C(5)–C(6)–C(7)	113·4 (1)	113·4 (2)
C(6)–C(7)–C(8)	110·0 (1)	109·3 (2)
C(6)–C(7)–C(11)	118·3 (1)	118·6 (2)
C(8)–C(7)–C(11)	102·3 (1)	102·3 (2)
C(7)–C(8)–O(9)	105·0 (1)	105·0 (3)
C(8)–O(9)–C(10)	110·2 (1)	110·3 (2)
O(9)–C(10)–O(12)	120·4 (1)	121·0 (3)
O(9)–C(10)–C(11)	110·9 (1)	110·7 (3)
O(12)–C(10)–C(11)	128·6 (1)	128·4 (3)
C(10)–C(11)–C(13)	109·6 (1)	109·6 (2)
C(10)–C(11)–C(7)	101·6 (1)	101·9 (2)
C(7)–C(11)–C(13)	114·6 (1)	115·6 (2)
C(11)–C(13)–C(14)	114·7 (1)	114·9 (3)

Discussion

The pilocarpine cation as the hydrochloride salt assumes an extended conformation as shown in Fig. 2 and in the torsion angles in Table 3; this conformation is different from that found previously for pilocarpine. In the trichlorogermanate crystals (Fregerslev & Rasmussen, 1968, hereafter FR) there are two observations of the pilocarpine cation; they are nearly identical. The conformations of the pilocarpine cation as observed in the two crystal structures are depicted in Fig. 3 with the imidazole rings of the two structures superimposed.

There are three contributions to the differences in conformation evident in Fig. 3. The first is a difference in the C(5)–C(6)–C(7)–C(8) torsion angle of about 120°; this causes the lactone rings of the two structures to be nearly perpendicular to each other. In both structures the conformation of the lactone ring is an envelope with the atom corresponding to C(7) (in this determination) at the flap. The second conformational difference can be viewed as a flip of this atom, C(7), from one side of the envelope plane to the other. As a result, in the hydrochloride structure the ethyl

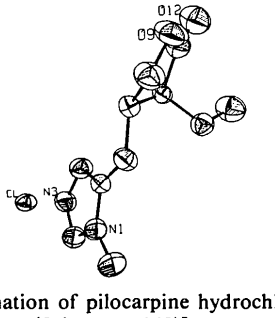


Fig. 2. The conformation of pilocarpine hydrochloride [*ORTEP* (Johnson, 1965)].

Table 3. *Selected torsion angles* (°) *describing the pilocarpine conformations*

	Hydrochloride (77 K)	Trichlorogermanate* Mol. 1	Mol. 2
C(2)–N(1)–C(5)–C(6)	176·7 (1)	177·2	173·4
N(3)–C(4)–C(5)–C(6)	−176·3 (1)	−179·6	−175·7
C(4)–C(5)–C(6)–C(7)	−4·2 (2)	−7·4	−18·2
N(1)–C(5)–C(6)–C(7)	179·8 (1)	167·6	165·3
C(5)–C(6)–C(7)–C(8)	168·4 (1)	−71·7	−72·3
C(5)–C(6)–C(7)–C(11)	−74·5 (1)	177·6	177·7
C(6)–C(7)–C(11)–C(13)	−31·6 (1)	−37·0	−41·1
C(6)–C(7)–C(11)–C(10)	−149·7 (1)	88·9	87·3
C(6)–C(7)–C(8)–O(9)	157·0 (1)	87·2	83·8
C(7)–C(11)–C(13)–C(14)	−171·4 (1)	−64·4	−58·4
C(7)–C(11)–C(10)–O(12)	−162·8 (1)	−163·0	−160·5
C(7)–C(11)–C(10)–O(9)	18·2 (1)	20·1	20·0
C(7)–C(8)–O(9)–C(10)	−20·4 (1)	−22·0	−22·8

* Calculated from the data of Fregerslev & Rasmussen (1968).

group is axial and the atom bonded to the imidazole ring is equatorial so that C(6) is −0·02 Å and C(13) is −1·41 Å on the opposite side of the plane from the flap, C(7), while in the FR structure, both the atom bonded to the imidazole and the ethyl group are on the same side of the envelope plane as the flap. A third difference in conformation between the two crystal structures appears in the ethyl side chain position relative to C(7). The methyl C atom is *gauche* to C(7) in the FR structure and *anti* in the hydrochloride salt.

These conformational differences change the separation of the tertiary N atom and the ether O atom from 5·11 Å in the FR structure to 6·162 (2) Å in the hydrochloride salt; yet, the separation of the same N atom, N(1), and the methyl C atom of the lactone-ring side chain has changed very little: from 5·71 (2) Å in the FR determination to 5·944 (2) Å for the structure reported here. Both conformations observed for pilocarpine differ from the *gauche* acetylcholine structure and from the folded conformation of muscarine as observed by Jellinek (1957). The folded conformations found for acetylcholine and muscarine gain stability from an electrostatic interaction between the positive N atom and the ether O atom which are approximately 3·0 Å apart; this interaction is sterically hindered in the pilocarpine molecule.

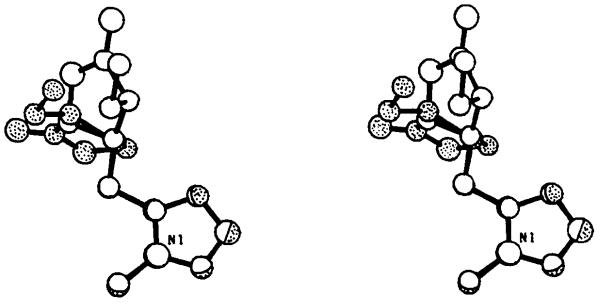


Fig. 3. A stereoscopic drawing [*PLUTO* (Motherwell, 1979)] of the superposition of the two solid-state conformations of the pilocarpine cation. The imidazole rings in the two structures were fit together by least squares. The atoms in the trichlorogermanate structure are stippled.

Examination of the bond distances in the imidazole ring indicates that protonation of the ring has produced a change in the π bonding of the N atoms. In the more precise low-temperature study, the C–N distances are equivalent whereas in a typical unprotonated imidazole ring the C–N distances differ by *ca* 0·035 Å (James & Matsushima, 1973). The least-squares plane of the five atoms of the imidazole ring of pilocarpine (at 77 K) has a χ^2 of 0·36 and a standard deviation of 0·0006 Å; all the atoms of the ring are within $1{\cdot}2\sigma$ of the plane. In contrast, in *N*-(β-D-ribofuranosyl)imidazole (James & Matsushima, 1973) the secondary N atom has partial tetrahedral character so C atoms bonded to it have significant deviations from the least-squares plane. Thus, the structural results indicate that the π electrons of the imidazole ring of the pilocarpine cation are extensively delocalized over the three atoms, N(1), C(2), N(3).

NMR spectra and structure–function relations

A study of the NMR spectra of pilocarpine in H_2O over the pH range 2·6–10·6 showed changes in the chemical shifts of the H(2), H(4) and C(15)H_3 signals as N(3) was titrated. The H(2) signal displayed the largest change over the pH range, 1·03 p.p.m., twice the change in the H(4) signal. This sensitivity to protonation can be explained by the presence of π-electron density in the bonds between H(2) and the site of protonation. The C(15)H_3 signal was a doublet throughout the pH range studied; a double-resonance experiment indicated the coupling was between H(2) and the methyl H atoms with a coupling constant of approximately 0·6 Hz. In this case as well, the interaction of H(2) and C(15)H_3 can be due to multiple-bond character between the two sites. The NMR results indicate, therefore, that the electronic effects evident in the crystal, from bond distances and planarity, are also present in solution at physiological pH. The chemical shift of the H(2) signal as a function of pH is shown in Fig. 4. An approximate pK_a value of 7·0 for the deprotonation of the imidazole ring can be derived from this plot. This pK_a value indicates that over 70% of the pilocarpine molecules would be uncharged at a physiological pH of 7·4. This low concentration of charged species may limit the binding of these molecules.

The spectroscopic and structural data show a major electronic difference between the structure of pilocarpine and the postulated characteristics of muscarinic agonists. The pilocarpine molecule does not have a pyramidal N atom that carries a full positive charge. Instead the molecule has a diffuse positive charge shared between two planar N atoms. This finding

agrees with the theoretical work of Pullman, Courrière & Coubeils (1971) that found the positive charge in acetycholine to be diffusely spread among the three methyl groups of the $-N^+(CH_3)_3$ moiety forming a globe of charge.

With two different observed conformations for the pilocarpine molecules the predictive power of the three geometric models for muscarinic agonists can be analyzed. The shapes of the pilocarpine molecules in the two structure determinations do not fit Kier's (1967, 1968) model. Neither example of the pilocarpine conformation is folded to produce an N–O distance of nearly 3·0 Å as required by the model. The Pauling model (Chothia, 1970; Baker, Chothia, Pauling & Petcher, 1971) has better agreement with the structural data. In both pilocarpine structures the tertiary N atom to methyl C atom distances (5·7 and 5·9 Å) are nearly the muscarinic-face N to methyl separation, 5·4 Å. Yet the atoms between the extremes are in different positions in the two structures (see Fig. 3). This congruity between experimental and postulated structures requires that the cationic site be at N(1), in disagreement with the delocalization of positive charge discussed above.

The Schulman, Sabio & Disch (1983) model is more difficult to evaluate because of the ambiguity in the placement of the anionic site, *P*, without a $-N(CH_3)_3$ group. One obvious choice for an anionic receptor site is the position of the Cl^- ion in the pilocarpine hydrochloride structure. The Cl–N(3) distance is approximately 3·0 Å and in the crystal the two atoms form a hydrogen bond. With this Cl position and an electrostatic site, *Q*, 1·2 Å from O(9), the only geometric parameter of pilocarpine hydrochloride that fits this third model is the methyl group to anionic site distance of 8·3 Å. The dihedral angle *Q*–O(9)–N(3)–Cl is too small at 70·6° and the *Q*–Cl distance is too long. If a point, *P*, for the anionic site is placed in an axial position 3·0 Å from N(1) either above or below the plane, the fit to the model is worse. A different correspondence between the theory and the X-ray structure is obtained with the FR structure.

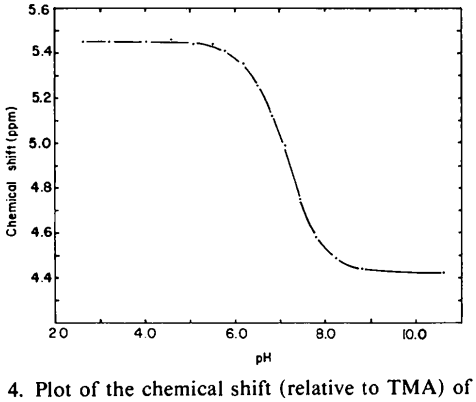


Fig. 4. Plot of the chemical shift (relative to TMA) of the signal from H(2) as a function of pH.

One choice of anionic site (*P*) on the tertiary N atom above the imidazole ring produces a *P*–*Q* distance of 6·49 Å, close to the proposed range of 6·6–6·8 Å. The *Q*–O–N–*P* angle is 97·1° a value 3° less than the range required by the theory. However, the *P* to methyl C atom distance is too short at 5·26 Å rather than 8·5 Å. In sum, neither of the observed conformations of pilocarpine fits all components of the Schulman, Sabio & Disch model. At best this lack of correspondence between observations and model indicates that all the features of this model are not required for partial agonist activity. Further testing of the fit of partial and full agonist structures might clarify this question.

These two conformations of pilocarpine show that the molecule is flexible and has at least two accessible conformations. Acetylcholine is also a flexible molecule, and the calculations of Schulman, Sabio & Disch found alternate conformations for muscarine when appropriate receptor sites were included in the calculations. Since the observed conformations of pilocarpine do not completely fit any static model for the muscarinic receptor, it is possible that flexibility is integral to the activity of muscarinic agents. This explanation can be assessed only when data on the relative energies of alternate conformations are available. Another possible explanation for the discrepancy between pilocarpine and the models is that partial agonist activity may be achieved through binding to only part of the receptor site. Further structural analysis of partial agonists may establish the minimum fit of such compounds to the models mentioned here.

The bond distances and angles for the two structures are typical for these ring systems with the exception of the C–N distances previously discussed. The differences in bond distances between the two temperatures must be due in part to the decrease in thermal motion at the lower temperature. This decrease in thermal motion is exemplified by an average decrease in isotropic thermal parameter of 2·8 $Å^2$.

A stereodiagram of the packing of the molecules has been deposited (see deposition footnote). There is a hydrogen bond between the Cl^- ion and N(3) with a distance of 3·035 (2) Å (at 298 K). The molecules are packed in a loose herringbone arrangement along the *c* axis, the only close intermolecular contact being that between O(12) and C(14) of the molecule at $(\bar{x}, y+\frac{1}{2}, 1-z)$ of 3·259 (5) Å (at 298 K). The *c* unit-cell length shows the greatest change with temperature, a decrease of 0·190 Å (3%), which is evident in the decrease of this intermolecular contact distance to 3·170 (2) Å at 77 K. The packing in the **b** direction is governed by the stacking of the imidazole rings and in the **a** direction by the Cl^- ion contacts. This packing arrangement appears to impose few restraints on the conformation of the molecule.

The authors thank Martin Cowie for the design of the compressed-gas plumbing for the low-temperature device and D. L. Rabenstein for assistance with the NMR spectroscopy. This work was supported by grants from the Medical Research Council of Canada to MNGJ and PWC and from the Alberta Heritage Foundation for Medical Research to PWC.

References

BAKER, L. W., CHOTHIA, C. H., PAULING, P. & PETCHER, T. J. (1971). *Nature (London)*, **230**, 439–445.
BIRDSALL, N. J. M., BURGEN, A. S. V. & HULME, E. C. (1978). *Mol. Pharmacol.* **14**, 723–736.
CHOTHIA, C. (1970). *Nature (London)*, **225**, 36–38.
CODDING, P. W. & JAMES, M. N. G. (1973). Am. Crystallogr. Assoc. Summer Meet., Storrs, CT, June, Abstr. 02.
CROMER, D. T. (1968). *Acta Cryst.* A**24**, 321–324.
EELES, W. T. (1953). *Acta Cryst.* **6**, 809–810.
FREGERSLEV, S. & RASMUSSEN, S. E. (1968). *Acta Chem. Scand.* **22**, 2541–2556.
JAMES, M. N. G. & MATSUSHIMA, M. (1973). *Acta Cryst.* B**29**, 838–846.
JELLINEK, F. (1957). *Acta Cryst.* **10**, 277–280.
JOHNSON, C. K. (1965). *ORTEP.* Report ORNL-3794. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
KIER, L. B. (1967). *Mol. Pharmacol.* **3**, 487–494.
KIER, L. B. (1968). *Mol. Pharmacol.* **4**, 70–76.
MAKRIYANNIS, A., THEARD, J. M. & MAUTNER, H. G. (1979). *Biochem. Pharmacol.* **28**, 1911–1915.
MASON, R. & ROBERTSON, G. B. (1966). *Adv. Struct. Res. Diffr. Methods*, **2**, 57.
MOTHERWELL, S. (1979). *PLUTO.* Univ. Chemical Laboratory, Cambridge, England.
PULLMAN, B., COURRIÈRE, PH. & COUBEILS, J. L. (1971). *Mol. Pharmacol.* **7**, 397–405.
REED, K. W., MURRAY, W. J., ROCHE, E. B. & DOMELSMITH, L. N. (1981). *Gen. Pharmacol.* **12**, 177–185.
SCHULMAN, J. M., SABIO, M. L. & DISCH, R. L. (1983). *J. Med. Chem.* **26**, 817–823.
STEWART, J. M., KUNDELL, F. A. & BALDWIN, J. C. (1970). The *XRAY*70 system. Univ. of Maryland, College Park, Maryland.

Acta Cryst. (1984). B**40**, 434–436

Polytypism, Twinning, and Disorder in 2,2-Aziridinedicarboxamide

BY K. FICHTNER AND H. GRELL

Zentralinstitut für physikalische Chemie, Akademie der Wissenschaften der DDR, Rudower Chaussee 5, 1199 *Berlin, German Democratic Republic*

(*Received* 5 *December* 1983; *accepted* 8 *March* 1984)

Abstract

Some phenomena of $C_4H_7N_3O_2$ [Brückner (1982). *Acta Cryst.* B**38**, 2405–2408] are explained on the basis of the OD theory of Dornberger-Schiff. The symbol of the OD groupoid family is $Pba(2)|(n)x, y|(\bar{4})x', y'$ with parameters $x \approx \pm\frac{1}{6}$, $y \approx 0$ (or $x \approx 0$, $y \approx \pm\frac{1}{6}$); $x' \approx \pm\frac{1}{4}$, $y' \approx \pm\frac{1}{4}$. The net constants are $a = b \approx 12{\cdot}38$ Å; the distance between next-but-one layers is $c_o \approx 7{\cdot}55$ Å.

Introduction

The structures of two crystalline forms of the title compound have been reported by Brückner (1982): form *A*, $P4_12_12$, $a = 12{\cdot}382\ (2)$, $c = 30{\cdot}211\ (3)$ Å, $V = 4632$ Å^3, $Z = 32$; form *B*, $P1$, $a = 12{\cdot}381\ (2)$, $b = 12{\cdot}391\ (2)$, $c = 15{\cdot}829\ (2)$ Å, $\alpha = 74{\cdot}3\ (1)$, $\beta = 83{\cdot}1\ (1)$, $\gamma = 90{\cdot}0\ (1)°$, $V = 2312$ Å^3, $Z = 16$ (axes of form *B* interchanged for better comparison with form *A*). The two crystals are formed by different stacking of layers; the layer symmetry is not extended to the whole packing (Brückner, 1982, p. 2405).

These are strong indications that the two crystals are order–disorder (OD) structures. The OD theory of Dornberger-Schiff (Dornberger-Schiff, 1956, 1979, 1982; Dornberger-Schiff & Grell-Niemann, 1961) explains the existence of different stacking variants of a layered structure by partial symmetry operations, which are valid for any single layer but do not extend to its adjacent layers. The different structures are built up of the same kind of layer and the same pairs of layers; they differ, however, in triples and higher *n*-tuples of layers.

The aim of this paper is to explain polymorphism, twinning and apparent disorder and to describe the stacking possibilities of $C_4H_7N_3O_2$.

The family of OD structures according to form *A*

Fig. 1 shows a layer of form *A* consisting of hydrogen-bonded molecules arranged in rows along the *x* direction (*P* layer). There are also layers with rows along the *y* direction (*Q* layer). The unit cell consists of eight layers with the following sequences in the *z* direction: $P_1P_2Q_3Q_4P_5P_6Q_7Q_8$. Obviously, there are two types of layer pairs: *PP* and *QQ* on the one hand, and *PQ* and *QP* on the other. Consecutive pairs of the same type are transformed into each other by the 4_1 space-group transformation. The layers have a square net,

0108-7681/84/040436-03$01.50