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On the Conformational Varieties of Acetylcholine in the Crystals of its Halides

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Different conformations of acetylcholine are observed in the crystals of its chloride (*trans–gauche*, *tg*) and bromide (*gauche–gauche*, *gg*). Quantum-mechanical computations predict the *tg* conformation to be intrinsically preferred for the free molecule. Therefore there is obviously an influence of the crystalline environment upon the intramolecular conformation. The intermolecular lattice energy has been computed for the experimental crystals and a number of hypothetical ones, obtained by varying the conformation of acetylcholine or by exchanging the two anions. The computations point to two kinds of result: (*a*) if we modify the conformation of acetylcholine without drastically altering the lattice configuration each experimental crystal structure appears as the most stable one; (*b*) if, however, we allow important changes in the lattice configurations the bromide-type structure appears as the most stable, for both the Cl^- and Br ions and, moreover, with a *gg* conformation for acetylcholine. The structure of the acetylcholine chloride crystal thus seems to correspond to a local minimum of the energy hypersurface. It is proposed that this situation may be explained by considering the crystallization process and the properties of microcrystals. It appears possible that: (*a*) the two different crystal structures associated with the chloride and the bromide could be stable for the corresponding microcrystals; and/or (*b*) a structure of the chloride type would be energetically preferred for microcrystals in both cases but a higher probability of crossing over the potential barrier in the case of Br^- would make possible the transition to the bromide-type structure in the course of crystal growth.

1. Introduction

The theoretical and experimental study of the conformation of acetylcholine has been the subject of a large number of investigations (for a review see, for example, Pullman, 1977). These studies centred on the values of the two essential torsion angles (Fig. 1) {τ_1 [C(6)–O(1)–C(5)–C(4)] and τ_2 [O(1)–C(5)–C(4)–N^+]} which define the overall conformation of this important intercellular affector of the nervous transmission system.

Experimentally an interesting problem is raised by the different conformations adopted by acetylcholine in the crystals of its different halides: acetylcholine is *trans–gauche* ($\tau_1 = -166{\cdot}9$, $\tau_2 = 84{\cdot}7°$) in the crystal of its chloride (Herdklotz & Sass, 1970) and *gauche–gauche* ($\tau_1 = 78{\cdot}9$, $\tau_2 = 78{\cdot}4°$) in the crystal of its bromide (Sørum, 1959; Svinning & Sørum, 1975). Recent work has shown that it is also *gauche–gauche* ($\tau_1 = \pm 83$, $\tau_2 = \pm 89°$) in the crystal of its iodide (Jagner & Jensen, 1977).

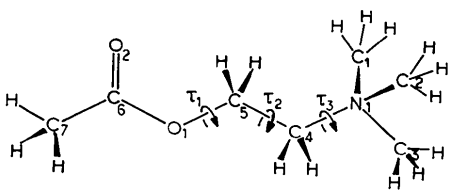

Fig. 1. Atom numbering and torsion angles of interest in acetylcholine.

From the theoretical viewpoint, quantum-mechanical computations carried out by the PCILO method for the first two halides (Pullman & Courriere, 1972) have shown that the global energy minimum for acetylcholine based on its geometry in the crystal of the chloride corresponds well to a *trans–gauche* conformation, close to the experimental one. In contrast, the conformational-energy map, constructed with the geometry of acetylcholine as in the crystal of its bromide, indicates that the experimental *gauche–gauche* conformation corresponds only to a local energy minimum, about 2 kcal mol^{-1} above the global one (associated with a different *gauche–gauche* conformation). The calculations also indicate that fundamentally the structure of acetylcholine derived from the crystal of its chloride is slightly more stable (1–2 kcal mol^{-1}) than that derived from the crystal of the bromide.

The aim of this paper is to account for the effect of the crystalline environment on the conformation of acetylcholine. This work is thus a continuation of our previous studies on similar problems related to phenethylamines (Caillet, Claverie & Pullman, 1976) and indolalkylamines (Caillet, Claverie & Pullman, 1977). *A priori*, the present case appears especially delicate, since the conformational change of acetylcholine and the structural changes in the crystal occur merely by changing the co-crystallizing ion (Cl^- or Br^-).

2. Method

The computation of the crystal lattice energies has been carried out with the same procedure as in our previous studies (Caillet & Claverie, 1974, 1975; Caillet, Claverie & Pullman, 1976, 1977, 1978). We shall not repeat here the detailed description of this procedure. We simply recall that the interaction energy is obtained as a sum of three long-range contributions (electrostatic, polarization, dispersion) and a short-range repulsive one; the electrostatic, dispersion and short-range contributions are pair-wise additive (between molecules) and these contributions pertaining to a given pair of molecules are obtained as a sum of atom–atom terms. The polarization energy is not pair-wise additive but is obtained as the sum of the polarization energies of each molecule, polarized by the electric field due to all other molecules.

The peculiar problem in the present study was to determine appropriate parameters for the atom–atom potentials involving one of the ions Cl^- or Br^-. Indeed, since the only difference between the two crystals to be studied is the nature of the ion co-crystallizing with acetylcholine, the difference between the crystal structures may ultimately be attributed to the change of the interaction involving the negative ions. Hence it is necessary to represent these interactions as accurately as possible in order to elucidate the structural differences in which we are interested. The values used previously for Cl^- (Caillet, Claverie & Pullman, 1977) were obtained by a rough extrapolation from the parameters of the S atom, but no such simplification seemed possible for Br^-. We therefore decided to reconsider the problem from a broader viewpoint. In the present paper we shall give only a brief summary of our procedure for obtaining the parameters, and the relevant values; a full account will be published elsewhere.

Basically we use the theoretical energy values computed by Kim & Gordon (1974) for the interactions between rare-gas atoms (He,Ne,Ar,Kr) themselves, and between them and the monovalent ions (Li^+,Na^+,K^+; F^-,Cl^-). The dispersion and short-range repulsion are represented by means of a Buckingham-type formula

$$E(i,j) = K_{ij}\left[-\frac{A}{z^6} + C\exp(-\alpha z)\right] \quad (1)$$

where

$$z = R_{ij}/R^0_{ij}. \quad (2)$$

$A = -0{\cdot}214$, $C = 47\,000$ [corresponding to $E(i,j)$ expressed in kcal mol^{-1}] and $\alpha = 12{\cdot}35$ do not depend on the atomic species (i,j) involved. Thus we only need to determine the two parameters K_{ij} and R^0_{ij} for each pair of species (i,j). As a general rule, we try to express them in terms of atomic parameters k_i, R^W_i:

$$R^0_{ij} = \sqrt{[(2R^W_i)(2R^W_j)]} \quad (3)$$

$$K_{ij} = k_i k_j \quad (4)$$

[concerning (3), see, for example, Good & Hope (1970), and concerning (4) see, for example, Kitaigorodskii, Mirskaya & Nauchitel' (1969)]. In the present work we first determined pair parameters K_{ij}, R^0_{ij}, and then atomic parameters suitable at least for the interactions between the ions and the Ne atom (considered as representative of the second-row atoms C, N, O). The procedure basically consists of determining the distance R^{min}_{ij} and the energy E^{min}_{ij} corresponding to the dispersion + repulsion-energy curve deduced from the results of Kim & Gordon (1974) (these authors give the total interaction energy, from which the dispersion + repulsion part is obtained by subtracting the polarization energy of the rare gas by the ion). Then, from the two values R^{min}_{ij} and E^{min}_{ij}, the two parameters R^0_{ij} and K_{ij} are easily obtained for each pair (i,j). In fact we used values of E^{min}_{ij} modified with respect to the theoretical values, the correction factors being obtained from a comparison between the theoretical and experimental values of E^{min}_{ij} in the case of the rare-gas–rare-gas interactions. The detailed results are given in Tables 1 and 2. Practically, we propose for the interactions between the ions and the (first- and) second-row atoms:

$$R^W_{Na^+} = 1{\cdot}2,\ R^W_{K^+} = 1{\cdot}46,\ R^W_{F^-} = 1{\cdot}9,\quad R^W_{Cl^-} = 2{\cdot}5 \quad (5)$$

and

$$k_{Na^+} = 1{\cdot}4,\quad k_{K^+} = 2{\cdot}9,\quad k_{F^-} = 0{\cdot}92,\quad k_{Cl^-} = 1{\cdot}1. \quad (6)$$

The interaction energy of Br^- with the rare gases is not given by Kim & Gordon (1974). Rather than perform the relatively involved computation according to their method (the results of which would have required some modification concerning the minimum-energy values, as mentioned above), we preferred to proceed in a simpler way by extrapolation from the parameters obtained above for the first two halogen ions F^- and Cl^-, and by

Table. 1. *Values of the parameters R^0_{ij} for the interactions between ions and rare gases, and corresponding values (in parentheses) of the parameters R^W_i for the ions [$2R^W_i = (R^0_{ij})^2/2R^W_j$, where i corresponds to the ion and j to the rare gas]*

	He	Ne	Ar	Kr
Na^+	2·506	2·548	3·103	3·336
	(1·228)	(1·172)	(1·448)	(1·578)
K^+	2·765	2·822	3·151	3·317
	(1·495)	(1·438)	(1·493)	(1·559)
F^-	2·998	3·246	3·239	3·359
	(1·759)	(1·903)	(1·578)	(1·599)
Cl^-	3·425	3·731	3·504	3·548
	(2·295)	(2·515)	(1·847)	(1·785)

also using the known interaction energies between Kr (which is isoelectronic with Br^-) and the other rare gases. We are thus led to propose:

$$R^W_{Br^-}(Ne) = 2{\cdot}8, \quad k_{Br^-}(Ne) = 1{\cdot}306 \qquad (7)$$

where this value of k_{Br^-} corresponds to $K(Br^- \cdots Ne) = 1{\cdot}256$, and the label Ne means that these parameters are suitable for interactions of Br^- with Ne and, therefore, second-row atoms (we also use them with the H atom), but they should eventually be modified for interactions of Br^- with atoms beyond the second row.

Similar calculations performed for the interactions with He and Ar instead of Ne led us to the values $K(Br^-\text{–He}) = 0{\cdot}5123$ [hence $k_{Br^-}(He) = 0{\cdot}864$] and $K(Br^-\text{–Ar}) = 2{\cdot}992$ [hence $k_{Br^-}(Ar) = 1{\cdot}372$]. It must be emphasized, however, that these evaluations were made by using the same value (2·8) for $R^W_{Br^-}$, which corresponds in principle to interactions with Ne. It would be possible to evaluate slightly modified values of $R^W_{Br^-}$ adapted to He and Ar, respectively, hence obtaining correspondingly modified values of the parameters K and k for the interactions $(Br^- \cdots He)$ and $(Br^- \cdots Ar)$, but such refinements were not needed for the present applications, where we used the values of $R^W_{Br^-}$ and k_{Br^-} given by equation (7).

3. Results and discussion

As in our previous papers on the effect of crystalline environment on molecular conformations (Caillet, Claverie & Pullman, 1976, 1977) we proceed by calculating the minimum energy of the crystal, adopting different conformations for acetylcholine.

(*A*) *Crystalline conformations*

(1) *Acetylcholine bromide*; *gauche–gauche* (*gg*) *conformation.* During the process of minimization, we maintain the cell angles α and γ at their initial values of 90°. The angle β varies. Thus the symmetry elements remain those for a monoclinic crystal lattice. The energy value varies very slowly and there is a minimum at −151 kcal mol^{-1}. The acetylcholine cation is slightly displaced from its experimental position and the crystal is slightly enlarged with respect to its initial dimensions (Table 3); the energy reduction with respect to the initial (experimental) crystal configuration is not large (from −146·26 to −151 kcal mol^{-1}), as should be expected.

(2) *Acetylcholine chloride*; *trans–gauche* (*tg*) *conformation.* In this case, with the three angles α, β and γ maintained at 90°, the minimum energy (−136·9 kcal mol^{-1}) is very close to the value corresponding to the experimental configuration (−134 kcal mol^{-1}). Again,

Table 2. *Values of the parameters K_{ij} for the interactions between ions and rare gases, and corresponding values (in parentheses) of the parameters k_i for the ions ($k_{ion} = K_{ion-rare\,gas}/k_{rare\,gas}$)*

	He	Ne	Ar	Kr
Na^+	0·504	1·724	2·1286	2·086
	(0·849)	(1·426)	(1·0007)	(0·813)
K^+	1·015	3·556	4·943	5·544
	(1·709)	(2·941	(2·324)	(2·162)
F^-	0·535	1·160	4·277	5·258
	(0·902)	(0·959)	(2·011)	(2·050)
Cl^-	0·658	1·376	5·684	7·840
	(1·107)	(1·138)	(2·672)	(3·056)

Table 3. *Detailed geometrical results of the energy minimizations concerning acetylcholine*

The direction cosines of the rotation axis and the translation coordinates are given with respect to an orthonormal lattice cell **a, b, c**.

Experimental crystalline conformation of acetylcholine bromide (*gg*): $\tau_1 = 78{\cdot}9$, $\tau_2 = 78{\cdot}44°$

Cell parameters	$a = 11{\cdot}13$ A	$\alpha = 90{\cdot}0°$	
	$b = 13{\cdot}46$	$\beta = 108{\cdot}6$	
	$c = 7{\cdot}30$	$\gamma = 90{\cdot}0$	
Rotation angle	13·9°	Translations	0·39 Å
Rotation axis	−0·46		−0·62
	0·89		0·03
	0·0		

Experimental crystalline conformation of acetylcholine chloride (*tg*): $\tau_1 = -166{\cdot}9$, $\tau_2 = 84{\cdot}7°$

Cell parameters	$a = 10{\cdot}5$ A	$\alpha = 90{\cdot}0°$	
	$b = 15{\cdot}1$	$\beta = 90{\cdot}0$	
	$c = 6{\cdot}38$	$\gamma = 90{\cdot}0$	
Rotation angle	6·3°	Translations	0·17 Å
Rotation axis	0·3		−0·11
	−0·08		0·80
	0·9		

Hypothetical crystalline conformation of acetylcholine bromide (*tg*): $\tau_1 = -166{\cdot}9$, $\tau_2 = 84{\cdot}7°$

Cell parameters	$a = 11{\cdot}6$ A	$\alpha = 90{\cdot}0°$	
	$b = 15{\cdot}3$	$\beta = 117{\cdot}39$	
	$c = 7{\cdot}5$	$\gamma = 90{\cdot}0$	
Rotation angle	3·20°	Translations	−0·05 Å
Rotation axis	−0·17		1·05
	−0·13		0·03
	−0·97		

Hypothetical crystalline conformation of acetylcholine chloride (*gg*): $\tau_1 = 78{\cdot}9$, $\tau_2 = 78{\cdot}44°$

Cell parameters	$a = 10{\cdot}5$ A	$\alpha = 90{\cdot}0°$	
	$b = 19{\cdot}6$	$\beta = 90{\cdot}0$	
	$c = 7{\cdot}3$	$\gamma = 90{\cdot}0$	
Rotation angle	34·6°	Translations	0·49 Å
Rotation axis	−0·27		−0·88
	0·20		−0·13
	0·94		

the cell is slightly enlarged, but the acetylcholine cation is only very slightly displaced.

(*B*) *Hypothetical crystalline conformations*

(1) *trans–gauche conformation in the bromide crystal.* In this case we change the conformation of acetylcholine from *gg* to *tg*. We have maintained the cell angles at their initial values. The cell is enlarged in the **b** direction, which corresponds to a lengthening of the molecule accompanying the new conformation. The energy minimum amounts to $-145{\cdot}3$ kcal mol^{-1} with relatively important displacements of the two molecules in the **b** direction.

(2) *gauche–gauche conformation in the chloride crystal.* In this case the minimization leads to a shortening of the cell in the **b** direction and to an energy of $-136{\cdot}7$ kcal mol^{-1}.

When we consult the conformational-energy map obtained for free acetylcholine by the *ab initio* self-consistent-field molecular-orbital method with an STO 3G basis set (Pullman & Port, 1973), we can see that the two conformations studied above do not correspond to the global minimum. The conformation of the chloride is on a plateau embodying this minimum but about 2 kcal mol^{-1} above it, while the conformation of the bromide is 4 kcal mol^{-1} above that minimum. When we take into account the contribution of these conformational energies, we obtain the results presented in Table 4.

Thus, by considering only conformational changes of acetylcholine (from *tg* to *gg* or conversely) in the two kinds of crystal configurations (chloride or bromide), we actually find in both cases that the experimental structure is the most stable.

However, other structural changes are possible: we may consider hypothetical crystal structures for acetylcholine chloride similar to the experimental structure of acetylcholine bromide, and conversely. Such structures may be easily generated by replacing the Br^- ion of an acetylcholine bromide structure by a Cl^- ion, and conversely. Thus, from the four structures considered in Table 4, we generate four structures which, after the minimization of the lattice energy, lead to the results given in Table 5.

Table 4. *Lattice energy and conformational energy for crystals of acetylcholine*

Symbols: exp: experimental; hyp: hypothetical.

Crystal		Conformation of the acetylcholine cation τ_1 (°)	τ_2 (°)	Total lattice energy (kcal mol^{-1})	Conformational energy with respect to the STO 3G minimum (kcal mol^{-1}) ($\tau_1 = 150$, $\tau_2 = 60°$)	Lattice energy + conformational energy (kcal mol^{-1})
Acetylcholine bromide (hyp)	*tg*	$-166{\cdot}9$	$-84{\cdot}7$	$-145{\cdot}3$	1·5	$-143{\cdot}8$
Acetylcholine bromide (exp)	*gg*	78·9	78·44	-151	3	-148
Acetylcholine chloride (exp)	*tg*	$-166{\cdot}9$	84·7	$-136{\cdot}9$	1·5	$-135{\cdot}4$
Acetylcholine chloride (hyp)	*gg*	78·9	78·44	$-136{\cdot}7$	3	$-133{\cdot}7$

Table 5. *Lattice energy and conformational energy for hypothetical crystals of acetylcholine (obtained by replacing* Cl^- *by* Br^- *and conversely in the structures considered in Table* 4)

Hypothetical crystal		Conformation of the acetylcholine cation τ_1 (°)	τ_2 (°)	Total lattice energy (kcal mol^{-1})	Conformational energy (kcal mol^{-1})	Lattice energy + conformational energy (kcal mol^{-1})
Acetylcholine bromide (with the acetylcholine chloride crystal configuration)	*tg*	$-166{\cdot}9$	84·7	$-126{\cdot}85$	1·5	$-125{\cdot}35$
Acetylcholine bromide (with the acetylcholine chloride crystal configuration)	*gg*	78·9	78·44	$-125{\cdot}7$	3	$-122{\cdot}7$
Acetylcholine chloride (with the acetylcholine bromide crystal configuration)	*tg*	$-166{\cdot}9$	84·7	$-149{\cdot}1$	1·5	$-147{\cdot}6$
Acetylcholine chloride (with the acetylcholine bromide crystal configuration)	*gg*	78·9	78·44	$-197{\cdot}0$	3	$-194{\cdot}0$

We now obtain the rather remarkable result that, whatever the conformation of acetylcholine (*tg* or *gg*), the 'bromide-type' crystal structure systematically appears markedly more stable (lower negative energy values) than the 'chloride-type' structure for *both* the Cl^- and Br^- ions. (In the course of the minimization corresponding to the fourth line of Table 5 the lattice energy remained almost stable at -160 kcal mol^{-1} for some time, before finally falling into the potential well at -197 kcal mol^{-1}; but the previous statement would remain perfectly valid even by retaining the intermediate almost stabilized value of -160 kcal mol^{-1}.)

These results present no probelm for the acetylcholine bromide crystal: they confirm the greater stability of the experimental crystal structure, and may be considered as a further example of the possibility that the requirement for higher crystal stability may easily override the requirement for higher conformational stability of the molecules of the crystal, as emphasized previously (Caillet, Claverie & Pullman, 1976, 1977, 1978). A problem now appears for the experimental acetylcholine chloride crystal: why does it exhibit a structure with a more stable conformation of acetylcholine (*tg*) but a less favourable lattice energy, instead of changing to a structure similar to that of acetylcholine bromide, involving a less stable conformation of acetylcholine (*gg*) but a much more favourable lattice energy?

A possible answer to this question may involve the explicit consideration of the crystallization process. The free acetylcholine molecules should exhibit a *trans–gauche* conformation, according to the quantum-mechanical calculations (Pullman & Port, 1973; Port & Pullman, 1973). It seems plausible, therefore, that when microcrystals begin to appear, they first contain acetylcholine molecules with such a conformation. In fact, for a macrocrystal, even by restricting ourselves to the *tg* conformation, we find the bromide-type structure more stable than the chloride type ($-147{\cdot}6$ and $-135{\cdot}4$ kcal mol^{-1} for the Cl^- ion, $-143{\cdot}7$ and $-126{\cdot}8$ kcal mol^{-1} for the Br^- ion respectively). The situation may well be different for a microcrystal, and it is already interesting that the difference between the two structures is less for Cl^- ($-12{\cdot}2$ kcal mol^{-1}) than for Br^- ($-16{\cdot}9$ kcal mol^{-1}). These differences, which correspond essentially to the different sizes of the ions, suggest the possibility that a microcrystal of acetylcholine chloride could preferentially exhibit the chloride-type structure rather than the bromide-type; then, upon continuous growth of such a microcrystal, we would get a macrocrystal exhibiting the same structure, corresponding to a *local minimum* of the energy hypersurface, instead of its *absolute* minimum. That such a situation may occur is proved by the existence for *p*-cresol (Perrin & Thozet, 1974) of several crystal structures: one at most may be at the absolute energy minimum, the others necessarily correspond to local minima of the energy hypersurface.

In an attempt to assess the relative stability of such a local minimum in our problem, we considered some orientational and conformational changes of acetylcholine with respect to the chloride-type structure, and then performed restricted minimizations of the energy. More precisely, we considered two 'paths' in the conformation–configuration space, starting from the acetylcholine chloride structure (denoted Cl–*tg*) towards the bromide-type crystal configuration, either with the *tg* or *gg* conformation of acetylcholine (denoted respectively Br–*tg* and Br–*gg*). For this purpose we first determined the parameters of the geometrical transformation which carries acetylcholine from the Cl–*tg* position to those for Br–*tg* and Br–*gg*. In fact, we are interested only in the rotation part as the translation is found to be small, and anyway the translational degrees of freedom will be left free in the subsequent minimization. These rotation parameters (components of a vector defining the rotation axis **A**, and rotation angle ω) are:

$$\mathbf{A}\ (0{\cdot}6552; -0{\cdot}4882; 0{\cdot}5765), \quad \omega = 169°. \qquad (8)$$

This rotation is first applied to the whole acetylcholine molecule in both cases (Cl–*tg* → Br–*tg* and Cl–*tg* → Br–*gg*). In the second case, the conformational change must be performed afterwards, namely a rotation of the cationic head C(4)N(1)C(1)C(2)C(3) around O(1)–C(5) by an angle $\Delta\tau_1 = 246°$ (from $-166{\cdot}9$ to $78{\cdot}9°$).

For both changes (Cl–*tg* to Br–*tg* and Cl–*tg* to Br–*gg*), we define continuous paths by considering the rotations with angles $\lambda\omega$ and $\lambda\Delta\tau_1$, where λ varies from 0 to 1 [always with the same rotation axes **A** and O(1)–C(5)]. For every given rotation (fixed λ), we may minimize the lattice energy with respect to the lengths (a,b,c) of the unit-cell vectors and the translational degrees of freedom of both acetylcholine and the halogen ion. In principle we should also minimize with respect to the angles of the unit cell (which are all equal

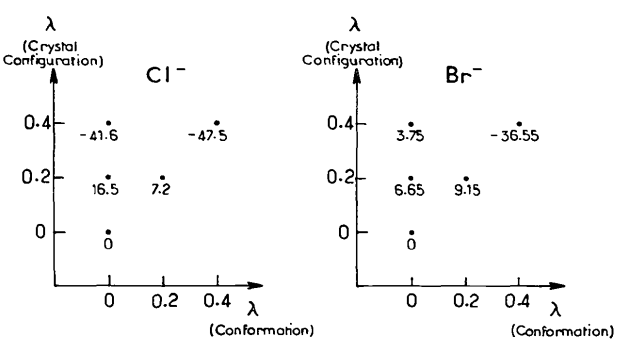

Fig. 2. Relative total energy (lattice + conformational) in kcal mol^{-1} for crystals of acetylcholine chloride and bromide in the neighbourhood of the structure given by experiment for acetylcholine chloride (this structure is taken in both cases as the origin for energies; its actual energy is $-136{\cdot}9$ kcal mol^{-1} with Cl^- and $-128{\cdot}35$ kcal mol^{-1} with Br^-).

to 90° in the initial structure Cl–*tg*, *i.e.* when $\lambda = 0$), but this would imply further difficulties since the symmetry elements would then be modified. Since we intend to consider only some structures not too far from Cl–*tg*, we decided to keep for simplicity $\alpha = \beta = \gamma = 90°$. Then, along each of the two paths defined above (namely from Cl–*tg* to Br–*tg* and from Cl–*tg* to Br–*gg*), we calculated the energy (minimized as indicated above) for $\lambda = 0{\cdot}2$ and $\lambda = 0{\cdot}4$, and for both ions Cl^- and Br^- (in practice it is not possible to consider a large number of values of λ, owing to the relatively large amount of computer time required by each minimization process).

The results are given in Fig. 2. For the sake of clarity, the energy of the initial structure Cl–*tg* has been taken as the reference in both cases; the actual energy values for this reference structure are respectively −136·9 for Cl^- and −128·35 kcal mol^{-1} for Br^-. In all cases leaving the Cl–*tg* structure (towards either Br–*tg* or Br–*gg*) requires crossing a potential barrier, which actually implies the possibility for a crystal to remain trapped in the Cl–*tg* structure if it began to grow with this structure. However, from a quantitative point of view, the barrier corresponding to the path Cl–*tg* to Br–*tg* (namely changing the lattice configuration without changing the conformation of acetylcholine) appears much higher with the Cl^- ion (16·5 kcal mol^{-1} at $\lambda = 0{\cdot}2$) than with the Br^- ion (6·65 kcal mol^{-1} at $\lambda = 0{\cdot}2$). The larger size of the Br^- ion may be a partial explanation: the molecules may lie at slightly greater distances and therefore be less 'geared' one with another; hence the possibility for easier rearrangement. Along the other path Cl–*tg* to Br–*gg* (both lattice configuration and acetylcholine conformation modified simultaneously), the barrier values (at $\lambda = 0{\cdot}2$) are intermediate between the previous ones, and in the reverse order (7·2 kcal mol^{-1} with Cl^- and 9·15 kcal mol^{-1} with Br^-). The results are anyway less significant since the other possibility for conformational change, namely using $\Delta\tau_1 = -114°$ instead of 246°, should also be considered. A more accurate analysis would also require smaller steps for the path parameter λ. From the results displayed in Fig. 2, it seems safer to draw only the qualitative conclusion that the barrier to be crossed for leaving the Cl–*tg* structure appears somewhat lower with the Br^- ion (6·55 kcal mol^{-1}) than with the Cl^- ion (7·2 kcal mol^{-1}). Thus, these results may be taken as a hint that leaving the Cl–*tg* structure could be easier in the case of acetylcholine bromide than in the case of acetylcholine chloride.

4. Conclusion

The problem of the structure of crystals of acetylcholine with various halogen counter-ions appears to us rather difficult. We study in the present work the cases of Cl^- and Br^- but it must be remembered that the experimental structure of acetylcholine iodide has also been determined recently (Jagner & Jensen, 1977). The difficulty of the problem is not unexpected, if we note that the only difference between these various crystals is the nature of the negative counter-ion. According to theoretical calculations, the most stable structure of isolated acetylcholine is *trans–gauche* (*tg*), while both *trans–gauche* and *gauche–gauche* (*gg*) conformations are observed in crystals. In fact, Jagner & Jansen (1977) have compiled (see their Table 3) structural data for a number of crystals of choline esters; these data suggest that in the presence of the Cl^- ion the *tg* conformation is preferred, while with larger ions (Br^- and I^-) it is preferably *gg*.

In our theoretical study of the crystals of acetylcholine chloride and bromide, two kinds of results appear: (1) If we modify the conformation of acetylcholine (*tg* → *gg* for chloride, and *gg* → *tg* for bromide) without altering drastically the lattice configuration, each experimental crystal structure (*tg* with Cl^-, *gg* with Br^-) actually appears as the most favourable one. (2) If we allow important changes of the lattice configuration, a different situation occurs; namely, an acetylcholine bromide type structure appears to be the most stable with both the Cl^- and the Br^- ion. Moreover, in both cases also, the *gg* conformation of acetylcholine leads to the most stable structure (this actually agrees with the experimental structure of acetylcholine bromide, as pointed out above).

Of course, we cannot claim that our theoretical formulae are exactly accurate, but the rather large energy differences between the chloride-type and bromide-type structures make it unlikely that this result could be qualitatively wrong.

Thus, if we accept the qualitative validity of our theoretical data, we must conclude that the acetylcholine chloride crystal structure corresponds to a *local* minimum of the energy hypersurface, while the acetylcholine bromide structure would correspond to a deeper minimum (the absolute one?). The question which now arises concerns the factors which lead to the choice of one or the other of these structures, and the investigation of this problem seems to require the study of the crystallization process, and more especially of the structure of microcrystals. It is indeed clear that if a microcrystal may exist with some structure of the acetylcholine chloride type, the crystal growth may proceed with this structure, finally leading to a crystal 'trapped' in this 'local-minimum' structure. Thus two factors appear: the energetic factor (relative stability of the various possible structures of microcrystals) and the entropic factors (among which we may consider more especially the probability of jumping over the potential barriers surrounding the various minima of the energy hypersurface). As a contribution to the study of this last point, we considered some crystal structures in the neighbourhood of the acetylcholine chloride structure

and found some indication that the height of the potential barrier surrounding this local minimum could indeed be smaller in the case of the Br^- ion than in the case of the Cl^- ion, so that the probability of leaving this local minimum under the effect of thermal fluctuations would indeed be larger in the former case.

Thus, two non-exclusive possibilities exist for explaining the different crystal structures of acetylcholine chloride and bromide: (*a*) different structures would be stable for the corresponding microcrystals and (*b*) a structure of the chloride type would be *energetically* preferred for *microcrystals* in both cases, but a higher probability of crossing the (lower) potential barrier in the case of Br^- would make possible the transition to the bromide-type structure (energetically more stable for a macrocrystal) in the course of crystal growth. If we consider that a lower barrier in the case of Br^- is related to the larger size of this ion with respect to Cl^- (this larger size resulting in molecules less geared one with another, and therefore in easier molecular rearrangements of the lattice), we could expect, for a crystal containing I^-, properties similar to those exhibited by crystals containing Br^-, in agreement with the structural data of Jagner & Jensen (1977). This work also gives further hints concerning the existence of several possible structures and the difficulty for the crystal of 'choosing' between them in some instances: acetylcholine iodide actually crystallizes with a partially disordered structure (ordered layers whose mode of stacking is disordered), statistical disorder exists in the crystals of acetylcholine resorcylate and acetylcholine (+)-ditartrate, and two conformations of acetylcholine (one *trans–gauche* and one *gauche–gauche*) even coexist in the crystal of bis(acetylcholine) tetrabromodioxouranium. Such facts may be qualitatively understood if there actually exist several locally stable structures of the crystal lattice. Examples of such situations have been reported by Perrin & Thozet (1974) for *p*-cresol. Altogether the conformation of acetylcholine in these various structures confirms the situation found in our previous studies on adrenaline (Caillet, Claverie & Pullman, 1976), serotonin and 5-methylated bufotenine (Caillet, Claverie & Pullman, 1977), namely that the requirement of minimizing the lattice energy overrides the requirement of minimizing the conformational energy, so that there is nothing surprising in the existence of different conformers in different crystal structures. The more difficult problem seems to be to understand the existence of these various lattice structures, rather than the conformational changes which appear as mere consequences of the changes of these structures.

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