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Conformational Analysis of Acetylcholine and Related Choline Esters

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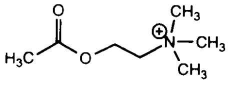
Abstract

The crystal structures of carbamoylcholine [2-(carbamoyloxy)-*N*,*N*,*N*-trimethylethanaminium] chloride, bromide and iodide, methoxycarbonylcholine [2-(methoxycarbonyloxy)-*N*,*N*,*N*-trimethylethanaminium] iodide, acetylcholine [2-(acetyloxy)-*N*,*N*,*N*-trimethylethanaminium] chloride and succinylcholine {2,2′-[(1,4-dioxo-1,4-butanediyl)bis(oxy)]bis(*N*,*N*,*N*-trimethylethanaminium)} iodide have been redetermined at 105 K in order to obtain detailed and accurate information on the geometry of choline esters and to elucidate the conformationally dependent changes of geometry. The conformational flexibility and the preferred conformations are elucidated based on results obtained from X-ray crystallographic studies and molecular mechanics (MM2) calculations. The usefulness of molecular mechanics calculations for quaternary ammonium ions is discussed.

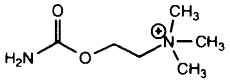
1. Introduction

Acetylcholine is an endogenous neurotransmitter and functions in many parts of both the central and the peripheral nervous system. Over the years a multitude of studies has dealt with acetylcholine with the purpose of obtaining information on the detailed mechanism of the interaction of acetylcholine with the cholinergic receptors. The three-dimensional structure of the receptors is not known and therefore models for the interaction between the neurotransmitter and the receptor are of great interest. The crystallographic studies of salts of choline esters were performed in order to obtain information on the geometry of the compounds and preferred contacts to the surrounding molecules or ions. This information can contribute to the reliability of the receptor models.

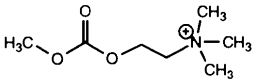
Structure determinations of acetylcholine, carbamoylcholine and methoxycarbonylcholine salts have previously been performed, but they are not very accurate as X-ray data were collected at room temperature and large displacement parameters were observed (Jensen, 1984, and references therein, *cf.* Table 1). The crystal structures of the compounds selected for this paper have been redetermined at low temperature in order to obtain more accurate information on the geometry of the choline esters. The six compounds are carbamoylcholine chloride (CARCHL), carbamoylcholine bromide (CARBRO), methoxycarbonylcholine iodide (MECOLI), carbamoylcholine iodide (CARIOD), acetylcholine chloride (ACHCHL) and succinylcholineiodide (SUXIOD).



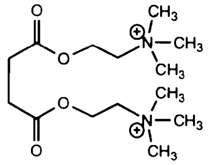
Acetylcholine



Carbamoylcholine



Methoxycarbonylcholine



Succinylcholine

(I)

These compounds were selected to include different choline esters and different conformations. Further, no large displacement parameters should have been found in the originally determined crystal structures. In the known crystal structures a larger variation in conformation has been observed for methoxycarbonylcholine and especially for carbamoylcholine than for acetylcholine itself. Acetylcholine has never been observed in the all-*trans* conformation. The different number of conformations observed in crystal structures may have two main reasons. Carbamoylcholine and methoxycarbonylcholine

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may, in reality, be more flexible ions than acetylcholine, or the packing forces may induce the differences. In order to address this question, molecular mechanics calculations have been performed.

Preliminary molecular mechanics calculations (MM2) have been used earlier in order to gain knowledge on the favourable conformations of acetylcholine and its analogues (Jensen, unpublished results). Programs for molecular mechanics calculations have, however, not been created to handle ionic compounds. Electrostatic interactions are in MM2 normally treated as dipole–dipole interactions. It is, however, possible to use individual point charges for all atoms and to replace the energy contribution from dipoles by the energy contribution from charge–charge interactions. Different sets of point charges for acetylcholine can be found in the literature or they can be calculated by different methods. *A priori*, however, it seems to be a difficult problem to decide which point charges to use. We found it of interest to examine if reliable results for acetylcholine, methoxycarbonylcholine and carbamoylcholine could be obtained by MM2 calculations, using different sets of point charges.

2. Experimental

2.1. *X-ray structure analyses*

The preparation of the six salts and the single crystals followed previously described procedures (for references see Table 1). ACHCHL is extremely hygroscopic and the crystal was therefore taken directly from the warm plate of a hot-plate microscope and into a cold stream of nitrogen. The crystal data and some details of the data collection and refinements are given in Table 1. The temperature (105 K) was recorded with a thermocouple; variations were within 1 K. Corrections for the observed decline were performed. Absorption corrections, based on measured crystal geometry, were made for CARBRO, MECOLI, CARIOD and SUXIOD. All H atoms were found in difference maps. The structures were refined by least-squares minimizing $\sum w(|F_o| - k|F_c|)^2$. The positional parameters for all atoms (except H atoms of CARBRO) and anisotropic displacement parameters for non-H atoms were varied. The weighting schemes used were chosen so that the average values of $\sum w(|F_o| - k|F_c|)^2$ for groups of reflections were approximately equal. A few very strong reflections with a high negative value of $(|F_o| - k|F_c|)$ were ignored in the refinements. The space groups of ACHCHL and SUXIOD are chiral. Refinements using the two alternative enantiomers gave no significant differences for the geometry, and as knowledge about the chirality of the crystals used has no interest, the final coordinates have arbitrarily been given so that they have close relations to those published for the structures studied at room temperature. Two papers on the structure determination at room temperature of SUXIOD have been published, one is cited in Table 1, the other by Mndzhoyan, Avoyan, Ayetisian & Arutyumian (1972). Calculations were performed by means of *XRAY*76 (Stewart *et al.*, 1976). For all structures, features in the final difference Fourier map were only found close to the halogenide ion.

2.2. *Molecular mechanics calculations*

The MM2(91) program (Burkert & Allinger, 1982) was accessed through the InStar Software (MacMimic, 1991) system on a Macintosh IIci. All molecular mechanics calculations were performed using the standard value of 1.5 for the dielectric constant. New force-field parameters had to be introduced in MM2 in order to permit calculations for the carbamoyl group and the carbonic acid ester group of the methoxycarbonylcholine ion. The geometry of these moieties was essentially unchanged throughout the calculations. The force-field parameters chosen were evaluated on the basis of their ability to reproduce relevant geometry as retrieved from the Cambridge Structural Database, Version 5.05, April 1993 (Allen *et al.*, 1991). Detailed information on the introduced parameters has been deposited.* In order to study the flexibility of the three ions, two torsion angles have been analysed systematically by the torsional drive technique, which is available in the MM2(91) program. The torsion angles C—O—C—C and O—C—C—N have been varied from 0 to 360° in increments of 10°. By this technique one torsion angle at a time is changed in discrete steps and the geometry is optimized with respect to all other variables in order to minimize the energy. The results have been visualized through a depiction of the potential energy surface, shown as a function of the above-mentioned torsion angles.

For one set of calculations (*A*) the electrostatic contribution was represented by the default dipole–dipole interactions (and thus no contribution from the charge of the ammonium group was included). For the remaining three sets of calculations (*B*, *C* and *D*), the electrostatic energy contribution was based on different sets of point charges. These sets of point charges were all calculated *via* the *SYBYL*6.03 molecular modelling system (Tripos Associates, Inc., 1992). Set (*B*) was calculated using the method described by Gasteiger & Marsili, (1980). Set (*C*) was calculated using the PM3 method (Stewart, 1990). Set (*D*) was calculated using the *ab initio* program *GAUSSIAN*80 UCSF, STO-3G basis set (Singh & Kollman, 1982).

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* Lists of structure factors, anisotropic displacement parameters, H-atom coordinates and complete geometry have been deposited with the IUCr (Reference: AB0341). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Experimental details*

	ACHCHL	CARBRO	CARCHL	CARIOD	MECOLI	SUXIOD
Crystal data						
Chemical formula	$C_7H_{16}ClNO_2$	$C_6H_{15}BrN_2O_2$	$C_6H_{15}ClN_2O_2$	$C_6H_{15}IN_2O_2$	$C_7H_{16}INO_3$	$C_{14}H_{30}I_2N_2O_4$
Chemical formula weight	181.66	227.11	182.65	274.10	289.12	544.21
Cell setting	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$Pnam$	$Pnam$	$P2_1/c$	$Pnam$	$P2_1$
a (Å)	9.890 (2)	10.350 (4)	10.179 (3)	5.919 (2)	10.267 (1)	12.803 (3)
b (Å)	15.324 (6)	13.028 (2)	12.781 (4)	12.573 (4)	15.305 (7)	8.165 (2)
c (Å)	6.302 (3)	6.816 (1)	6.713 (2)	13.643 (2)	6.873 (2)	9.612 (2)
β (°)	90.0	90.0	90.0	94.46 (2)	90.0	98.09 (2)
M.p. (K)	422–423	477–478	476–481[a]	466–468[a]	418–419[b]	523–528[c]
V (Å^3)	955 (1)	919.1 (8)	873.3 (8)	1012.2 (8)	1080.0 (9)	994.8 (6)
Z	4	4	4	4	4	2
D_x (Mg m^{-3})	1.263 (1)	1.641 (1)	1.389 (1)	1.793 (2)	1.778 (2)	1.817 (1)
D_m (Mg m^{-3}; flotation, 293 K)	*	1.60[d]	1.35[a]	1.74[a]	1.72[b]	1.76[c]
F(000)	392	464	392	536	568	532
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
No. of reflections for cell parameters	18	18	18	18	18	20
θ range (°)	18–22	18–22	18–22	18–22	18–22	18–22
μ (mm^{-1})	0.355	4.39	0.392	3.15	2.92	3.14
Temperature (K)	105	105	105	105	105	105
Crystal form	Prism	Prism	Prism	Prism	Prism	Needle
Crystal size (mm)	0.67 × 0.38 × 0.38	0.42 × 0.34 × 0.28	0.38 × 0.38 × 0.33	0.35 × 0.23 × 0.13	0.32 × 0.28 × 0.17	0.50 × 0.33 × 0.28
Crystal colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Data collection						
Diffractometer	Enraf–Nonius CAD-4	Enraf–Nonius CAD-4	Enraf–Nonius CAD-4	Enraf–Nonius CAD-4	Enraf–Nonius CAD-4	Enraf–Nonius CAD-4
Data collection method	ω–2θ	ω–2θ	ω–2θ	ω–2θ	ω–2θ	ω–2θ
Absorption correction	None	Empirical	None	Empirical	Empirical	Empirical
T_{min}	–	0.26	–	0.39	0.44	0.43
T_{max}	–	0.40	–	0.62	0.64	0.85
No. of measured reflections	6474	5077	5366	9121	5555	10024
No. of independent reflections	5627	3790	3799	8293	4691	8560
No. of observed reflections	4803	2736	3299	6671	3787	7740
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.03	0.07	0.02	0.02	0.02	0.07
θ_{max} (°)	45	44	45	45	45	45
Range of h, k, l	$-14 \rightarrow h \rightarrow 19$ $0 \rightarrow k \rightarrow 30$ $0 \rightarrow l \rightarrow 12$	$-13 \rightarrow h \rightarrow 20$ $0 \rightarrow k \rightarrow 25$ $0 \rightarrow l \rightarrow 13$	$-14 \rightarrow h \rightarrow 20$ $0 \rightarrow k \rightarrow 25$ $0 \rightarrow l \rightarrow 13$	$0 \rightarrow h \rightarrow 11$ $0 \rightarrow k \rightarrow 24$ $-26 \rightarrow l \rightarrow 26$	$-9 \rightarrow h \rightarrow 20$ $0 \rightarrow k \rightarrow 30$ $0 \rightarrow l \rightarrow 13$	$-25 \rightarrow h \rightarrow 24$ $0 \rightarrow k \rightarrow 16$ $0 \rightarrow l \rightarrow 19$
No. of standard reflections	3	3	3	3	3	3
Frequency of standard reflections	10 000	10 000	10 000	10 000	10 000	10 000
Intensity decay (%)	–	–	–	–	5	–
Refinement						
Refinement on	F	F	F	F	F	F
R	0.034	0.039	0.028	0.037	0.023	0.034
wR	0.038	0.038	0.042	0.043	0.027	0.040
S	3.3	1.4	1.3	1.0	0.6	0.7
No. of reflections used in refinement	4803	2724	3290	6642	3777	7734
No. of parameters used	149	64	88	145	95	288
H-atom treatment	Refined from difference maps	Calculated	Refined from difference maps	Refined from difference maps	Refined from difference maps	Refined from difference maps
Weighting scheme	$\sum w(\lvert F_o\rvert - k\lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - k\lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - k\lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - k\lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - k\lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - k\lvert F_c\rvert)^2$
$(\Delta/\sigma)_{max}$	0.07	0.03	0.06	0.09	0.05	0.03
$\Delta\rho_{max}$ (e Å^{-3})	0.7	1.3	0.7	2.6	1.1	2.7
$\Delta\rho_{min}$ (e Å^{-3})	−0.3	−1.6	−0.7	−2.4	−1.3	−2.7
Source of atomic scattering factors	*XRAY*76 (Stewart *et al.*, 1976)	*XRAY*76 (Stewart *et al.*, 1976)	*XRAY*76 (Stewart *et al.*, 1976)	*XRAY*76 (Stewart *et al.*, 1976)	*XRAY*76 (Stewart *et al.*, 1976)	*XRAY*76 (Stewart *et al.*, 1976)

References: (*a*) Jensen (1975); (*b*) Jensen (1979); (*c*) Jensen (1970); (*d*) Barrans (1971). * D_m not determined as crystals were extremely hygroscopic (Herdklotz & Sass, 1970).

Table 2. *Fractional atomic coordinates and equivalent isotropic displacement parameters* (Å²)

$U_{eq} = (1/3)\Sigma_i\Sigma_jU_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j.$

	x	y	z	U_{eq}
ACHCHL				
C1	0.0683 (1)	0.63609 (7)	0.7220 (2)	0.0196 (2)
C2	0.1224 (1)	0.56264 (7)	0.5900 (2)	0.0154 (1)
O3	0.2273 (1)	0.56407 (7)	0.4926 (2)	0.0243 (1)
O4	0.0389 (1)	0.49281 (5)	0.5915 (1)	0.0144 (1)
C5	0.0931 (1)	0.41692 (7)	0.4858 (2)	0.0175 (1)
C6	−0.0148 (1)	0.34800 (6)	0.4608 (2)	0.0139 (1)
N7	−0.1063 (1)	0.35610 (4)	0.2698 (1)	0.0108 (1)
C8	−0.2003 (1)	0.43239 (6)	0.2901 (2)	0.0157 (1)
C9	−0.0278 (1)	0.36363 (8)	0.0676 (2)	0.0182 (1)
C10	−0.1879 (1)	0.27333 (5)	0.2604 (2)	0.0141 (1)
Cl	0.15355 (2)	0.16582 (1)	0.23729 (3)	0.01449 (3)
CARBRO				
N1	0.4929 (2)	−0.1127 (1)	1/4	0.0150 (3)
C2	0.4148 (2)	−0.0304 (2)	1/4	0.0106 (2)
O3	0.2976 (2)	−0.0326 (1)	1/4	0.0139 (2)
O4	0.4835 (2)	0.0588 (1)	1/4	0.0125 (2)
C5	0.4023 (2)	0.1484 (2)	1/4	0.0118 (2)
C6	0.4977 (2)	0.2374 (2)	1/4	0.0104 (2)
N7	0.4355 (2)	0.3417 (2)	1/4	0.0089 (2)
C8	0.3551 (1)	0.3584 (1)	0.0698 (2)	0.0129 (2)
C10	0.5425 (2)	0.4201 (2)	1/4	0.0121 (3)
Br	0.82314 (2)	0.84196 (2)	1/4	0.01104 (4)
CARCHL				
N1	1.00135 (6)	0.37568 (4)	1/4	0.0117 (1)
C2	0.92247 (6)	0.45971 (4)	1/4	0.0084 (1)
O3	0.80264 (5)	0.45784 (4)	1/4	0.0115 (1)
O4	0.99180 (5)	0.55103 (4)	1/4	0.0106 (1)
C5	0.90859 (6)	0.64188 (4)	1/4	0.0099 (1)
C6	1.00422 (5)	0.73326 (4)	1/4	0.0087 (1)
N7	0.93935 (5)	0.83905 (4)	1/4	0.0072 (1)
C8	0.85724 (5)	0.85530 (3)	0.06741 (7)	0.0115 (1)
C10	1.04687 (6)	0.91989 (5)	1/4	0.0104 (1)
Cl	0.82700 (1)	0.15491 (1)	1/4	0.01077 (2)
CARIOD				
N1	0.9126 (3)	−0.0552 (2)	0.6124 (1)	0.0204 (3)
C2	0.7550 (3)	0.0208 (1)	0.5914 (1)	0.0163 (3)
O3	0.7243 (3)	0.0716 (1)	0.5146 (1)	0.0190 (3)
O4	0.6280 (3)	0.0332 (1)	0.6669 (1)	0.0187 (3)
C5	0.4595 (3)	0.1161 (2)	0.6555 (1)	0.0169 (3)
C6	0.3244 (3)	0.1123 (2)	0.7430 (1)	0.0163 (3)
N7	0.4457 (3)	0.1462 (1)	0.8417 (1)	0.0164 (2)
C8	0.6178 (4)	0.0643 (2)	0.8798 (2)	0.0240 (4)
C9	0.5554 (5)	0.2526 (2)	0.8359 (2)	0.0280 (4)
C10	0.2716 (4)	0.1536 (2)	0.9132 (2)	0.0240 (3)
I	−0.05588 (2)	0.15533 (1)	0.13640 (1)	0.01593 (2)
MECOLI				
C11	0.0120 (2)	0.4048 (1)	1/4	0.0169 (2)
O1	−0.0236 (1)	0.4964 (1)	1/4	0.0170 (1)
C2	0.0768 (2)	0.5512 (1)	1/4	0.0126 (1)
O3	0.1904 (1)	0.5323 (1)	1/4	0.0192 (1)
O4	0.0277 (1)	0.6322 (1)	1/4	0.0150 (1)
C5	0.1226 (2)	0.7020 (1)	1/4	0.0135 (1)
C6	0.0341 (1)	0.7814 (1)	1/4	0.0121 (1)
N7	0.1019 (1)	0.8688 (1)	1/4	0.0113 (1)
C8	0.1838 (1)	0.8803 (1)	0.0716 (2)	0.0182 (1)
C10	−0.0038 (2)	0.9364 (1)	1/4	0.0153 (2)
I	0.14735 (1)	0.17931 (1)	1/4	0.01289 (2)
SUXIOD				
C1	0.4478 (3)	0.8868 (4)	0.4711 (3)	0.0172 (4)
C2	0.4180 (2)	0.9405 (4)	0.6103 (3)	0.0142 (4)
O3	0.4626 (2)	0.8953 (4)	0.7230 (3)	0.0203 (4)
O4	0.3335 (2)	1.0403 (3)	0.5950 (2)	0.0160 (3)
C5	0.2933 (2)	1.0882 (4)	0.7221 (3)	0.0159 (4)
C6	0.2079 (2)	0.9737 (4)	0.7571 (3)	0.0144 (4)
N7	0.0988 (2)	1.0018 (3)	0.6768 (2)	0.0129 (3)
C8	0.1009 (2)	1.0019 (5)	0.5206 (3)	0.0173 (4)
C9	0.0525 (3)	1.1598 (4)	0.7201 (4)	0.0188 (5)
C10	0.0304 (3)	0.8645 (5)	0.7137 (5)	0.0206 (5)
C1′	0.4177 (2)	1.0038 (2)	0.3489 (3)	0.0163 (4)
C2′	0.4269 (2)	0.9347 (4)	0.2062 (3)	0.0151 (4)
O3′	0.4255 (3)	1.0151 (3)	0.1007 (3)	0.0244 (5)
O4′	0.4318 (2)	0.7699 (3)	0.2066 (3)	0.0155 (4)
C5′	0.4277 (2)	0.6929 (4)	0.0708 (3)	0.0153 (4)
C6′	0.3153 (3)	0.6598 (4)	0.0047 (3)	0.0136 (4)
N7′	0.2643 (2)	0.5091 (3)	0.0591 (2)	0.0125 (3)
C8′	0.2637 (2)	0.5169 (5)	0.2146 (3)	0.0176 (5)
C9′	0.3192 (3)	0.3574 (4)	0.0245 (4)	0.0177 (5)
C10′	0.1521 (2)	0.5054 (5)	−0.0124 (3)	0.0185 (4)
I1	0.23534 (2)	0.53086 (3)	0.58424 (2)	0.01576 (3)
I2	0.12239 (1)	1.0	0.14290 (2)	0.01425 (3)

3. Results and discussion

3.1. *X-ray structure analysis*

The final atomic coordinates and equivalent isotropic displacement parameters are listed in Table 2 for the non-H atoms of the six compounds, carbamoylcholine chloride (CARCHL), carbamoylcholine bromide (CARBRO), methoxycarbonylcholine iodide (MECOLI), carbamoylcholine iodide (CARIOD), acetylcholine chloride (ACHCHL) and succinylcholine iodide (SUXIOD). For the room-temperature study of CARBRO (Barrans, 1971) the X-ray structure is described in the space group *Pna*2₁, while the room-temperature structure of CARCHL (Jensen, 1975) has the space group *Pnam*. The low-temperature structures of both compounds are satisfactorily described in space group *Pnam*. No fundamental changes in the molecular structures as a result of the change in temperature has been observed for any of the six compounds. Bond lengths, bond angles and torsion angles are shown in Table 3, which for comparison also contains results from three other crystal structures of choline ester salts, determined at low temperature. In Fig.1 the ions are shown with the atom labelling. The ions have been oriented in the same way to facilitate comparison. It is of interest to examine if the bond lengths and angles vary as a function of the conformation of the choline ester. No such variation has been observed for the ester moieties. The C5—C6 bond is *ca* 0.01 Å longer in the ions with the fully extended conformation (CARCHL, CARBRO and MECOLI) than in the more folded ions. The C6—N7 bond is marginally shorter (~0.005 Å) in the extended conformers than in those which are partly folded, where it furthermore tends to be a bit shorter than in the conformations which are fully folded. The extended conformation with the two torsion angles C—O—C—C and O—C—C—N antiperiplanar is named the *TT* conformation. The fully folded conformation with the two torsion angles *gauche* is named the *GG* conformation. The partly folded molecules with the two torsion angles either *gauche* or antiperiplanar are named *GT* and *TG* conformations.

Table 3. *Bond lengths (Å), bond angles (°) and selected torsion angles (°)*

	CARCHL	CARBRO	MECOLI	CARIOD	ACHCHL	MECOPI*	ETCOPI†	SUXIOD	‡	ACPICR*
C12—C11							1.502 (1)			
C11—*X*1			1.449 (2)			1.444 (1)	1.457 (1)	1.521 (4)		
*X*1—C2	1.341 (1)	1.342 (3)'	1.328 (2)	1.343 (3)	1.498 (2)	1.333 (1)	1.332 (1)	1.507 (5)	1.503 (4)	1.486 (8)
C2—O3	1.220 (1)	1.213 (3)	1.202 (2)	1.223 (2)	1.206 (1)	1.203 (1)	1.204 (1)	1.209 (4)	1.206 (4)	1.203 (7)
C2—O4	1.364 (1)	1.362 (3)	1.338 (2)	1.351 (2)	1.351 (1)	1.339 (1)	1.337 (1)	1.346 (4)	1.348 (4)	1.351 (6)
O4—C5	1.437 (1)	1.438 (3)	1.447 (2)	1.439 (3)	1.443 (1)	1.450 (1)	1.450 (1)	1.444 (4)	1.443 (4)	1.452 (5)
C5—C6	1.521 (1)	1.523 (3)	1.518 (2)	1.510 (3)	1.509 (1)	1.511 (1)	1.510 (1)	1.513 (4)	1.513 (4)	1.508 (8)
C6—N7	1.505 (1)	1.504 (3)	1.508 (2)	1.515 (2)	1.511 (1)	1.511 (1)	1.511 (1)	1.515 (3)	1.521 (4)	1.516 (6)
N7—C8	1.498 (1)	1.499 (2)	1.497 (2)	1.500 (3)	1.499 (1)	1.499 (1)	1.499 (1)	1.505 (4)	1.498 (3)	1.502 (7)
N7—C9§	1.498 (1)	1.499 (2)	1.497 (2)	1.493 (3)	1.496 (1)	1.500 (2)	1.499 (1)	1.503 (5)	1.485 (4)	1.502 (7)
N7—C10	1.505 (1)	1.507 (3)	1.500 (2)	1.500 (3)	1.505 (1)	1.503 (2)	1.502 (1)	1.496 (5)	1.503 (3)	1.508 (7)
C12—C11—*X*1							107.95 (7)			
C11—*X*1—C2			114.5 (1)			114.21 (7)	113.67 (7)	115.8 (3)	115.2 (3)	
*X*1—C2—O3	125.66 (5)	125.7 (2)	126.9 (2)	126.0 (2)	125.19 (10)	126.74 (8)	126.52 (8)	124.1 (3)	124.7 (3)	125.4 (5)
*X*1—C2—O4	112.06 (5)	111.5 (2)	107.0 (1)	110.6 (2)	111.91 (8)	107.32 (7)	107.92 (7)	112.3 (2)	112.4 (3)	111.6 (5)
O3—C2—O4	122.28 (5)	122.8 (2)	126.1 (1)	123.4 (2)	122.91 (10)	125.94 (8)	125.57 (8)	123.6 (3)	122.8 (3)	123.0 (4)
C2—O4—C5	112.73 (5)	112.8 (2)	115.5 (1)	116.1 (1)	114.11 (8)	114.49 (7)	114.23 (7)	116.6 (2)	115.9 (2)	115.1 (4)
O4—C5—C6	104.08 (5)	103.9 (2)	100.8 (1)	108.3 (1)	110.50 (8)	107.52 (6)	107.67 (7)	112.1 (2)	111.7 (2)	113.3 (4)
C5—C6—N7	114.16 (4)	114.2 (2)	115.7 (1)	117.0 (1)	116.64 (8)	115.42 (6)	115.59 (7)	115.9 (2)	115.5 (2)	116.2 (4)
C6--N7—C8	111.68 (3)	111.6 (1)	111.3 (1)	110.5 (2)	111.58 (7)	112.23 (6)	111.94 (7)	111.4 (2)	111.8 (2)	111.3 (4)
C6—N7—C9§	111.68 (3)	111.6 (1)	111.3 (1)	111.7 (2)	111.99 (8)	109.92 (8)	110.04 (7)	111.1 (2)	111.0 (2)	111.2 (4)
C6—N7—C10	107.32 (4)	107.3 (1)	106.1 (1)	107.7 (1)	106.46 (7)	108.16 (7)	108.30 (7)	107.1 (2)	106.8 (2)	107.4 (4)
C8—N7—C9§	109.81 (3)	110.0 (1)	110.0 (1)	110.6 (2)	109.52 (8)	109.68 (6)	109.58 (7)	109.8 (2)	109.2 (2)	109.3 (4)
C8—N7—C10	108.09 (3)	108.0 (1)	109.0 (1)	107.9 (2)	109.14 (7)	108.58 (7)	108.62 (7)	109.3 (3)	108.6 (2)	109.2 (4)
C9§—N7—C10	108.09 (3)	108.0 (1)	109.0 (1)	108.2 (2)	108.02 (8)	108.17 (8)	108.28 (7)	108.1 (3)	109.5 (2)	108.3 (4)
C12—C11—*X*1—C2							174.21 (8)	−167.8 (3)		
C11—*X*1—C2—O4			180			−175.9 (1)	175.33 (7)	27.1 (4)	17.3 (4)	
*X*1—C2—O4—C5	180	180	180	177.2 (2)	−173.6 (1)	−175.8 (1)	178.17 (7)	175.2 (3)	172.7 (2)	176.4 (4)
C2—O4—C5—C6	180	180	180	175.8 (1)	−170.0 (1)	−176.6 (1)	174.59 (7)	−92.0 (3)	−88.2 (3)	78.1 (5)
O4—C5—C6—N7	180	180	180	70.0 (2)	84.7 (1)	85.9 (1)	−83.60 (9)	−80.2 (3)	−79.4 (3)	78.8 (5)
C5—C6—N7—C8	−61.70 (3)	−61.8 (1)	61.6 (1)	−71.6 (2)	−70.2 (1)	−51.2 (1)	49.93 (9)	53.7 (3)	57.6 (3)	−56.1 (5)
C5—C6—N7—C9§	61.70 (3)	61.8 (1)	−61.6 (1)	52.0 (2)	53.0 (1)	71.1 (1)	−72.17 (9)	−69.0 (3)	−64.5 (3)	66.1 (5)
C5—C6—N7—C10	180	180	180	170.8 (2)	170.8 (1)	−171.0 (1)	169.65 (7)	173.1 (3)	176.3 (3)	−175.6 (4)

* Frydenvang, Grønborg & Jensen (1988). † Frydenvang, Trickey & Jensen (1994). ‡ The last column for SUXIOD originates in the atoms named C1′–C10′. § For CARCHL, CARBRO and MECOLI C9 is symmetry related to C8.

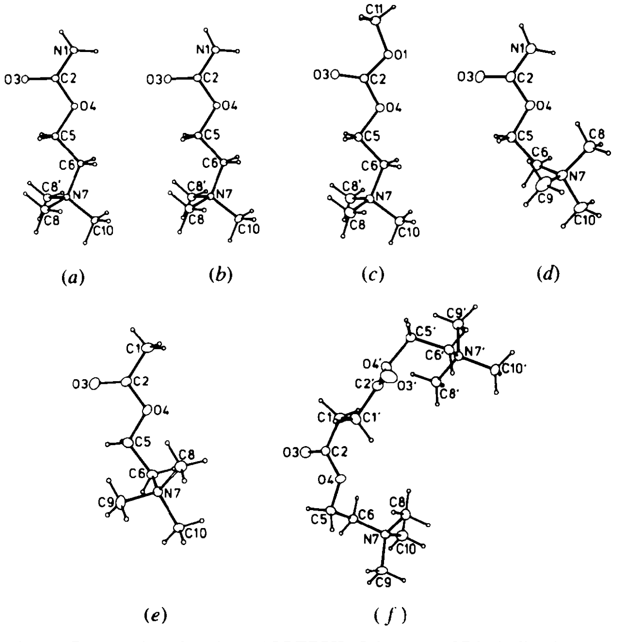


Fig. 1. Perspective drawings (*ORTEP*II; Johnson, 1976) indicating the atom labelling. Atoms are represented by displacement ellipsoids at the 50% probability level. (*a*) CARCHL, (*b*) CARBRO, (*c*) MECOLI, (*d*) CARIOD, (*e*) ACHCHL and (*f*) SUXIOD.

The most pronounced variation as a function of conformation is found for the angle O4—C5—C6, for which values of 104.08 (5), 103.9 (2) and 100.8 (1)° are found for CARCHL, CARBRO and MECOLI, respectively (*TT* conformation), 108.3 (1), 107.52 (6), 107.67 (7) and 110.5 (8)° for CARIOD, MECOPI (methoxycarbonylcholine picrate), ETCOPI (ethoxycarbonylcholine picrate) and ACHCHL (*TG* conformation), and finally the values 112.1 (2), 111.7 (2) and 113.3 (4)° are seen in SUXIOD and ACPICR (*GG* conformations). This variation parallels the situation in acetals and the phenomenon has been widely discussed and studied by different methods (Cossé-Barbi & Dubois, 1987; Ettlinger, Watson & Jaroszewski, 1994; Nørskov-Lauritsen, Larsen, Ettlinger & Jaroszewski, 1982; Ettlinger & Jaroszewski, 1994). The C5—C6—N7 angle is marginally (1°) more open in folded rather than fully extended ions.

Only two of the torsion angles, C2—O4—C5—C6 and O4—C5—C6—N7, show considerable variation. Most choline esters have in crystals been found to be in one or both conformations, often called *TG* and *GG*, as shown in Fig. 2, and the present selection with three out of nine structures being in the *TT* conformation is far from typical.

3.2. *Interionic contacts*

In order to identify the forces responsible for the unfolded conformation the packing characteristics have been examined in the crystal structures, in which the *TT* conformation was observed. The coordinates given for the asymmetric units of CARCHL, CARBRO and MECOLI have close relations to those published for the room-temperature studies of the compounds (*cf.* Table 2). This tends to hide the fact that the CARCHL crystals are isomorphous with those of CARBRO and that the MECOLI structure is of the same type. Packing diagrams demonstrating the similarity of the structures of CARCHL and MECOLI are shown in Figs. 3(*a*) and (*b*). Packing diagrams for other structures are not shown in the present paper, but may be found in the originally published papers on the room-temperature structures (Table 1).

3.3. *Surroundings of the choline ester moiety*

The carbamoylcholine ions of CARCHL and CARBRO participate in two hydrogen bonds, N—H···X^-, where X^- is a halogenide ion (Table 4). Further close contacts to halogenide ions are observed between the methylene group and the methyl groups of the quaternary ammonium group. As the carbamoylcholine ion is situated at a mirror plane, the contacts to halogenide ions outside the mirror plane will be found twice and a total of nine C—H···X^- contacts are formed from each carbamoylcholine ion, involving five different halogenide ions. A number of these contacts may be classified as C—H···X^- hydrogen bonds, according to the criteria suggested for this type of contact (Taylor & Kennard, 1982). Furthermore, the three methyl groups take part in close contacts to carbonyl O atoms (N—C···O), one contact for each methyl group. These electrostatic contacts are among the strongest attractive contacts in the crystal structure of CARCHL and they are almost of the same strength in CARBRO. Each carbonyl oxygen has close contacts to three N—CH_3 groups in different ions.

Contrary to the crystal packing of CARCHL, no classical hydrogen bonds are possible in the crystal packing of MECOLI. The hydrogen bonds in CARCHL and

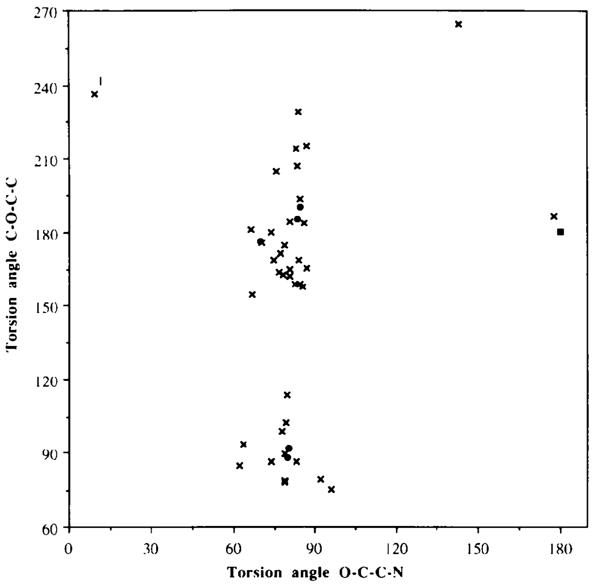
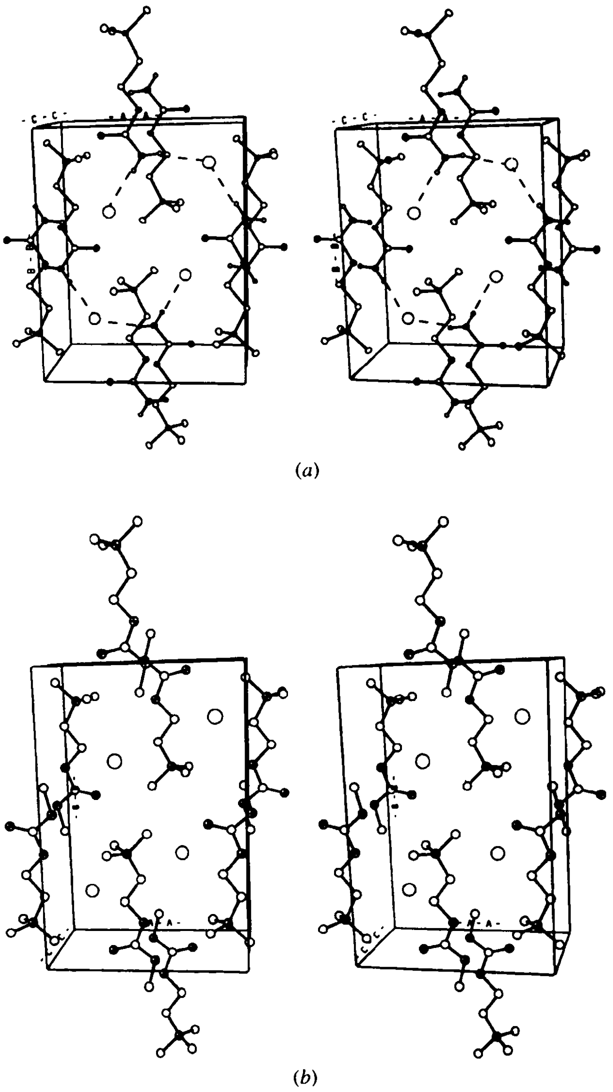


Fig. 2. The variations observed for the two torsion angles C—O—C—C and O—C—C—N (°). Results retrieved from the Cambridge Structural Database are indicated by x and results from the present structure determinations by ● (1; ACTART10, not reliable due to disorder; Jensen, 1982); ■ = ● + x.


(*a*)

(*b*)

Fig. 3. Steredrawings of the unit cells of (*a*) CARCHL and (*b*) MECOLI. N and O atoms are drawn with principal ellipsoids added. H atoms connected to N atoms are shown and the hydrogen bonds are indicated for CARCHL. (*a*) *x* horizontal, *y* vertical and *z* into the plane of the paper. (*b*) *x* horizontal, *y* vertical and *z* out of the plane of the paper.

Table 4. *Selected intermolecular contacts* (Å)

D—Y···A	D···A	Y···A	D—Y···A
CARCHL			
N1—H11···Cl$^{-\mathrm{i}}$	3.333 (1)	2.45 (2)	178 (1)
N1—H12···Cl$^{-\mathrm{ii}}$	3.338 (1)	2.47 (2)	162 (2)
C6—H6···Cl$^{-\mathrm{iii}}$	4.032 (1)	3.18 (1)	148 (1)
C8—H81···Cl$^{-\mathrm{iii}}$	3.858 (1)	2.93 (1)	155 (1)
C10—H101···Cl$^{-\mathrm{iii}}$	3.719 (1)	2.83 (1)	160 (1)
C8—H82···Cl$^{-\mathrm{iv}}$	3.823 (1)	3.04 (1)	136 (1)
C10—H103···Cl$^{-\mathrm{v}}$	3.746 (1)	2.73 (2)	164 (1)
N7—C8···O3$^{\mathrm{iv}}$		2.984 (1)	161.68 (7)
N7—C10···O3$^{\mathrm{vi}}$		3.037 (1)	167.62 (9)
CARBRO			
N1—H11···Br$^{-\mathrm{vii}}$	3.466 (2)	2.58	170
N1—H12···Br$^{-\mathrm{viii}}$	3.469 (2)	2.62	157
C6—H6···Br$^{-\mathrm{ix}}$	4.112 (1)	3.23	147
C8—H81···Br$^{-\mathrm{ix}}$	3.986 (2)	3.06	153
C10—H101···Br$^{-\mathrm{ix}}$	3.819 (1)	2.86	158
C8—H82···Br$^{-\mathrm{x}}$	3.869 (2)	3.07	138
C10—H103···Br$^{-\mathrm{xi}}$	3.843 (2)	2.87	165
N7—C8···O3$^{\mathrm{xii}}$		3.044 (2)	160.4 (1)
N7—C10···O3$^{\mathrm{xiii}}$		3.020 (3)	166.4 (2)
MECOLI			
O1—C11···I$^{-\mathrm{i}}$		3.721 (2)	172.7 (2)
C11—H113···I$^{-\mathrm{vii}}$	3.959 (2)	3.10 (5)	164 (4)
C6—H6···I$^{-\mathrm{xiv}}$	3.955 (1)	3.02 (3)	165 (2)
C8—H81···I$^{-\mathrm{xiv}}$	4.156 (1)	3.27 (3)	154 (2)
C10—H101···I$^{-\mathrm{xiv}}$	4.137 (1)	3.26 (3)	154 (2)
C5—H5···I$^{-\mathrm{xv}}$	4.184 (1)	3.24 (3)	179 (2)
C8—H82···I$^{-\mathrm{xv}}$	4.166 (2)	3.45 (3)	131 (2)
C10—H103···I$^{-\mathrm{v}}$	4.029 (2)	3.06 (4)	147 (3)
N7—C8···O3$^{\mathrm{xii}}$		3.459 (2)	144.3 (1)
N7—C10···O3$^{\mathrm{xi}}$		3.176 (2)	145.0 (1)
CARIOD			
N1—H11···I$^{-\mathrm{xv}}$	3.660 (2)	2.92 (5)	172 (5)
N1—H12···O3$^{\mathrm{xvi}}$	2.916 (3)	2.03 (5)	178 (4)
C8—H83···I$^{-\mathrm{xvii}}$	3.972 (2)	3.05 (5)	174 (4)
N7—C10···I$^{-\mathrm{xviii}}$		3.789 (3)	167.0 (1)
N7—C8···I$^{-\mathrm{xv}}$		3.811 (3)	156.6 (1)
C6—H62···I$^{-\mathrm{xix}}$	4.141 (2)	3.22 (5)	159 (4)
C8—H81···I$^{-\mathrm{xix}}$	4.309 (3)	3.46 (5)	150 (4)
C10—H102···I$^{-\mathrm{xix}}$	4.121 (3)	3.19 (5)	160 (4)
C5—H51···I$^{-\mathrm{xx}}$	4.090 (2)	3.23 (5)	156 (4)
C9—H91···I$^{-\mathrm{xx}}$	3.931 (3)	3.12 (5)	148 (4)
C6—H61···I$^{-\mathrm{xxi}}$	3.870 (2)	3.01 (5)	157 (4)
C5—H52···O3$^{\mathrm{xv}}$	3.402 (2)	2.67 (5)	130 (4)
C9—H93···O3$^{\mathrm{xxii}}$	3.358 (8)	2.56 (5)	147 (4)
ACHCHL			
C6—H61···Cl$^{-\mathrm{i}}$	3.555 (1)	2.68 (2)	156 (2)
C9—H92···Cl$^{-\mathrm{i}}$	3.694 (2)	2.88 (2)	151 (2)
C10—H101···Cl$^{-\mathrm{i}}$	3.767 (1)	2.92 (2)	148 (2)
C6—H62···Cl$^{-\mathrm{xxiii}}$	3.797 (1)	2.97 (2)	148 (2)
C8—H81···Cl$^{-\mathrm{xxiii}}$	3.630 (2)	2.73 (2)	153 (2)
C10—H102···Cl$^{-\mathrm{xxiii}}$	3.658 (2)	2.77 (2)	153 (2)
C8—H83···Cl$^{-\mathrm{xxiv}}$	3.918 (2)	3.08 (2)	152 (2)
C9—H93···Cl$^{-\mathrm{xxiv}}$	3.716 (1)	2.82 (2)	160 (2)
C10—H103···Cl$^{-\mathrm{xxiv}}$	3.632 (2)	2.78 (2)	157 (2)
C1—H11···Cl$^{-\mathrm{xxv}}$	3.659 (2)	2.82 (2)	144 (2)
C8—H82···Cl$^{-\mathrm{xxv}}$	3.595 (2)	2.76 (2)	148 (2)
N7—C9···O3$^{\mathrm{xxvi}}$		3.207 (2)	129.2 (1)
SUXIOD			
C5—H52···I1$^{-\mathrm{v}}$	3.885 (3)	2.93 (9)	160 (7)
C9—H92···I1$^{-\mathrm{v}}$	4.150 (4)	3.14 (10)	172 (7)
C6—H62···I2$^{-\mathrm{xvii}}$	4.018 (3)	3.04 (8)	169 (6)
C6—H61···I1$^{-\mathrm{i}}$	4.015 (3)	3.08 (9)	161 (6)
C10—H101···I1$^{-\mathrm{i}}$	4.096 (4)	3.26 (9)	162 (8)
N7—C8···I2$^{-\mathrm{i}}$		3.679 (3)	176.7 (3)
N7—C9···I2$^{-\mathrm{xxvii}}$		3.911 (4)	165.9 (2)
N7—C10···I2$^{-\mathrm{xxviii}}$		3.917 (4)	172.3 (3)
C1′—H11′···I2$^{-\mathrm{i}}$	4.014 (3)	3.22 (7)	140 (6)
C6′—H61′···I2$^{-\mathrm{i}}$	4.063 (3)	3.04 (10)	168 (7)
C6′—H62′···I1$^{-\mathrm{xxiv}}$	4.163 (3)	3.28 (9)	153 (7)
C10′—H102′···I1$^{-\mathrm{xxix}}$	4.169 (3)	3.26 (8)	160 (6)
C9′—H93′···I2$^{-\mathrm{viii}}$	4.119 (4)	3.23 (10)	174 (8)
N7′—C8′···I1$^{-\mathrm{i}}$		3.624 (3)	174.5 (3)
N7′—C10′···I2$^{-\mathrm{xxx}}$		3.561 (3)	173.5 (3)
N7′—C9′···O3$'^{\mathrm{vii}}$		3.149 (5)	153.0 (2)
C5′···O3$^{\mathrm{xxxi}}$	3.321 (4)		
C5′···O3$'^{\mathrm{xxxii}}$	3.039 (5)		

Symmetry codes: (i) x, y, z; (ii) $\frac{1}{2} + x, \frac{1}{2} - y, z$; (iii) $2 - x, 1 - y, z$; (iv) $\frac{3}{2} - x, \frac{1}{2} + y, -z$; (v) $x, y + 1, z$; (vi) $\frac{1}{2} + x, \frac{3}{2} - y, z$; (vii) $x - \frac{1}{2}, \frac{1}{2} - y, z$; (viii) $x, y - 1, z$; (ix) $\frac{3}{2} - x, y - \frac{1}{2}, -z$; (x) $1 - x, 1 - y, -z$; (xi) $x - \frac{1}{2}, \frac{3}{2} - y, z$; (xii) $\frac{1}{2} - x, \frac{1}{2} + y, z - \frac{1}{2}$; (xiii) $\frac{1}{2} + x, \frac{1}{2} - y, \frac{1}{2} - z$; (xiv) $-x, 1 - y, -z$; (xv) $1 - x, -y, 1 - z$; (xvi) $2 - x, -y, 1 - z$; (xvii) $x + 1, y, z + 1$; (xviii) $x, y, z + 1$; (xix) $-x, -y, 1 - z$; (xx) $1 + x, \frac{1}{2} - y, \frac{1}{2} + z$; (xxi) $x, \frac{1}{2} - y, \frac{1}{2} + z$; (xxii) $x, \frac{1}{2} - y, \frac{1}{2} + z$; (xxiii) $x - \frac{1}{2}, \frac{1}{2} - y, 1 - z$; (xxiv) $x - \frac{1}{2}, \frac{1}{2} - y, -z$; (xxv) $-x, y + \frac{1}{2}, \frac{1}{2} - z$; (xxvi) $\frac{1}{2} - x, 1 - y, z - \frac{1}{2}$; (xxvii) $-x, \frac{1}{2} + y, 1 - z$; (xxviii) $-x, y - \frac{1}{2}, 1 - z$; (xxix) $x, y, z - 1$; (xxx) $-x, y - \frac{1}{2}, -z$; (xxxi) $1 - x, y - \frac{1}{2}, 1 - z$; (xxxii) $1 - x, y - 1, z$.

CARBRO are replaced by two contacts, C11···I$^-$. One of these is of pure electrostatic character, while the other may be classified as a hydrogen bond [C11—H113···I$^-$ ($x - \frac{1}{2}, \frac{1}{2} - y, z$)]. Calculations have indicated (Johansen, Rettrup & Jensen, 1980) that the C11 methyl groups carry a non-negligible positive partial charge. The quaternary ammonium group has contacts to five iodide ions and, as for the previously described structures, some of the contacts may be seen as C—H···X^- hydrogen bonds. Contacts involving the carbonyl oxygen atom are much weaker in the MECOLI structure than in the structures of CARCHL and CARBRO.

The crystal structures of MECOPI and ETCOPI are isomorphous. Two crystal forms have been characterized for ethoxycarbonylcholine iodide (Jensen, 1981), but none of them are isomorphous with MECOLI, a fact which may relate to the difference in charge of C11 in MECOLI and the methyl group of the ethoxy group in ETCOLI (ethoxycarbonylcholine iodide).

While the structures of CARCHL and CARBRO are isomorphous, the structure of CARIOD is completely different. This may be due to the size of I$^-$, as this would prevent the formation of the strong attractive contacts described for CARCHL and CARBRO. The $\mathrm{NH_2}$ group in CARIOD is involved in two hydrogen bonds, one N—H···I$^-$ and one N—H···O ($2 - x, -y, 1 - z$). The N—H···O contact is one of a pair related by a centre of symmetry, as often found for amides. The methylene and methyl groups of the quaternary ammonium moiety have a number of rather weak contacts to iodide ions. Only one weak N—$\mathrm{CH_3}$···O contact is found.

The carbonyl O atom of the carbamoyl group participates in the formation of strong hydrogen bonds. No such contacts could be formed to the carbonyl O atoms of ACHCHL or SUXIOD, as no hydrogen-bond donor is present. It is, however, remarkable that hydrogen bonds to the acetyl O atoms have never been observed in any of the numerous known structures of acetylcholine salts (Jensen, 1984, and references therein).

3.3.1. *The surroundings of the quaternary ammonium group.* The preferred position of the halogenide ions or other negatively charged groups relative to the quaternary ammonium group in choline esters and other compounds has been thoroughly studied (Kokkinidis, 1981; Gieren & Kokkinidis, 1981; Rosenfield Jr & Murray-Rust, 1982; Snyder, 1985), and a shallow energy minimum has been proposed, indicating a preference for the situation where a negatively charged group is approximately equidistant from three and opposite to the fourth C atom attached to N. In the present six structures (and in many other crystal structures of choline ester salts) a variety of arrangements has been observed, with one, two or three contacts between a halogenide ion and the C atoms of a quaternary ammonium group. This may indicate that the preference for the last mentioned geometry may be of minor importance for contacts between the quaternary ammonium group and a biomacromolecule.

3.3.2. *Molecular mechanics calculations.* The calculated potential energy map for acetylcholine, using the default dipoles of the MM2 program in the energy calculations, has been published by Kooijman, Kanters & Kroon (1990). This map is closely similar (but not identical) to the map for carbamoylcholine shown in Fig. 4. Several shallow minima are found, corresponding to the *GG*, *TG*, *GT* and *TT* conformations. As the full range 0–360° is shown for both the torsion angles C2—O4—C5—C6 and O4—C5—C6—N7, the symmetry-related conformations *-G-G*, *T-G* and *-GT* are also seen. Due to steric interactions between the quaternary ammonium group and the ester moiety, the *-GG* conformation cannot be achieved without high-energy investments and it seems unrealistic that this conformation should be relevant in any biological system. Potential energy maps obtained through analogous calculations, but with the use of different sets of point charges, are very similar to that shown in Fig. 4. Results of MM2 calculations performed for the carbamoylcholine ion and for the methoxycarbonylcholine ion are similar to those obtained for acetylcholine. The same minima are found in all calculations.

The geometry of the ions has been optimized for every observed energy minimum. The results are, as shown in Fig. 5, not totally independent of the charges used, although energy minimization leads to essentially the same conformations. Full details on the optimized geometries have been deposited.*

The absolute values of the steric energy as calculated by the MM2 program depend, as expected, to a very high degree on the point charges used. It can be mentioned as an example that the calculated dipole/charge contributions to the steric energy of the *TT* conformation of the acetylcholine ion are 8.4, 26.4, –212.1 and 82.0 kJ mol^{-1} for dipoles, Gasteiger charges, PM3 charges and STO-3G charges, respectively.

The relative levels of the steric energy for different conformations, calculated using a certain set of charges, may be compared. As the contributions to the steric energy (compression, bending, stretch–bend,

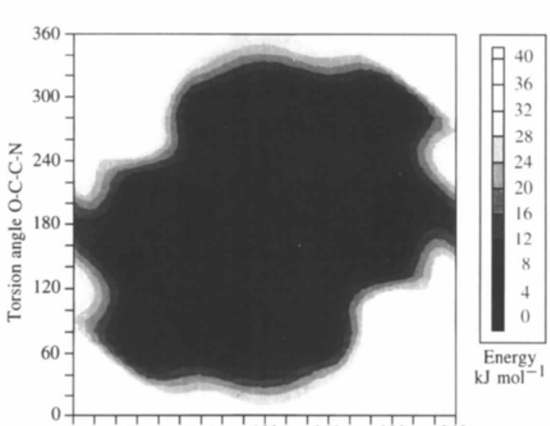


Fig. 4. Calculated potential energy surface for carbamoylcholine as a function of the two torsion angles C—O—C—C and O—C—C—N. Electrostatic interactions were calculated using dipole–dipole interactions (general for calculations labelled *A*).

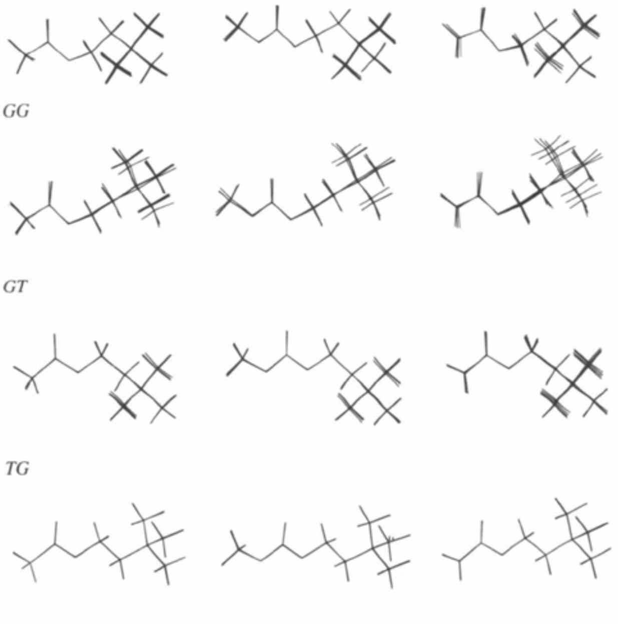


Fig. 5. The calculated minimum energy conformations of the three ions acetylcholine (ACH), methoxycarbonylcholine (MET) and carbamoylcholine (CAR). For each of the conformations, *gauche–gauche* (*GG*), *gauche–trans* (*GT*), *trans–gauche* (*TG*) and *trans–trans* (*TT*), calculations were performed using dipole–dipole interactions (*A*) and three different sets of point charges (*B*, *C* and *D*). Using the molecular modelling program *SYBYL*6.03 (Tripos Associates, 1992), the four similar conformations were superimposed.

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* See footnote on p. 185.

van der Waals 1,4 interactions, torsional, electrostatic and other energy contributions) are interdependent, it is without meaning to analyse the energy of the single contributions. It may, however, be mentioned, that the calculated changes in electrostatic contribution alone do not determine the relative energies for the four conformations *GG*, *TG*, *GT* and *TT*.

The relative energies of the low-energy conformations of acetylcholine, carbamoylcholine and methoxycarbonylcholine using dipoles and different sets of point charges are compared in Fig. 6. The variation in steric energy from the most stable to the least stable conformation is in all cases less than 7 kJ mol^{-1} and in most cases less than 4 kJ mol^{-1}, indicating that all three ions are very flexible.

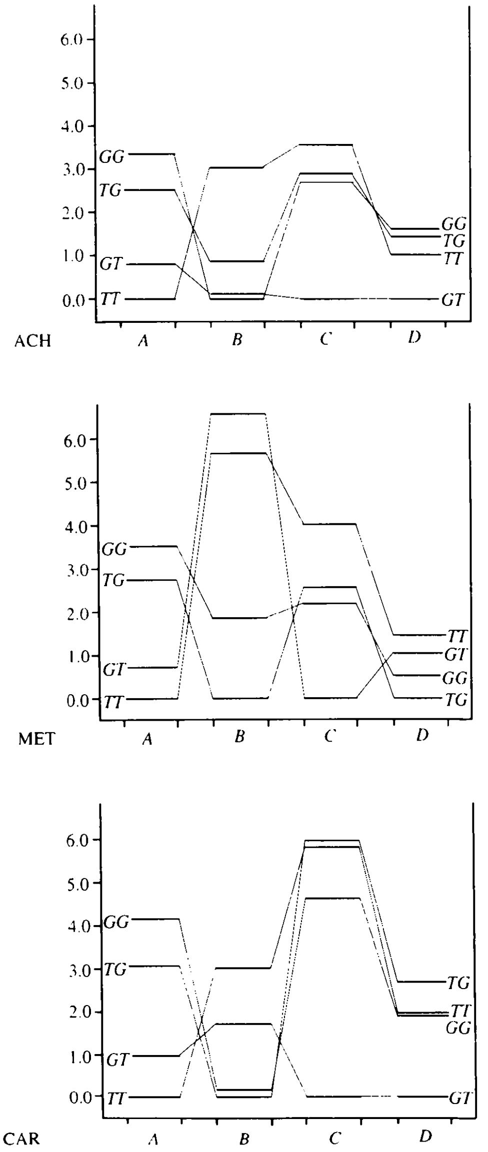

Fig. 6. The relative energies (kJ mol^{-1}) of the different conformations using dipole–dipole interactions (*A*) or one of the three sets of point charges for charge–charge interactions (*B*, *C* and *D*). ACH = acetylcholine, MET = methoxycarbonylcholine and CAR = carbamoylcholine.

It seems impossible to draw conclusions concerning the relative stability of the four conformations *GG*, *TG*, *GT* and *TT*. The calculated energy differences are small and would hardly be a solid basis for conclusions, no matter if the compounds in question were charged or uncharged. Furthermore, the relative position of the energy levels for the four conformations change when different sets of charges are used. It also seems impossible to conclude whether MM2 calculations for ions such as acetylcholine (ions having one delocalized charge) should preferably be performed using point charges rather than dipoles. From a more philosophical viewpoint, one may argue that the program has been created with dipole–dipole interactions and, therefore, these should be used. On the other hand, it seems unsatisfactory that the charge is totally disregarded. In the present case, where the charge is highly delocalized, the results obtained form calculations using point charges are hardly less reliable than those from standard MM2 calculations.

4. Concluding remarks

Crystallographic results have formed an important basis for the parameters in the MM2 program and molecular geometry is generally very well reproduced. We have examined if the variation of the O4—C5—C6 angle, which is observed in the solid state, has also been reproduced in the MM2 calculations. A variation is observed whether dipoles or charges are used in the calculations. The trend is the same as observed in the crystal structures: the magnitude of the angle decreases when the ion becomes less folded. However, the variation is underestimated, with changes of only ~2° between the values for *GG*, *TG* and *TT*. The value calculated for the *GG* conformation is very close to the experimental findings.

Choline esters are very flexible ions and for the acetylcholine, carbamoylcholine and methoxycarbonylcholine ions the presence of more than one conformation should always be considered possible in biological media. The calculated relative energies do not suggest that two of the conformations, *TG* and *GG*, should be found more often in the solid state than the other two conformations, *TT* and *GT*. As seen from the results presented in this paper, *TT* has been found in some cases. A conformation which can be classified as similar to *GT* has only been observed once (*cf.* Fig. 2) and, in addition, for the highly substituted acetylcholine ion in threo-α,β-dimethylacetylcholine iodide (Shefter, Sackman, Stephen Jr & Smissman, 1970).

It is tempting to propose as an explanation for the discrepancy between results from molecular mechanics calculations and crystallographic results that the stability of a *G* conformation of the O—C—C—N fragment is underestimated in the MM2 calculations.

No indications are found in the results of the molecular mechanics calculations proposing that the acetylcholine ion should be less flexible than the carbamoylcholine and the methoxycarbonylcholine ions. Acetylcholine ions do not participate in hydrogen bond formation or in other directional contacts. Acetylcholine may be classified as very tolerant and unselective with regard to contacts to neighbours and this may be the reason why the acetylcholine ion has never been observed in the extended conformation in a crystal structure. The quaternary ammonium head has, as mentioned earlier, contacts of almost the same strength to neighbours in many directions relative to the N—C bonds. It is further important to notice that the quaternary ammonium ion always participates in a number of weak contacts to surrounding ions and to partially charged atoms, *e.g.* the carbonyl O atoms of amides, rather than in one single strong contact. Contacts of types other than ion–ion interactions are important for the interaction between acetylcholine and the relevant biomacromolecules, and crystallographic results for acetylcholine esterase (Sussman *et al.*, 1991) and the phosphocholine-binding immunoglobulin FAB fragment (Satow, Cohen, Padlan & Davies, 1986) confirm that a favourable contact is observed between the quarternary ammonium group and an aromatic system.

Proposals concerning a cholinergic pharmacophore (*e.g.* Schulman, Sabio & Disch, 1983) have included a single counterion to the quaternary ammonium group. Crystallographic results indicate that a reliable proposal concerning the optimal position of this counterion is hard to obtain, and it should at least be kept in mind that very little energy is needed in order to change a proposed arrangement.

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