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X-ray Crystal Structure and Conformation of a Spin-Labeled Acetylcholine, DL-4-[*N*,*N*-Dimethyl-*N*-(ethan-2'-olacetate)amino]-2,2,6,6-tetramethylpiperidine-1-oxyliodide

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SUMMARY

MCPHAIL, ANDREW T., ABOU-DONIA, MOHAMED B. & ROSEN, GERALD, M. (1976) X-ray crystal structure and conformation of a spin-labeled acetylcholine, DL-4-[*N*,*N*-dimethyl-*N*-(ethan-2'-olacetate)amino]-2,2,6,6-tetramethylpiperidine-1-oxyliodide. *Mol. Pharmacol.*, 12, 590-597.

The detailed molecular geometry of the title compound, a muscarinic blocking agent, has been established by single-crystal X-ray analysis. Crystals are orthorhombic, space group *Pbca*, $a = 18.00(1)$, $b = 11.42(1)$, $c = 18.42(1)$ A, $Z = 8$. The structure was solved by the heavy-atom method, and atomic parameters were refined by full-matrix, least-squares calculations to R 0.049 over 1082 statistically significant reflections from diffractometer measurements. Comparison of the results with those for known muscarinic agents revealed that whereas the O–C–C–N^+ torsion angle at 78° is similar to those in other potent muscarinic agents, the C–O (ester)–C–C torsion angle at 99° lies outside the range predicted for muscarinic agonists.

INTRODUCTION

Acetylcholine, a synaptic transmitter, interacts with three distinct receptor types: preganglionic autonomic, postganglionic parasympathetic, and somatic motor. In addition, this neurohumoral agent is a substrate for the hydrolytic action of acetylcholinesterase. To account for the observed variety of activities, it has been postulated that acetylcholine is capable of existence in several rotameric states, and several groups (1-4) have undertaken studies to determine which conformer predominates. Nuclear magnetic resonance investigations by Partington *et al.* (1) suggested that acetylcholine favors the *gauche* (synclinal) conformation. From consideration of the results of X-ray crystallographic studies, Herdklotz and Sass (5) and Chothia and Pauling (6) arrived at the same conclusion. On the other hand, Genson and Christofferson (7), using a recent self-consistent field approach *ab initio*, reached the conclusion that acetylcholine exists preferentially as the *anti* conformer. However, these latter results have been brought into question by the more recent reinvestigation by Port and Pullman (8), which led these authors to conclude that, indeed, acetylcholine ought to exist predominantly in the *gauche* form.

In our continuing studies of the cholinergic receptor, it was advantageous to prepare a spin label of acetylcholine (I) so that drug-receptor interaction might be better

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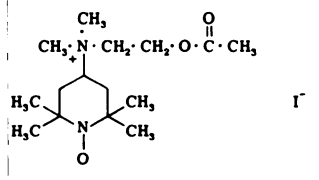


(I)

investigated. It was felt that whereas the spin-labeled acetylcholine might prove to be a weak agonist, it might also retain sufficient biological activity for such studies. To our surprise, the drug proved to be a very effective muscarinic blocking agent with a high degree of specificity (9). This result strongly suggested that the spin-labeled acetylcholine must exist predominantly in the "muscarinic conformation."

In the present work a complete single-crystal X-ray structure analysis of the spin-labeled acetylcholine has been undertaken to establish the detailed conformation of this species.

EXPERIMENTS AND RESULTS

Preparation of the spin-labeled acetylcholine, DL-4-[*N*,*N*-dimethyl-*N*-(ethan-2'-olacetate)amino]-2,2,6,6-tetramethylpiperidine-1-oxyl iodide, has been described elsewhere (9).

Crystal data. $C_{15}H_{30}N_2O_3I$, M = 413.3. Orthorhombic, a = 18.00(1), b = 11.42(1), c = 18.42(1) A, U = 3786 A^3, D_m (flotation) = 1.44 g cm^{-3}, Z = 8, D_c = 1.450 g cm^{-3}, $F(000)$ = 1688. MoK$_\alpha$ radiation, λ = 0.7107 A; μ (MoK$_\alpha$) = 17.3 cm^{-1}. Space group *Pbca* (D_{2h}^{15}) from systematic absences, $0kl$ when $k \neq 2n$, $h0l$ when $l \neq 2n$, $hk0$ when $h \neq 2n$.

Preliminary cell dimensions and space group data were obtained from precession and Weissenberg photographs. A crystal of dimensions approximately 0.20 × 0.08 × 1.00 mm was oriented on a glass fiber so that the needle b axis was parallel to the ϕ axis of an Enraf-Nonius CAD 3 automated diffractometer (zirconium-filtered MoK$_\alpha$ radiation; takeoff angle, 3°). Improved cell parameters were obtained by least-squares treatment of the θ, χ, and ϕ values of 40 accurately centered reflections widely separated in reciprocal space. One octant of intensity data up to θ = 25° was collected using the θ-2θ scanning technique with scan widths of (0.90 + 0.45 tan θ) degrees. Attenuators were inserted automatically to ensure that counting rates did not exceed 2500 counts/sec; attenuation factors were derived experimentally. Stationary background measurements were made at each end of the scan range for a time equal to half the scan period. A standard reflection remeasured after each batch of 99 reflections showed no significant variation of intensity. From these measurements, 1082 reflections, for which $I < 2.0\sigma(I)$, where $\sigma(I)$ = (scan count + total background count)$^{\frac{1}{2}}$, were used in the structure analysis. Absorption corrections were established experimentally from the ϕ dependence of the intensity of the axial 0 8 0 reflection measured at χ = 90°, and the data were corrected for Lorentz, polarization, and absorption effects.

Structural analysis. The structure was solved by the heavy-atom method. Initial coordinates for the iodine atom were derived from the three-dimensional Patterson map. Positions for the other non-hydrogen atoms were obtained from the iodine-phased

$$R = \frac{\sum||F_o| - |F_c||}{\sum|F_o|} = 0.46$$

Fourier synthesis, which contained a pseudo-mirror plane of symmetry owing to the approximately zero value of the iodine z coordinate. Several cycles of full-matrix, least-squares adjustment of the positional and isotropic thermal parameters reduced R to 0.082. Subsequent refinement, using anisotropic temperature factors, led to convergence at R = 0.061. A difference Fourier synthesis was then evaluated, and significant positive electron density was found at the positions calculated for 27 out of the total of 30 hydrogen atoms; hydrogen atoms of the acetate methyl group could not be placed unambiguously. Inclusion of the contributions for 27 hydrogen atoms, all with isotropic temperature factors B = 4.0 A^2, reduced R to 0.052, and further least-squares refinement of the non-hydrogen atom parameters converged at R = 0.049, at which point all parameter shifts were less than one-half their stand-

ard deviations. Final atomic coordinates and temperature factor parameters are summarized in Tables 1 and 2.

In the structure-factor calculations, scattering factors for neutral carbon, nitrogen, oxygen, and iodine were taken from the compilation by Cromer and Waber (10), and corrections for anomalous dispersion of iodine were included (11). For hydrogen, the values of Stewart, Davidson, and Simpson (12) were used. In the least-squares calculations $\sum w\,\Delta^2$, $\Delta = |F_0| - |F_c|$, was minimized, with weights w assigned according to the scheme $\sqrt{w} = 1$ for $|F_0| \leqq 50.0$ and $\sqrt{w} = 50.0/|F_0|$ for $|F_0| > 50.0$; this gave no systematic dependence of $\langle w\,\Delta^2 \rangle$ when analyzed in ranges of $|F_0|$ and $\sin\theta$.

DISCUSSION

In the present investigation we observed that the cholinergic activity of spin-labeled acetylcholine (I) (13) suppressed contraction of frog sartorius muscle by about 80% at a very high concentration (1 mM). This result indicated that the spin-labeled acetylcholine is a weak nicotinic cholinolytic agent, and was verified by determining that the same concentration of spin-labeled probe was required to block the miniature end plate potential in the same preparation. At lower concentrations (10 μM) this compound displayed no cholinolytic activity. On the other hand, the spin-labeled probe proved to be a potent muscarinic antagonist (affinity constant, 50 nM) against the cholinergic receptors of isolated frog heart. We have demonstrated elsewhere (14) that acetylcholinesterase could not hydrolyze the spin-labeled probe and that this compound was a reversible, competitive inhibitor for the enzyme.

In view of the rarity of compounds with the selective pharmacological activity of spin-labeled acetylcholine, we felt that it would be advantageous to determine its conformation.

The synthetic procedure yields a racemic mixture, and so the crystals contain enantiomer pairs. A view of the solid-state conformation adopted by one enantiomer of the spin-labeled acetylcholine and the

TABLE 1

Fractional atomic coordinates and anisotropic temperature factor parameters,[a] with estimated standard deviations (in parentheses)

Atom	x	y	z	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
	× 10⁴	× 10⁴	× 10⁴	× 10⁴	× 10⁴	× 10⁴	× 10⁴	× 10⁴	× 10⁴
C(1)	−1366(9)	−2711(13)	−2397(9)	43(6)	134(17)	55(7)	13(17)	36(9)	59(21)
C(2)	−1801(7)	−3639(12)	−2019(5)	33(4)	106(13)	11(3)	32(13)	1(7)	−1(12)
O(3)	−1566(5)	−4528(9)	−1770(7)	30(3)	115(9)	84(5)	29(10)	−13(7)	69(13)
O(4)	−2536(5)	−3362(7)	−1986(4)	28(3)	109(9)	28(3)	24(8)	12(5)	41(9)
C(5)	−3010(6)	−4200(10)	−1613(6)	24(4)	82(11)	25(4)	16(12)	−3(7)	11(13)
C(6)	−3172(6)	−3840(11)	−834(5)	24(4)	83(11)	13(3)	17(11)	15(6)	15(11)
N(7)	−3761(5)	−2886(8)	−722(5)	18(3)	62(7)	22(3)	−3(8)	−4(5)	0(9)
C(8)	−3546(6)	−1830(10)	−1145(6)	33(5)	68(11)	19(3)	7(12)	9(7)	22(10)
C(9)	−4502(7)	−3302(13)	−975(6)	27(5)	154(16)	15(4)	−14(14)	0(7)	−18(13)
C(10)	−3748(6)	−2588(10)	108(4)	26(4)	80(9)	8(3)	−1(10)	−6(5)	17(9)
C(11)	−4198(6)	−1470(10)	274(6)	27(4)	52(9)	22(3)	11(11)	9(6)	4(10)
C(12)	−4097(6)	−1114(10)	1059(6)	21(4)	64(9)	17(3)	−18(10)	−7(6)	−13(11)
C(13)	−3317(7)	−582(12)	1182(7)	36(5)	86(12)	28(4)	−47(13)	−10(8)	−33(13)
C(14)	−4682(7)	−208(11)	1256(7)	25(4)	78(11)	29(4)	31(11)	14(8)	6(13)
N(15)	−4195(5)	−2132(8)	1556(5)	25(4)	80(9)	15(3)	−9(9)	3(5)	−18(10)
O(16)	−4271(5)	−1925(8)	2238(4)	42(4)	113(9)	9(2)	11(10)	9(5)	−6(8)
C(17)	−3973(6)	−3369(10)	1357(5)	26(4)	72(9)	8(3)	−23(11)	6(6)	22(9)
C(18)	−3170(6)	−3601(12)	1597(6)	22(4)	125(13)	19(4)	30(12)	5(6)	28(13)
C(19)	−4504(7)	−4220(11)	1788(7)	35(5)	89(12)	26(4)	−1(13)	−10(8)	11(13)
C(20)	−4099(6)	−3553(10)	538(6)	24(4)	71(9)	18(4)	−20(11)	−7(6)	27(11)
I	1431.9(4)	2055.7(8)	10.1(6)	26.0(3)	95.8(7)	51.3(3)	−7(1)	−14(1)	2(1)

[a] In the form $B \sin^2\theta/\lambda^2 = b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl$.

TABLE 2

Calculated fractional coordinates for hydrogen atoms, labeled according to carbon atom to which they are bonded

Atom	x	y	z
	$\times 10^3$	$\times 10^3$	$\times 10^3$
H(5A)	−275	−502	−161
H(5B)	−352	−426	−188
H(6A)	−266	−354	−60
H(6B)	−334	−459	−54
H(8A)	−356	−202	−170
H(8B)	−395	−117	−107
H(8C)	−302	−156	−96
H(9A)	−452	−351	−154
H(9B)	−491	−266	−91
H(9C)	−464	−407	−68
H(10)	−318	−245	28
H(11A)	−406	−80	−8
H(11B)	−478	−168	18
H(13A)	−323	−30	174
H(13B)	−324	16	85
H(13C)	−294	−122	105
H(14A)	−459	7	182
H(14B)	−461	54	93
H(14C)	−521	−57	121
H(18A)	−300	−466	146
H(18B)	−308	−347	216
H(18C)	−284	−300	129
H(19A)	−435	−506	164
H(19B)	−443	−408	235
H(19C)	−506	−402	164
H(20A)	−386	−437	37
H(20B)	−467	−357	42

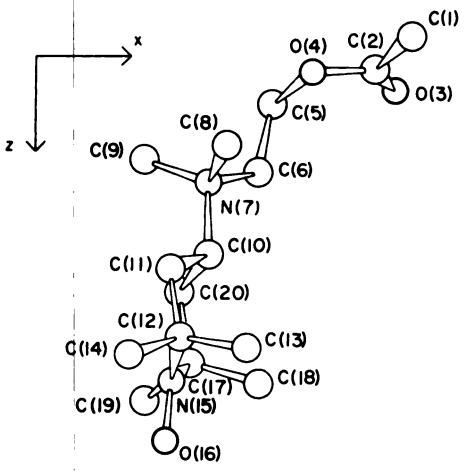


FIG. 1. *Atom numbering scheme and conformation of spin-labeled acetylcholine*

Hydrogen atoms have been omitted for clarity.

atom numbering scheme used are shown in Fig. 1. Interatomic distances and angles are presented in Table 3. Torsion angles for the enantiomer corresponding to L(+)-muscarine are given in Table 4; all ensuing discussion of these parameters refers to this enantiomer.

The cation contains two biologically interesting structural features: (a) the conformation adopted in the acetylcholine residue, and its relationship to other compounds displaying muscarinic activity, and (b) the geometry of the 2,2,6,6-tetramethylpiperidinoxyl moiety, which is finding increasing use as a spin label to probe biological structure-function relationships.

In the acetylcholine residue the O(4)—C(5)—C(6)—N^+(7) torsion angle assumes a value of 78°, and consequently the oxygen and nitrogen atoms are in an approximately *gauche* conformation about the C(5)—C(6) bond, with $N^+(7) \cdots O(4)$ = 3.25 A. Thus these values in the spin-labeled acetylcholine lie in the ranges associated with potent muscarinic activity, 70–137° for the O—C—C—N^+ torsion angle and 3.09–3.69 A for the $O \cdots N^+$ distance (5, 15–22).[2] One consequence of the adoption of the *gauche* O—C—C—N^+ form and a staggered, or approximately staggered, conformation of the *N*-methyl C—H bonds with respect to the C—N^+ bonds is the formation of the well-recognized pseudo-chair O(4)—C(5)—C(6)—N^+(7)—C(8)—H(8A) arrangement and the close approach of the ester oxygen atom and 1 hydrogen atom of an *N*-methyl group. From the present study, the computed $O(4) \cdots H(8A)$ separation of 2.45 A in the cation is only slightly less than the sum of Pauling's van der Waals radii (23), 2.6 A, and would be closer to this value if the geometry at the *N*-methyl groups differed only slightly from the ideal arrangement assumed in the derivation of the hydrogen atom positions (see EXPERIMENTS AND RESULTS). A considerable amount of discussion as to whether methyl C—H ··· O

[2] R. W. Baker, C. H. Chothia, and P. J. Pauling, unpublished observations cited by Chothia and Pauling (21).

TABLE 3

Interatomic distances and angles, with estimated standard deviations (in parentheses)

A. Bond lengths			
	A		*A*
C(1)–C(2)	1.491(20)	C(10)–C(20)	1.497(15)
C(2)–O(3)	1.191(17)	C(11)–C(12)	1.514(15)
C(2)–O(4)	1.361(15)	C(12)–C(13)	1.545(17)
O(4)–C(5)	1.455(14)	C(12)–C(14)	1.521(17)
C(5)–C(6)	1.521(15)	C(12)–N(15)	1.489(14)
C(6)–N(7)	1.534(14)	N(15)–O(16)	1.286(11)
N(7)–C(8)	1.487(14)	N(15)–C(17)	1.514(14)
N(7)–C(9)	1.492(15)	C(17)–C(18)	1.535(16)
N(7)–C(10)	1.566(12)	C(17)–C(19)	1.577(16)
C(10)–C(11)	1.542(16)	C(17)–C(20)	1.540(14)
B. Valence angles			
C(1)–C(2)–O(3)	126.8°(12)	C(11)–C(12)–C(13)	110.8°(9)
C(1)–C(2)–O(4)	111.5°(11)	C(11)–C(12)–C(14)	109.0°(9)
O(3)–C(2)–O(4)	121.7°(11)	C(11)–C(12)–N(15)	111.3°(9)
C(2)–O(4)–C(5)	116.0°(9)	C(13)–C(12)–C(14)	109.1°(10)
O(4)–C(5)–C(6)	112.4°(9)	C(13)–C(12)–N(15)	109.0°(9)
C(5)–C(6)–N(7)	116.9°(9)	C(14)–C(12)–N(15)	107.6°(9)
C(6)–N(7)–C(8)	109.0°(8)	C(12)–N(15)–O(16)	118.0°(9)
C(6)–N(7)–C(9)	110.4°(9)	C(12)–N(15)–C(17)	123.3°(8)
C(6)–N(7)–C(10)	106.0°(7)	O(16)–N(15)–C(17)	115.8°(8)
C(8)–N(7)–C(9)	109.1°(9)	N(15)–C(17)–C(18)	110.0°(9)
C(8)–N(7)–C(10)	109.4°(8)	N(15)–C(17)–C(19)	107.1°(8)
C(9)–N(7)–C(10)	112.8°(8)	N(15)–C(17)–C(20)	108.9°(8)
N(7)–C(10)–C(11)	111.4°(8)	C(18)–C(17)–C(19)	108.7°(9)
N(7)–C(10)–C(20)	110.5°(8)	C(18)–C(17)–C(20)	113.4°(9)
C(11)–C(10)–C(20)	106.4°(8)	C(19)–C(17)–C(20)	108.6°(9)
C(10)–C(11)–C(12)	110.3°(8)	C(10)–C(20)–C(17)	110.9°(9)
C. Intercation distances < 3.7 A involving non-hydrogen atoms			
	A		*A*
O(3)···C(8^{II})[a]	2.88	O(3)···C(19^{IV})	3.58
C(9)···O(16^{I})	3.33	C(18)···C(13^{II})	3.59
O(4)···O(16^{I})	3.45	O(4)···C(18^{I})	3.62
C(14)···O(3^{III})	3.53	C(19)···C(9^{V})	3.67
C(8)···O(16^{I})	3.55		

[a] Roman numeral superscripts refer to the following transformations of the coordinates of Table 1:
I: $x, -\frac{1}{2} - y, -\frac{1}{2} + z$.
II: $-\frac{1}{2} - x, -\frac{1}{2} + y, z$.
III: $-\frac{1}{2} + x, -\frac{1}{2} - y, -z$.
IV: $-\frac{1}{2} - x, -1 - y, -\frac{1}{2} + z$.
V: $-1 - x, -1 - y, -z$.

interactions at this distance represent genuine hydrogen bonds has pervaded the literature (24, 25), with the lack of supporting observable spectroscopic evidence implying that they do not satisfy the usual criteria associated with such interactions. Moreover, classification of the distance found in compound I in terms of a van der Waals interaction would be in accord with Baur's (26) assignment of a smaller value, 1.0 A, compared with Pauling's 1.2 A, to the hydrogen atom van der Waals radius, which in turn would require O ··· H distances of less than 2.4 A in order to be considered genuine hydrogen-bonded interactions. On the other hand, the results of quantum mechanical calculations (3, 27) have emphasized that O ··· H interactions

TABLE 4

Torsion angles for enantiomer corresponding to L(+)-muscarine

The angle $A-B-C-D$ is defined as positive if, when viewed along the $B-C$ bond, atom A must be rotated clockwise to eclipse atom D.

C(1)–C(2)–O(4)–C(5)	−179°	C(10)–C(11)–C(12)–C(14)	−166°
O(3)–C(2)–O(4)–C(5)	2°	C(10)–C(11)–C(12)–N(15)	−48°
C(2)–O(4)–C(5)–C(6)	99°	C(11)–C(12)–N(15)–O(16)	−167°
O(4)–C(5)–C(6)–N(7)	78°	C(11)–C(12)–N(15)–C(17)	34°
C(5)–C(6)–N(7)–C(8)	−56°	C(13)–C(12)–N(15)–O(16)	71°
C(5)–C(6)–N(7)–C(9)	64°	C(13)–C(12)–N(15)–C(17)	−89°
C(5)–C(6)–N(7)–C(10)	−174°	C(14)–C(12)–N(15)–O(16)	−47°
C(6)–N(7)–C(10)–C(11)	168°	C(14)–C(12)–N(15)–C(17)	153°
C(6)–N(7)–C(10)–C(20)	−73°	C(12)–N(15)–C(17)–C(18)	91°
C(8)–N(7)–C(10)–C(11)	51°	C(12)–N(15)–C(17)–C(19)	−151°
C(8)–N(7)–C(10)–C(20)	169°	C(12)–N(15)–C(17)–C(20)	−34°
C(9)–N(7)–C(10)–C(11)	−71°	O(16)–N(15)–C(17)–C(18)	−69°
C(9)–N(7)–C(10)–C(20)	46°	O(16)–N(15)–C(17)–C(19)	49°
N(7)–C(10)–C(11)–C(12)	−173°	O(16)–N(15)–C(17)–C(20)	166°
C(20)–C(10)–C(11)–C(12)	67°	N(15)–C(17)–C(20)–C(10)	50°
N(7)–C(10)–C(20)–C(17)	170°	C(18)–C(17)–C(20)–C(10)	−72°
C(11)–C(10)–C(20)–C(17)	−68°	C(19)–C(17)–C(20)–C(10)	167°
C(10)–C(11)–C(12)–C(13)	74°		

arising from Coulombic attraction resulting from delocalization of the positive charge over the entire cationic head are important in stabilizing the *gauche* conformation over the *anti* form. Recent experimental studies on the position of conformational equilibrium in several acetylcholine analogues have suggested (28) that the dominant factor stabilizing the *gauche* form in 5-dimethylsulfonio-2-isopropyl-1,3-dioxanes is probably electrostatic attraction between the positively charged sulfur site and the negatively charged ring oxygen sites, and that the intramolecular methyl ··· oxygen interaction is, in fact, repulsive. Extension of these results to acetylcholine and its analogues would imply that the methyl ··· oxygen interaction is repulsive in these species also. The N—C—C angles in acetylcholine (15), 118°; xylocholine (29), 117°; and the spin-labeled acetylcholine, 117°, are all enlarged from the *n*-paraffin value (30-32), 112.5°.

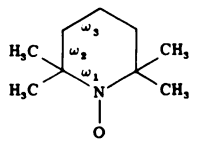
Whereas the C—O—CO—C ester group atoms are coplanar, as is normal for esters because of electron delocalization, the C(2)—O(4)—C(5)—C(6) torsion angle at 99° differs substantially from that for the preferred conformation at primary esters, where the predominant observed geometry is that in which the C=O bond approximately bisects the H—C_α—H angle to give a C–O—C_α—C_β angle of approximately 180° (33). The conformation found in the spin-labeled acetylcholine more closely resembles that encountered in secondary esters, where the C=O group is *syn*-oriented with respect to the C_α—H bond. By adoption of the conformation found here, the C—O—C—C angle lies closer to the muscarine iodide value (144°) than does the corresponding acetylcholine bromide angle of 79°, but it lies outside the range of 180° ± 35° suggested as necessary for potent muscarinic agonists from the results of a number of X-ray diffraction studies (22). Thus either these proposed limits are too narrow or the conformational difference in this region may distinguish between agonist and antagonist activities.

Endocyclic torsion angles for the 2,2,6,6-tetramethylpiperidine-l-oxyl moiety are presented in Table 5, along with those for the 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl radical calculated from the coordinates published by Berliner (34). Comparison of these values shows that replacement of the TEMPOL[3] hydroxyl group by the cholinester moiety to form the spin-labeled acetylcholine results in only a

[3] The abbreviation used is: TEMPOL, 2,2,6,6- tetramethyl-4-piperidinol-1-oxyl.

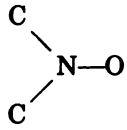
TABLE 5

Endocyclic torsion angles defining the piperidine-1-oxyl moieties of TEMPOL and spin-labeled acetylcholine, averaged assuming C_s symmetry



Angle	TEMPOL	Spin-labeled acetylcholine
ω_1	−30°	−34°
ω_2	45°	49°
ω_3	−62°	−68°

small perturbation in the ring geometry, producing a moderately more puckered conformation as reflected by the slightly larger torsion angles in the spin-labeled compound. In each of these molecules the heterocyclic ring adopts a chair form flattened at the nitroxide end in order to minimize nonbonded 1,3-diaxial interactions between the methyl groups, thereby resulting in C(13) ··· C(18) separations of 3.54 A, in contrast to the much shorter C(12) ··· C(17) distance of 2.64 A. Around the nitroxide nitrogen atom the sum of the valence angles, 357.1°, indicates that the



system is approximately planar, with the nitrogen atom lying 0.142 A from the least-squares plane through C(12), C(17), and O(16). These dimensions are in excellent agreement with the corresponding values in TEMPOL, 357.8° and 0.123 A, established by the reinvestigation of this structure by Berliner (34) following the earlier study by Lajzérowicz-Bonneteau (35).

Elongation of carbon-carbon single bonds at *tert*-butyl groups of substituted cyclohexane rings has been predicted on the basis of theoretical considerations (36, 37) and verified experimentally for these and other overcrowded molecules by the results of a limited number of X-ray diffraction studies (36, 38–43). A further conclusion arising from these calculations is that the minimum energy conformation at an equatorial *tert*-butyl group occurs with a twist of approximately ±17° away from a perfectly staggered form, and small twists of this type have been encountered in *trans*-1-methyl-4-*tert*-butylphosphorinan-4-ol (38). Since a normal C(sp^3)—N^+(sp^3) single bond length (1.510 A) is slightly shorter than a normal C(sp^3)—C(sp^3) bond length (1.535 A), an equatorial —$^+NR_3$ substituent (R = alkyl) should show similar deformations from ideal geometry; indeed, this was revealed by the present study. The ring C(10)—N^+(7) length at 1.566(12) A is slightly longer than the mean of the other three C(sp^3)—N^+(7) bond lengths, 1.504(14) A, and the unequal C—N^+—C—C torsion angles about the C(10)—N^+(7) bond show a mean rotation of 14° from the ideal staggered form. These departures from ideality in the spin-labeled acetylcholine minimize nonbonded steric interactions between hydrogen atoms of the C(11) and C(20) methylene groups and those of the quaternary nitrogen methyl and methylene groups. There is no clear evidence of any conformational transmission effects into the rest of the cholinester moiety as a consequence of these interactions.

The two shorter cation ··· cation separations in the crystal (Table 3) deserve comment. For the first of these, O(3) ··· C(8) 2.88 A, the ester carbonyl oxygen atom O(3) of the reference cation lies close to the local threefold axis at C(8) of a neighboring cation related in the crystal by the *b* glide plane operation and yields distances O(3) ··· H(8A) 2.86, O(3) ··· H(8B) 2.46, O(3) ··· H(8C) 2.86 A. Although the shortest of these three distances is less than the sum of Pauling's van der Waals radii, it is slightly greater than the corresponding sum when Baur's value for hydrogen is used (see above) and does not have the geometry typical of a genuine hydrogen bond. The total arrangement appears to represent a normal van der Waals interaction, in which the methyl hydrogen atoms "cap" the carbonyl oxygen atom and may be of significance with regard to drug ··· receptor interaction. The second short sep-

aration, C(9) ··· O(16) 3.33 A, is between a methyl group of the reference molecule and the nitroxide oxygen of a cation related by the *c* glide plane operation. The atoms involved, C(9), H(9A), O(16), and N(15), are approximately collinear, and thus the geometry differs significantly from that encountered in crystals of TEMPOL (34), where a genuine O—H ··· O(nitroxide) hydrogen bond is present and the N—O ··· H angle is 124.0°. Again we prefer to consider the interaction in the crystals of the spin-labeled analogue as belonging to the van der Waals class.

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