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Structure and Conformation of the Muscarinic Agonists 3-(3-Amino-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.2]octane and 1,2,5,6-Tetrahydro-1-methyl-3-pyridinecarboxaldehyde Oxime and Related Tertiary Amine, Quaternary Ammonium and Sulfonium Analogues

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Abstract

The crystal structures of two muscarinic agonists, 3-(3-amino-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.2]-octane [L-660863, $C_9H_{14}N_4O$, $M_r = 194.24$, triclinic, $P\bar{1}$, $a = 6.607$ (1), $b = 8.2157$ (8), $c = 9.287$ (1) Å, $\alpha = 105.52$ (1), $\beta = 93.88$ (1), $\gamma = 91.79$ (1)°, $V = 484.0$ (1) Å^3, $Z = 2$, $D_x = 1.33$ g cm^{-3}, λ(Mo $K\alpha$) = 0.71073 Å, $\mu = 0.9$ cm^{-1}, $F(000) = 208$, $R = 0.041$ for 1935 reflections with $I > 2.5\sigma(I)$] and 1,2,-5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde oxime monohydrochloride [Org 31956, $C_7H_{13}N_2O^+.Cl^-$, $M_r = 176.64$, triclinic, $P\bar{1}$, $a = 6.843$ (3), $b = 6.997$ (3), $c = 9.837$ (4) Å, $\alpha = 89.72$ (3), $\beta = 87.78$ (4), $\gamma = 75.69$ (4)°, $V = 456.0$ (3) Å^3, $Z = 2$, $D_x = 1.286$ g cm^{-3}, λ(Mo $K\alpha$) = 0.71073 Å, $\mu = 3.7$ cm^{-1}, $F(000) = 188$, $R = 0.046$ for 2195 reflections with $I > 2.5\sigma(I)$] have been determined. A model, based on these crystal structure determinations, Cambridge Structural Database statistics and molecular mechanics calculations, is presented in which the muscarinic agonists L-660863, Org 31956, 1-azabicyclo[2.2.2]octane-3-carboxylic acid methyl ester, arecoline, sulfonium-arecoline, sulfonium-isoarecoline and *N*-methylisoarecoline are matched. A common interaction mode for these reverse ester bioisosteres of acetylcholine is found, provided that the different interaction geometries of quaternary ammonium, protonated tertiary amino and sulfonium groups with a negatively charged receptor site are taken into account. The low muscarinic activity of *N*-methylarecoline and isoarecoline can also be explained using this model. Acetylcholine cannot be fitted into this model, which suggests that the discussed compounds bind to the muscarinic receptor site in a mode that is different from that of acetylcholine.

Introduction

The muscarinic cholinergic receptors mediate in a large number of physiological functions, such as smooth muscle contraction and relaxation, glandular secretion and a number of cardiac functions (Burgen, 1990). The main interest is nowadays directed towards the role of muscarinic receptors in the central nervous system (CNS). Over the past decades it has become clear that a number of muscarinic receptor subtypes exist. In the CNS the M_1 receptor subtype appears to be primarily involved with higher brain functions like learning and memory. The M_2 subtype is mainly involved with sensory and motor functions and vegetative processes (Cortés, Probst, Tobler & Palacios, 1986). Two widely occurring forms of senile cognitive decline, Alzheimer's disease

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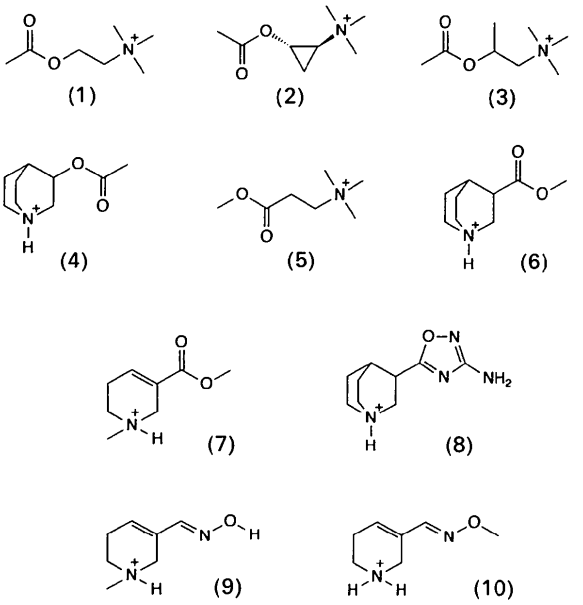
(AD) and senile dementia of the Alzheimer type, are believed to be related to a degradation of certain muscarinic nerve terminals, causing the loss of presynaptic M_2 receptor subtypes (Quirion *et al.*, 1989). One of the possible therapeutic strategies for treatment of AD involves stimulation of the cholinergic nerve system by muscarinic agonists acting directly at postsynaptic receptor sites. Since M_2 receptors may also serve as autoreceptors regulating the acetylcholine level, administration of M_2 selective agonists may result in a detrimental decrease of acetylcholine levels. Research is therefore concentrating on producing M_1 selective agonists that are able to penetrate the blood–brain barrier. A number of substances have been tested, such as arecoline, oxotremorine and pilocarpine. Owing to peripheral cholinergic activity of these compounds, side effects such as nausea, salivation, slight hypotension and flushing occur (Hollander, Mohs & Davis, 1986).

The muscarinic receptor is capable of accommodating a wide range of agonists. The natural ligand is the acetylcholine cation (1). The acetylcholine fragment has been incorporated in a number of potent synthetic muscarinic agonists such as 2-acetyloxycyclopropyltrimethylammonium or ACTM [(2) (Armstrong, Cannon & Long, 1968; Chotia & Pauling, 1970)] and β-methylacetylcholine [(3) (Beckett, Harper & Clithrow, 1963)]. In aceclidine (Lambrecht, 1976) the acetylcholine fragment is included in a quinuclidinyl cage, thereby restricting the flexibility of the O—C—C—N torsion angle. Aceclidine, however, is a tertiary amine, rather than a quaternary ammonium compound like acetylcholine. Aceclidine is expected to interact with the muscarinic receptor as a protonated species (4). Interestingly, the protonated tertiary amino analogue of acetylcholine displays only slight cholinergic activity (Stehle, Melville & Oldham, 1936). The reverse esters of acetylcholine [(5) (Welsh & Taub, 1951)] and aceclidine [(6) (Lambrecht & Mutschler, 1974)] also show muscarinic agonism. Arecoline (7) is another tertiary amine belonging to this reverse ester group.

The two compounds of which the crystal structures are presented here, L-660863 [(8), displayed in the protonated form] and Org 31956 (9), can be accommodated in the group of reverse ester bioisosteres, although neither actually displays an ester function. These molecules can be perfectly matched to those of representative reverse ester bioisosteres of acetylcholine, spatially as well as concerning the orientation of the functional groups. The structure–activity relationships of L-660863 and a large number of its derivatives were recently discussed (Saunders *et al.*, 1990; MacLeod *et al.*, 1990; Street *et al.*, 1990). Org 31956 is a compound based on the muscarinic agonist RU 35963 [(10) (Galliani, 1987)]. Bandoli, Dolmella, Moos, Nicolini & Ongaro (1991) reported the crystal structures of three related oxime compounds.



Experimental

Crystal structure determination of L-660863

Colourless plate-shaped crystals of L-660863 were obtained from Organon International BV. The crystals were grown by evaporation of a solution in methanol and diethyl ether. Data from a crystal of dimensions $1.2 \times 0.6 \times 0.2$ mm were measured at room temperature on an Enraf–Nonius CAD-4 diffractometer with Zr-filtered Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å). Unit-cell parameters were refined by least-squares fitting of 25 reflections with $13.9 < \theta < 19.5°$. The unit-cell parameters were checked for the presence of higher lattice symmetry (Spek, 1988). ω–2θ scan mode, $\Delta\omega = (0.65 + 0.35\tan\theta)°$, 2533 reflections measured up to $\theta = 27.5°$, $\pm h \pm k - l$ (maximum range 8, 10, 12). After merging equivalent reflections ($R_{int} = 0.026$), 1935 unique reflections remained with $I > 2.5\sigma(I)$. Three periodically measured standard reflections ($3\bar{1}0$, $01\bar{4}$, $\bar{1}02$) showed no significant decay in 33 h of X-ray exposure time. Lp correction, no absorption correction applied. Space group $P\bar{1}$ was discriminated from $P1$ during the structure determination process. The structure was solved by automated direct methods using *SHELXS*86 (Sheldrick, 1990). All non-H atoms were found in the best E map of the default run. H atoms were located on subsequent difference Fourier maps. In the final cycles of full-matrix least-squares refinement, using *SHELX*76 (Sheldrick, 1976), 170 parameters were refined, including an overall scale

factor, positional parameters for all atoms, overall isotropic thermal parameter for the H atoms and anisotropic thermal parameters for all non-H atoms. The refinement on F converged at $R = 0.041$ and $wR = 0.058$, where $w = 1/[\sigma^2(F_o) + 0.00012F_o^2]$, $S = 0.22$. $\Delta/\sigma = 0.084$ (av.) and 0.31 (max.) for all parameters; final residual electron density $-0.18 < \Delta\rho < 0.22$ e Å^{-3}.

Crystal structure determination of Org 31956

Colourless rod-shaped crystals of Org 31956 were obtained from Organon International BV. The crystals were grown by evaporation of a solution in 2-propanol and petroleum ether. Data from a crystal of dimensions 0.2 × 0.2 × 1.0 mm were measured at room temperature on an Enraf–Nonius CAD-4 diffractometer with Zr-filtered Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å). Unit-cell parameters were refined by least-squares fitting of 25 reflections with $14 < \theta < 21°$. The unit-cell parameters were checked for the presence of higher lattice symmetry (Spek, 1988). ω–2θ scan mode, $\Delta\omega = (0.60 + 0.35\tan\theta)°$, 5862 reflections measured up to $\theta = 30.3°$, $\pm h - k \pm l$ (maximum range 9, 9, 13). After merging equivalent reflections ($R_{int} = 0.012$), a unique set of 2195 reflections with $I > 2.5\sigma(I)$ remained. Three periodically measured standard relections ($10\bar{2}$, $\bar{2}20$, $02\bar{1}$) showed intensity variations less than 2% in 90 h of X-ray exposure time. Lp correction, no absorption correction applied. Space group $P\bar{1}$ was discriminated from $P1$ during the structure determination process. The structure was solved by automated direct methods using *SHELXS*86 (Sheldrick, 1990). All non-H atoms were found in the best E map of the default run. H atoms were located on a difference Fourier map. In the final cycles of full-matrix least-squares refinement, using *SHELX*76 (Sheldrick, 1976), 141 parameters were refined, including overall scale factor, positional parameters for all atoms, overall isotropic thermal parameters for H atoms (one parameter for the methyl and oxime H atoms, and one for the other H atoms) and anisotropic thermal parameters for all non-H atoms. The refinement on F converged at $R = 0.046$ and $wR = 0.065$, where $w = 1/[\sigma^2(F_o) + 0.00142F_o^2]$, $S = 0.25$. $\Delta/\sigma = 0.085$ (av.) and 0.88 (max.) for all parameters; final residual electron density between -0.32 and 0.49 e Å^{-3}.

Scattering factors were taken from Cromer & Mann (1968); anomalous-dispersion corrections from Cromer & Liberman (1970). Geometric calculations were performed with the *EUCLID* package (Spek, 1982).

Molecular mechanics calculations

In order to assess the conformational flexibility of these two compounds molecular mechanics calculations were performed, using the *MMP*2(85) and *MMP*2(87) force fields (Allinger, 1985, 1987). A number of parameters involving the five-membered heterocyclic aromatic ring, as present in L-660863, are not included in the *MM*2 force field. The missing parameters are all concerned with the aromatic O···N bond, represented by the *MM*2 types 41–37. The additional parameters we derived* are based on Cambridge Structural Database statistics and semi-empirical quantum-mechanical calculations performed with the *MOPAC* package (Stewart, 1989). The conjugated oxime group of Org 31956 also needed extra parametrization. We used the atom types 6 and 37 to represent the O and N atoms in this group. When dealing with conjugated oximes, as in Org 31956, the N atom is included in the π system. These parameters should not be used, however, to predict details concerning the geometry of the oxime group itself.

A number of test compounds containing heterocyclic five-membered rings or conjugated oximes were retrieved from the Cambridge Structural Database. All geometries could be reproduced within 0.02 Å or 3° for bond lengths and valence angles concerned with the new parameters, respectively. Relative lengths and angles were correctly calculated in all cases.

The experimental dipole moments of the heterocyclic five-membered-ring compounds furan, oxazole, isoxazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole (Hellwege, 1967) could be reproduced within reasonble limits. Since the directionality of the σ and π dipole moments is defined with regard to different sets of basis vectors in some versions of the *MM*2 program, care must be taken that both contributions are correctly added. The largest deviation of the calculated dipole moment from the observed value (0.4 D) was found for 1,3,4-oxadiazole, in which the new parameter for the dipole moment of the O···N bond is not involved; all other dipole moments were reproduced within 0.3 D (1 D ≡ 3.336×10^{-30} C m).

Discussion

Molecular structure and conformational aspects of L-660863 and related compounds

The final atomic parameters and U_{eq} values of the non-H atoms of L-660863 (8) are given in Table 1.* The intramolecular dimensions involving the non-H

* Lists of structure factors, anisotropic displacement parameters, H-atom coordinates, bond distances and bond angles for both compounds, and derived *MM*2 parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 55722 (46 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England. [CIF reference: AB0288]

Table 1. *Fractional atomic coordinates and equivalent isotropic thermal parameters of the non-H atoms of L-660863 (8)*

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

	x	y	z	U_{eq} (Å^2)
O(14)	0.9127 (1)	0.3241 (1)	0.0397 (1)	0.0467 (3)
N(1)	1.3363 (2)	0.2418 (2)	0.4383 (1)	0.0398 (3)
N(10)	0.9175 (2)	0.5099 (1)	0.2605 (1)	0.0412 (3)
N(12)	0.6157 (2)	0.6449 (2)	0.2176 (2)	0.0526 (4)
N(13)	0.7328 (2)	0.4161 (2)	0.0369 (1)	0.0451 (3)
C(2)	1.4966 (3)	0.1522 (3)	0.3533 (2)	0.0573 (5)
C(3)	1.4282 (3)	0.0795 (2)	0.1857 (2)	0.0519 (5)
C(4)	1.2095 (2)	0.1272 (2)	0.1591 (1)	0.0412 (4)
C(5)	1.2089 (2)	0.3215 (2)	0.2081 (1)	0.0372 (4)
C(6)	1.2806 (3)	0.3822 (2)	0.3780 (2)	0.0473 (4)
C(7)	1.1565 (3)	0.1268 (3)	0.4213 (2)	0.0582 (6)
C(8)	1.0735 (3)	0.0562 (2)	0.2569 (2)	0.0506 (5)
C(9)	1.0097 (2)	0.3894 (2)	0.1756 (1)	0.0361 (3)
C(11)	0.7478 (2)	0.5244 (2)	0.1702 (1)	0.0377 (4)

Table 2. *Bond lengths* (Å), *bond angles* (°) *and selected torsion angles* (°) *of L-660863, with e.s.d.'s in parentheses*

O(14)—N(13)	1.431 (2)	N(13)—C(11)	1.312 (2)
O(14)—C(9)	1.340 (2)	C(2)—C(3)	1.540 (2)
N(1)—C(2)	1.462 (2)	C(3)—C(4)	1.530 (2)
N(1)—C(6)	1.457 (2)	C(4)—C(5)	1.539 (2)
N(1)—C(7)	1.467 (2)	C(4)—C(8)	1.527 (2)
N(10)—C(9)	1.288 (2)	C(5)—C(6)	1.558 (2)
N(10)—C(11)	1.382 (2)	C(5)—C(9)	1.489 (2)
N(12)—C(11)	1.345 (2)	C(7)—C(8)	1.537 (2)

N(13)—O(14)—C(9)	106.2 (1)	C(4)—C(5)—C(9)	113.8 (1)
C(2)—N(1)—C(6)	108.9 (1)	C(6)—C(5)—C(9)	111.6 (1)
C(2)—N(1)—C(7)	109.5 (1)	N(1)—C(6)—C(5)	112.0 (1)
C(6)—N(1)—C(7)	108.4 (1)	N(1)—C(7)—C(8)	112.5 (1)
C(9)—N(10)—C(11)	102.9 (1)	C(4)—C(8)—C(7)	108.4 (2)
O(14)—N(13)—C(11)	102.6 (1)	O(14)—C(9)—N(10)	113.8 (1)
N(1)—C(2)—C(3)	112.3 (2)	O(14)—C(9)—C(5)	117.9 (1)
C(2)—C(3)—C(4)	108.5 (1)	N(10)—C(9)—C(5)	128.3 (1)
C(3)—C(4)—C(5)	107.1 (1)	N(10)—C(11)—N(12)	121.0 (1)
C(3)—C(4)—C(8)	109.2 (1)	N(10)—C(11)—N(13)	114.6 (1)
C(5)—C(4)—C(8)	108.8 (1)	N(12)—C(11)—N(13)	124.4 (1)
C(4)—C(5)—C(6)	108.0 (1)		

C(9)—O(14)—N(13)—C(11)	0.6 (1)	O(14)—N(13)—C(11)—N(10)	−1.2 (2)
N(13)—O(14)—C(9)—N(10)	0.2 (2)	N(1)—C(2)—C(3)—C(4)	0.9 (2)
C(2)—N(1)—C(6)—C(5)	57.8 (2)	C(2)—C(3)—C(4)—C(5)	59.0 (2)
C(6)—N(1)—C(7)—C(8)	59.5 (2)	C(8)—C(4)—C(5)—C(6)	57.0 (2)
C(7)—N(1)—C(2)—C(3)	57.9 (2)	C(3)—C(4)—C(8)—C(7)	57.6 (2)
C(11)—N(10)—C(9)—O(14)	−0.8 (2)	C(6)—C(5)—C(9)—N(10)	14.3 (2)
C(9)—N(10)—C(11)—N(12)	−176.8 (1)	N(1)—C(7)—C(8)—C(4)	1.0 (2)
		C(9)—C(5)—C(6)—N(1)	128.8 (1)

atoms are listed in Table 2. The molecular conformation and the atom-numbering scheme are shown in Fig. 1.

The heterocyclic five-membered ring of L-660863 is planar; the maximum deviation from the least-squares plane, including C(5) and N(12), amounts to 0.041 (3) Å. The orientation of the five-membered ring with respect to the quinuclidinyl fragment is indicated by the torsion angle τ[C(6)—C(5)—C(9)—N(10)] = ± 14.3 (2)°. The NH_2 group shows a slight deviation from planarity; the mean covalent angle around the central N atom is 118 (2)°. Allen *et al.* (1987) consider an N atom to have a planar sp^2 hybridization if the mean covalent angle is larger than 117.5°.

The molecular packing is shown in Fig. 2. The molecules are linked in an infinite one-dimensional chain (base vector [101]) by hydrogen bonds N(12)—H(121)···N(13)($1-x$, $1-y$, $-z$) with N—H = 0.85 (2), H···N = 2.28 (2) Å and N—H···N = 165 (2)°, and N(12)—H(122)···N(1)($2-x$, $1-y$, $1-z$) with N—H = 0.88 (2), H···N = 2.21 (2) Å and N—H···N = 165 (2)°. O(14) is not involved in any hydrogen bond, its closest contact (2.85 Å) is to H(81)($2-x$, $-y$, $-z$), nor is N(10). Electrostatic potential calculations suggest that N(10), but not O(14), should be considered as an acceptor candidate in receptor binding (Saunders *et al.*, 1990).

Saunders *et al.* (1990) reported the rotation of the five-membered ring around the C(5)—C(9) bond to be virtually without barrier, *i.e.* less than 4 kJ mol^{-1}. Our calculations also show low barriers, with a maximum relative steric energy of 7.95 kJ mol^{-1} for this torsion angle. In the *S* enantiomer, the absolute minimum was located at τ[C(6)—C(5)—C(9)—N(10)] = 135°; local minima were found at −20 and −135° with relative steric energies of 0.4 and 3.3 kJ mol^{-1}, respectively. Saunders *et al.* (1990) reported only two minima at −40 and +143°, without specifying the chirality of C(5). Only a few compounds containing an aliphatic side chain, with at least two C atoms, attached to a five-membered aromatic ring containing O and N could be located in the Cambridge Structural Database, so no meaningful statistics could be derived from these data. The observed torsion angles appear to favour conformations with the C—C—C—O angle staggered and the C—C—C—N angle eclipsed, which is in agreement with our calculations. The orientation of the five-membered ring in the crystal structure can be expected to be influenced by the molecular packing,

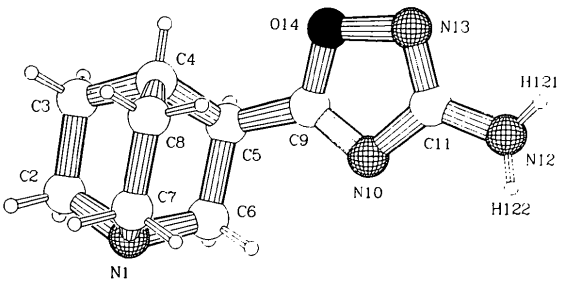


Fig. 1. Perspective view of L-660863 (8) with atomic labelling.

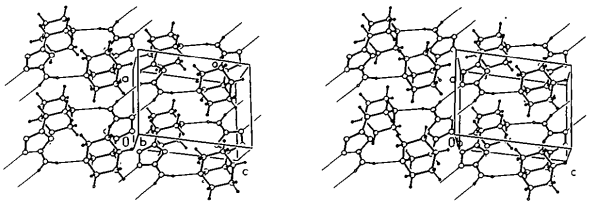


Fig. 2. Crystal packing of L-660863 (8), viewed along **b**.

for instance to optimize the position of the hydrogen-bond acceptors N(10) and N(13). Yet the X-ray conformation of L-660863 appears to be close to a local minimum.

The analogues of L-660863 in which one of the N atoms in the five-membered ring is replaced by a C atom show a decrease in activity. Replacement of N(13) with an sp^2 CH group has virtually no effect on the barrier height. After replacing N(10) with an sp^2 CH group, however, the shape of the potential function is changed. Minima now occur at -45, 35 and 135°, with steric energies of 0.0, 2.1 and 0.0 kJ mol^{-1}, respectively. The maximum energy barrier is 7.5 kJ mol^{-1}, which is equal to that of L-660863. The low energy barriers for these compounds show that a relatively low interaction energy with the receptor is sufficient to stabilize the investigated torsion angle at virtually any value. These results support the model of Saunders *et al.* (1990), which ascribes the loss of activity to the change of the electrostatic potential, rather than to conformational restrictions.

Within 12.6 kJ mol^{-1}, the torsion angle N(1)—C(2)—C(3)—C(4) of the quinuclidinyl fragment is allowed to vary between -35 and $+30$°. The energy function appears to be slightly asymmetrical, with the minimum located at *ca* -10°. The conformation observed in the crystal structure displays a value of 0.9 (2)° for this torsion angle. In the Cambridge Structural Database, torsion angles in quinuclidinyl compounds range up to an absolute value of 25°. Some examples of extreme torsion angles in this class of compounds are reported by Oleksyn, Lebioda & Ciechanowicz-Rutkowska (1978) and Karle & Karle (1981).

L-660863 shows a clear resemblance to methyl quinuclidine-3-carboxylate [1-azabicyclo[2.2.2]-octane-3-carboxylic acid methyl ester (6)], which also displays muscarinic activity. Activities of the discussed compounds are summarized in Table 3. Fig. 3 shows a match of these two compounds. The conformation of methyl quinuclidine-3-carboxylate was constructed by a modelling procedure and subsequent energy minimization. The match has been made using the protonated forms, in which the molecules are expected to interact with the receptor site. It appears to be possible to align both the N—H bonds of the protonated tertiary amino groups as well as the C=X bonds of the electron-rich parts of the molecules, with X = O in methyl quinuclidine-3-carboxylate and X = N in L-660863. A satisfactory spatial overlap is realized in this superposition of functional groups.

The torsion angle C—C—C=O in (*S*)-methyl quinuclidine-3-carboxylate, with the first two C atoms referring to an ethylene bridge of the quinuclidinyl group, shows conformational behaviour similar to that of the C(6)—C(5)—C(9)—N(10) torsion angle in L-660863, with the global minimum at 130°, and local minima at 0 and -130° with relative steric energies of 1.3 and 7.1 kJ mol^{-1}. The maximum barrier of 8.79 kJ mol^{-1} is not significantly higher than in L-660863.

Table 3. *Pharmacological activity of some of the compounds discussed*

The columns marked NMS and OXO-M give the pK_{app} value for the displacement of *N*-methylscopolamine and oxotremorine-M, expressed as affinity constants. Oxotremorine-M is a non-selective muscarinic agonist, *N*-methylscopolamine is an M_2 selective antagonist. The EPMR (equipotent molar ratio) is the ratio of the amount of the agonist studied to that of acetylcholine (ACh), needed to produce the same muscarinic effect.

	NMS (*a*)	OXO-M	EPMR (*b*) (relative to ACh)
L-660863 (8)	6.4	9.1 (*a*)	
analogue, N(10) replaced by CH	5.9	7.5 (*a*)	
analogue, N(13) replaced by CH	5.9	7.6 (*a*)	
Methyl quinuclidine-3-carboxylate (6)		7.4 (*c*)	
Org 31956 (9)		6.1 (*c*)	
Arecoline (7)	5.2	8.0 (*a*)	2
N-Methylarecoline (15)			140
Sulfonium-arecoline (13)			14
Sulfonium-isoarecoline (14)			16
Isoarecoline (16)			200
N-Methylisoarecoline (17)			16

Notes: (*a*) Saunders *et al.* (1990); (*b*) Jensen (1984); (*c*) de Boer (1991).

Molecular structure and conformational aspects of Org 31956 *and related compounds*

The final atomic parameters and U_{eq} values of the non-H atoms of Org 31956 (9) are given in Table 4. Geometrical parameters involving the non-H atoms are listed in Table 5. The molecular conformation and the atom-numbering scheme are shown in Fig. 4.

The unsaturated six-membered ring of Org 31956 is in a half-chair conformation, as is illustrated by the asymmetry parameter ΔC_2[N(1)—C(3)] = 4.5 (2)° (Duax & Norton, 1975). The oxime fragment and the conjugated double bond C(5)—C(6) are coplanar in an *E* conformation. The maximum deviation from

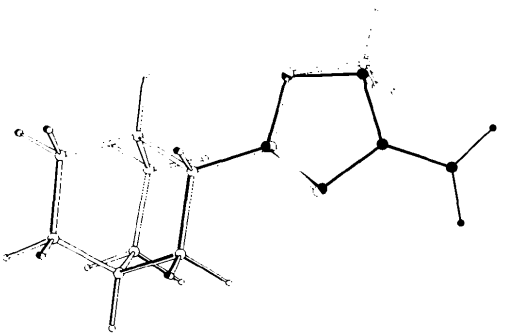


Fig. 3. Match of methyl quinuclidine-3-carboxylate [(6), open lines] with L-660863 [(8), solid lines].

Table 4. *Fractional atomic coordinates and equivalent isotropic thermal parameters of the non-H atoms of Org 31956 (9)*

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

	x	y	z	U_{eq} (Å^2)
O(10)	0.3658 (2)	0.7037 (2)	0.2406 (1)	0.0561 (4)
N(1)	0.1821 (2)	0.7197 (2)	−0.3129 (1)	0.0382 (3)
N(9)	0.3420 (2)	0.7261 (2)	0.0993 (1)	0.0435 (4)
C(2)	0.3269 (3)	0.7491 (3)	−0.4229 (2)	0.0522 (5)
C(3)	−0.0284 (3)	0.8387 (2)	−0.3342 (2)	0.0467 (4)
C(4)	−0.1728 (3)	0.7754 (3)	−0.2337 (2)	0.0501 (5)
C(5)	−0.0938 (3)	0.7508 (2)	−0.0938 (2)	0.0437 (4)
C(6)	0.0966 (2)	0.7457 (2)	−0.0674 (1)	0.0356 (4)
C(7)	0.2515 (2)	0.7597 (2)	−0.1764 (1)	0.0374 (4)
C(8)	0.1622 (2)	0.7253 (2)	0.0724 (2)	0.0403 (4)
Cl(1)	0.22435 (6)	0.27513 (5)	0.66177 (4)	0.0474 (1)

Table 5. *Bond lengths* (Å), *bond angles* (°) *and selected torsion angles* (°) *of Org* 31956, *with e.s.d.'s in parentheses*

O(10)—N(9)	1.409 (2)	C(3)—C(4)	1.512 (3)
N(1)—C(2)	1.487 (2)	C(4)—C(5)	1.492 (3)
N(1)—C(3)	1.497 (2)	C(5)—C(6)	1.330 (3)
N(1)—C(7)	1.492 (2)	C(6)—C(7)	1.498 (2)
N(9)—C(8)	1.270 (2)	C(6)—C(8)	1.459 (2)

C(2)—N(1)—C(3)	112.5 (1)	C(4)—C(5)—C(6)	123.0 (2)
C(2)—N(1)—C(7)	111.2 (1)	C(5)—C(6)—C(7)	122.7 (1)
C(3)—N(1)—C(7)	111.2 (1)	C(5)—C(6)—C(8)	120.0 (1)
O(10)—N(9)—C(8)	109.2 (1)	C(7)—C(6)—C(8)	117.2 (1)
N(1)—C(3)—C(4)	109.6 (1)	N(1)—C(7)—C(6)	110.7 (1)
C(3)—C(4)—C(5)	112.1 (2)	N(9)—C(8)—C(6)	120.6 (1)

C(2)—N(1)—C(3)—C(4)	170.1 (2)	C(3)—C(4)—C(5)—C(6)	−12.8 (2)
C(3)—N(1)—C(7)—C(6)	49.2 (2)	C(4)—C(5)—C(6)—C(7)	−1.4 (2)
O(1)—N(9)—C(8)—C(6)	−179.8 (2)	C(8)—C(6)—C(7)—N(1)	163.1 (1)
N(1)—C(3)—C(4)—C(5)	44.3 (2)	C(7)—C(6)—C(8)—N(9)	2.2 (2)

the least-squares plane through C(4), C(7) and the conjugated π system [including O(10)], is 0.023 (3) Å. The *N*-methyl group has taken an equatorial position.

The molecular packing is shown in Fig. 5. A cyclic dimer of the asymmetric unit is formed by hydrogen bonds N(1)—H(11)···Cl(1)(x, y, $z-1$) with N—H = 0.86 (1), H···Cl = 2.22 (1) Å and N—H···Cl = 167 (1)°, and O(10)—H(101)···Cl(1)($1-x$, $1-y$, $1-z$) with O—H = 0.87 (1), H···Cl = 2.19 (1) Å and O—H···Cl = 165 (1)°. N(9) is not involved in the hydrogen-bonding scheme.

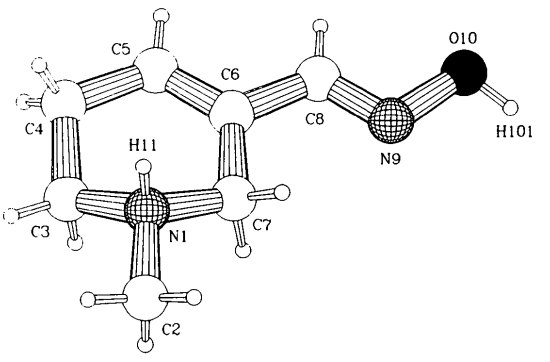


Fig. 4. Perspective view of Org 31956 (9) with atomic labelling.

The six-membered ring of Org 31956 can adopt two half-chair conformations. Fig. 6 shows the results of an *MM*2 calculation in which the torsion angles C(6)—C(7)—N(1)—C(3) and C(7)—N(1)—C(3)—C(4) were varied using the prudent ascent method (Hooft, Kanters & Kroon, 1991). The two minima correspond to the two half-chair conformations. There are two routes to invert the half-chair conformation, both involving a boat conformation at the saddle-point. If the half-chair conformation of Org 31956 is inverted, the *N*-methyl group changes from an equatorial to an axial position. The amine N atom is capable of pyramidal inversion in the unprotonated form, which transforms the axial *N*-methyl group back to an equatorial position. Ridell & Labaziewicz (1975) reported the energy barrier for this conversion to be 37.7 kJ mol^{-1}. After applying both a half-chair inversion and an amine pyramidal inversion, the mirror image of the molecule is obtained. The steric energy of the half-chairs being the same, the energy difference of +7.5 kJ mol^{-1} between the minima in Fig. 6 is solely produced by the conversion of the *N*-methyl group from an equatorial to an axial position. The appearance of the energy map drawn in Fig. 6 does not change significantly when such a map is calculated for Org 31956 with the conjugated double bond–oxime group in a *Z* conformation (see below) or arecoline.

However, if the torsion angles C(5)—C(6)—C(7)—N(1) and C(3)—C(4)—C(5)—C(6) of Org 31956 are varied, a map with a completely different appearance is obtained. This energy map displays parts of the two separate routes between the minima. As a consequence of the method used [see Hooft *et al.* (1991)] only the lowest energy conformations of each route, given the specified values for the driven torsion angles, are maintained in the final result. Therefore two neighbouring points in the energy map are not necessarily calculated consecutively. In this particular case a sharp ridge is displayed between the minima, which is not a genuine saddle-point. It is therefore impossible to determine the real barrier height between both minima from this particular energy map.

The energy difference between the *Z* and *E* conformations of the conjugated double-bond oxime fragment amounts to +9.6 kJ mol^{-1} with a barrier of

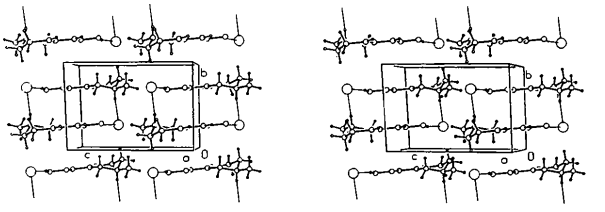


Fig. 5. Crystal packing of Org 31956 (9), viewed along **a**.

37.7 kJ mol^{-1}. In arecoline (7), which resembles Org 31956 closely in regard to the molecular structure (see below), this energy difference is also +9.6 kJ mol^{-1}. The barrier is 34.3 kJ mol^{-1}, which is somewhat lower than the value reported by Krogsgaard-Larsen, Jensen, Falch & Jørgensen (1989). Arecoline is most likely to interact in a *Z* conformation with the receptor, as was shown by means of rigidized analogues of arecoline (Krogsgaard-Larsen *et al.*, 1989). Therefore, when comparing Org 31956 with arecoline, we used the *Z* conformations of both molecules. A good match can be obtained, in which both the N—H and the C=*X* groups (*X* = O in arecoline and N in Org 31956) are matched, as well as the general shape of the molecules (see Fig. 7). Apparently the methoxy group of arecoline is not essential for activity. The activity of arecoline is, however, significantly higher than that of Org 31956, so it is reasonable to conclude that the methoxy group does enhance activity.

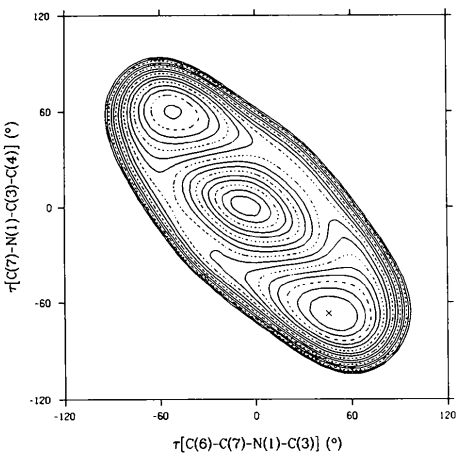


Fig. 6. Steric energy of Org 31956 (9), as a function of C(6)—C(7)—N(1)—C(3) and C(7)—N(1)—C(3)—C(4), calculated with *MM2P*(85). Contour lines are drawn at an interval of 4.184 kJ mol^{-1}, contour lines are dashed at an interval of 12.6 kJ mol^{-1}, with a longer dash applied to the 12.6 kJ mol^{-1} level. The conformation as observed in the crystal structure is indicated with ×.

Comparison of the conformation of L-660863 and Org 31956

To superimpose all of the discussed compounds, some changes in the hitherto assumed conformations have to be made. In order to give a good match of the N—H bonds, the *N*-methyl groups of Org 31956 and arecoline have to be positioned in axial conformations. With this position of the methyl group, the quinuclidinyl group of L-660863 and the six-membered ring of Org 31956 give a good spatial match. The chirality of C(5) in L-660863 determines which half-chair conformation of Org 31956 provides the best match (the free base of which is not chiral). Snow, Friedman, Baker & Saunders (1989) suggest L-660863 to be active in the *S* configuration. The electron-rich groups C=N and C=O, in L-660863 and methyl quinuclidine-3-carboxylate, respectively, have to be reoriented in order to give a good overlap with the equivalent groups of arecoline and Org 31956. This requires L-660863 and methyl quinuclidine-3-carboxylate to adopt a conformation indicated as an energy maximum in the torsional potential calculated for the isolated molecule. However, the relative energy with respect to the absolute minimum is only 8.8 or 3.8 kJ mol^{-1} for methyl quinuclidine-3-carboxylate and L-660863, respectively. The local minimum of arecoline with the conjugated system in the *Z* conformation lies 9.6 kJ mol^{-1} above the global minimum. Combined with the half-chair inversion necessary to bring the *N*-methyl group of this compound into an axial position, the steric energy of this conformation lies 17.2 kJ mol^{-1} above the global minimum which is rather high. It should be stressed that all compounds can adopt a conformation equivalent to the *E* conformation of arecoline, which is lower in energy than the conformations equivalent to an arecoline *Z* conformation. The reason to suggest that these energeti-

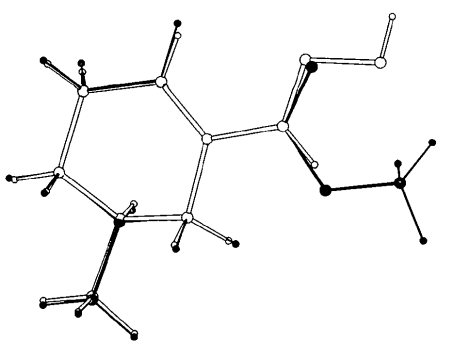


Fig. 7. Match of Org 31956 [(9), open lines] with arecoline [(7), solid lines].

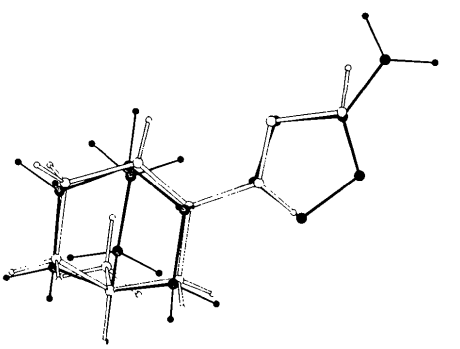
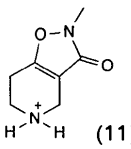


Fig. 8. Match of Org 31956 [(9), open lines] with L-660863 [(8), solid lines].

cally unfavourable conformations are the active ones is the activity of the arecoline analogue *O*-methyl THPO (12), in which the conjugated system is rigidized in a *Z* conformation. The inactive *N*-methyl THPO (11) displays a rigidized *E* conformation (Krogsgaard-Larsen *et al.*, 1989). These findings strongly suggest that the *Z* conformation is the active one. The interaction energy of the receptor ligand complex should be able to return this energy investment. Calculations with a dielectric constant $\varepsilon = 80$, which may give an indication of the situation in solution, display a slightly lower energy difference of 8.8 kJ mol^{-1} between *E* and *Z* conformations.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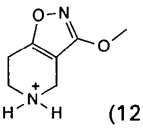

(12)

In norarecoline, which lacks the *N*-methyl group, both half-chair conformations are of equal energy. Despite the lower energy investment needed to adopt the active conformation, norarecoline is slightly less active than arecoline, which suggests that the *N*-methyl group is needed to stabilize the ligand in the receptor cavity.

In Fig. 8, the match between Org 31956 with *N*-methyl in axial position and the oxime group in a *Z* conformation with respect to the double bond, and L-660863 with the C(6)—C(5)—C(9)=N(10) torsion angle at 180° is displayed. Arecoline and methyl quinuclidine-3-carboxylate are omitted for clarity, but can be perfectly matched if equivalent restrictions are imposed. All four compounds in the obtained match have an electron-rich group in common, which is oriented in the direction of a postulated receptor site. Whether this receptor site involves a hydrogen-bond-donating moiety (Saunders & Freedman, 1989) or, for instance, an interaction with a π system cannot be decided by these models.

Comparison of L-660863 and Org 31956 with acetylcholine

One is tempted to superimpose acetylcholine on the match of four molecules described above. A spatial match of acetylcholine (1) on the combined model can indeed be found. In this match the ammonium group is fitted in such an orientation that the postulated receptor site towards which the N—H groups of the four discussed compounds form hydrogen bonds is in contact with the triangle formed by the three methyl groups of the acetylcholine quaternary head group (see below). However, in order to obtain a good spatial match, the acetylcholine molecule has to be stretched completely, *i.e.* both the C—O—C—C and the O—C—C—N torsion angles of acetylcholine have to adopt a value of 180°. Although there is considerable discussion in the literature (Schulman, Sabio & Dish, 1983; Kokkinidis & Gieren, 1984; Kooijman, Kanters & Kroon, 1990) concerning the active conformation of acetylcholine, none of these authors propose a completely stretched structure. While acetylcholine itself can easily adopt this conformation, other compounds such as muscarine cannot within 25.1 kJ mol^{-1} (Kooijman *et al.*, 1990). ACTM (2) cannot adopt this conformation without breaking the cyclopropane ring. In the superposition obtained, the functional groups of acetylcholine do not match very well with those of the other four compounds. Another argument opposing this match is the fact that α-methylacetylcholine can also be perfectly spatially matched on this model, while it is inactive. The active compound β-methylacetylcholine (3) has to be forced into an energetically unfavourable atomic arrangement and even then does not fit the model very well because of the conspicuous extension of the β-methyl group out of the overlapping volume. Therefore we concluded that acetylcholine cannot be fitted into this model. Apparently acetylcholine interacts in a different way or with other groups of the muscarinic receptor than do the inverse ester bioisosteres. If the postulated negative receptor site involves a carboxylate group, it might be feasible that acetylcholine interacts primarily with one O atom of this group and the reverse ester bioisosteres with the other.

Comparison of L-660863 and Org 31956 with sulfonium analogues of arecoline

In order to obtain rigid derivatives, which do not show amine pyramidal inversion, some sulfonium analogues of the discussed tertiary amino compounds were synthesized (Mutschler, Höltje, Lambrecht & Moser, 1983; Höltje, Lambrecht, Moser & Mutschler, 1983). However, care should be taken in comparing sulfonium analogues with tertiary amines. Using the Cambridge Structural Database, Britton & Dunitz (1980) showed the approach of a nucleophile towards and S^+ atom to be preferentially directed opposite to a C—S^+ bond. We repeated their search for sulfonium groups and expanded the investigation to include $C_3N^+H\cdots X^-$ and $C_4N^+\cdots X^-$ groups as well as $C_3S^+\cdots X^-$ interactions. The X^- atom could be either $O^{(-)}$, F^-, Cl^-, Br^- or I^-. The restriction on the C_4N^+ compounds was that at least one of the C atoms should be a methyl group, in order to allow the *X* atom sufficient space to approach the onium centre. The results of these statistics are presented in Fig. 9. In order to compare the interaction geometries of the three above-mentioned groups, we

defined geometrical parameters different from those of Britton & Dunitz. The geometrical mean of the three C atoms (in C_4N^+ the three unrestricted C atoms) is denoted by ⟨C⟩. Of interest is the angle φ between the vectors ⟨C⟩···A^+ and A^+···X^-, with $A^+ = N^+$ or S^+. An angle $\varphi = 0°$ denotes a linear interaction ⟨C⟩···A^+···X^-; $\varphi = 180°$ denotes the linear system X^-···⟨C⟩···A^+. The geometry reported by Britton & Dunitz in which the X^- ion is opposite a C—S^+ bond is indicated with $\varphi \simeq 60°$. Fig. 9(*b*) shows that the C_3N^+H group preferentially forms a linear hydrogen bond to X^-. The C_4N^+ and C_3S^+ groups are preferentially approached in the same way, *i.e.* opposite a C—N or C—S bond. Further analysis of the C_3S^+···X^- system shows that at $\varphi = 120°$ the improper dihedral angle C···⟨C⟩···S^+···X^- favours 'staggered' values. At $\varphi = 60°$ this torsion angle shows less preference, although some clustering around staggered values is still observed.

In conclusion one might state that, as far as intermolecular interactions are concerned, the sulfonium centre can be considered as an alternative of the quaternary ammonium group, rather than the protonated tertiary amino group. The sulfonium group allows a counter group a closer approach to the onium centre than does the quaternary ammonium group. In addition, the six-membered ring of the sulfonium analogue (13) of arecoline shows a different energy map to that of arecoline. While in the latter only the two half-chair conformations are present as local minima, the sulfonium compound shows besides the half-chair minima two other minima in which the ring has adopted a boat conformation (see Fig. 10), so sulfonium-arecoline has more minima, albeit at considerably higher energy values (*ca* 20.9 kJ mol^{-1}). Sulfonium-isoarecoline (14), in which the ester group is linked to position 4 of the six-membered ring, shows virtually the same energy map as sulfonium-arecoline. In Fig. 11 a tentative superposition of sulfonium-arecoline, sulfonium-isoarecoline and arecoline is given, taking into account the above considerations concerning the interaction of a sulfonium group with the postulated negative receptor site. An O atom is included in Fig. 11 to indicate the position of the postulated negatively charged receptor site. The best match could be obtained with sulfonium-arecoline in a boat conformation. A postulated negatively charged receptor site, represented by an O atom, is included in the match. The N—H···O geometry is defined by N—H···O = 170° and N···O = 2.7 Å. For sulfonium-arecoline the S···O distance is 3.3 Å and $\varphi = 45°$, which is within the range found in the Cambridge Structural Database (see Fig. 9*a*). Sulfonium-isoarecoline can also interact in a boat conformation, with S···O = 3.2 Å and $\varphi = 65°$. The boat conformation adopted by sulfonium-isoarecoline is the alternative boat to that displayed by sulfonium-arecoline, with the *S*-methyl group in an equatorial position. In Fig. 11 sulfonium-isoarecoline is presented in the *E*

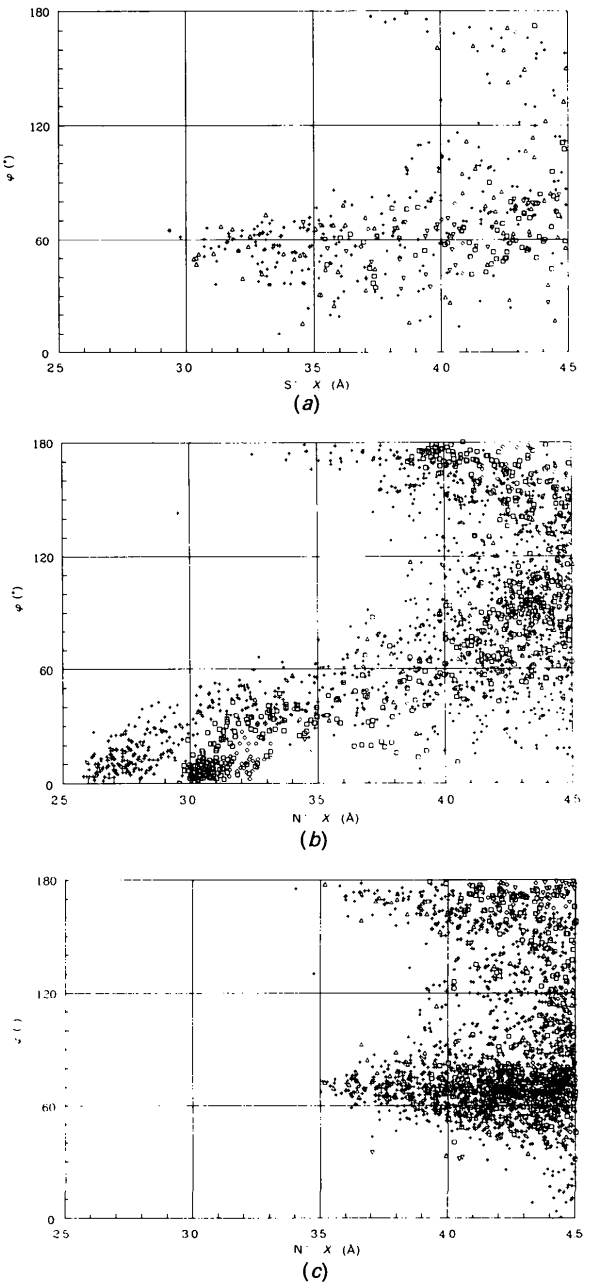
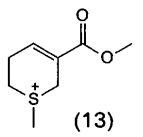


Fig. 9. Crystal structure statistics of the intermolecular geometry of (*a*) the C_3S^+···X^-, (*b*) the C_3N^+H···X and (*c*) the C_4N^+···X^- systems with at least one methyl group bonded to the quaternary nitrogen. X can be Br (indicated with ◇ in the diagrams), Cl^- (□), F^- (△), I^- (▽) or $O^{(-)}$ (+). φ is the angle between the vectors ⟨C⟩···A^+ and A^+···X^-, where A is S or N. ⟨C⟩ denotes the centroid of the three C atoms, in C_4N^+ the three C atoms which are not restricted to a methyl group. Further description is given in the text.

conformation. The molecule can also be fitted upon arecoline with the conjugated ester group of sulfonium-isoarecoline in a *Z* conformation. In this case the six-membered ring has to adopt another boat conformation, which is the alternative boat to that presented in Fig. 11. Since the *E* conformation is 9.6 kJ mol^{-1} lower in energy than the *Z* conformation, we have included the former in Fig. 11.



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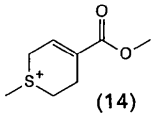


(14)

Comparison of L-660863 and Org 31956 with quaternary arecoline analogues

In view of the statistics presented it is not surprising that *N*-methylarecoline (15) is not a potent muscarinic agonist (Table 3). Mutschler *et al.* (1983) stressed the more bulky appearance of the molecule as the main reason for its lack of muscarinic activity. However, inspection of Fig. 9(*c*) makes it clear that in order to obtain a favourable interaction geometry, the onium group should be translated as well as rotated with respect to the tertiary amino group of arecoline. As a result the electron-rich ester group is displaced, diminishing the interaction of the molecule with the receptor. Schulman *et al.* (1983) have presented an explanation for the relative activities of the enantiomers of aceclidine and *N*-methylated aceclidine which was also based on the geometry of the interaction with the receptor.

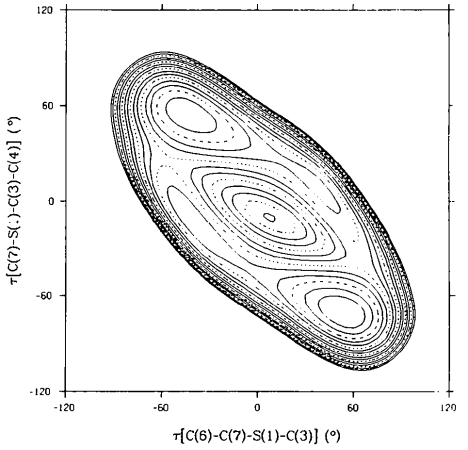


Fig. 10. Steric energy of sulfonium-arecoline (13), as a function of two ring torsion angles, equivalent to C(6)—C(7)—N(1)—C(3) and C(7)—N(1)—C(3)—C(4) of Org 31956, calculated with *MM2P*(85). Contour lines are drawn at an interval of 4.184 kJ mol^{-1}, contour lines are dashed at an interval of 12.6 kJ mol^{-1}, with a longer dash applied to the 12.6 kJ mol^{-1} level.

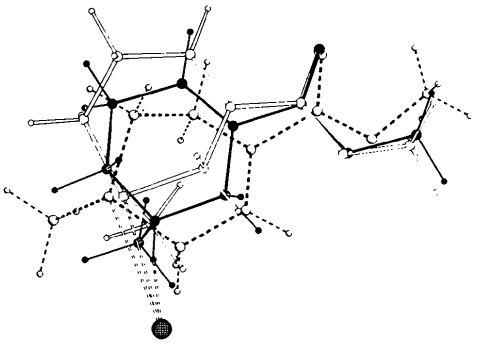


Fig. 11. Match of arecoline [(7), solid lines], sulfonium-arecoline [(13), open lines] and sulfonium-isoarecoline [(14), dashed lines] with a postulated negatively charged receptor site (hatched).

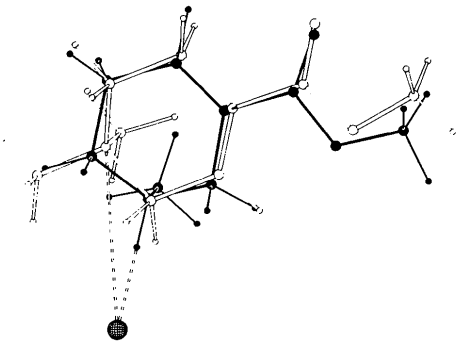
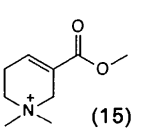
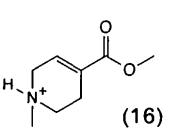


Fig. 12. Match of arecoline [(7), solid lines] and *N*-methylisoarecoline [(17), open lines] with a postulated negatively charged receptor site (hatched).

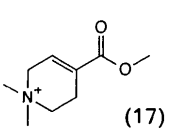
Isoarecoline (16) is known to be more active as a muscarinic agonist in the methylated form (17); see Table 3. This can also be explained using the above-mentioned statistics. A match of *N*-methylisoarecoline with arecoline is shown in Fig. 12. An O atom, representing the postulated negatively charged receptor site, is included. Arecoline is capable of forming a hydrogen bond towards this O, whereas isoarecoline is in a reasonably good quaternary-ammonium position, in agreement with the statistics presented in Fig. 9(*c*) (arecoline: $\varphi = 5°$, $d = 2.73$ Å; *N*-methylisoarecoline: $\varphi = 71°$, $d = 3.69$ Å). The N—H group of protonated isoarecoline is clearly not capable of forming a hydrogen bond to the depicted O in Fig. 12 without disrupting the alignment of the ester groups of both molecules. Like sulfonium-isoarecoline (14), *N*-methylisoarecoline can also be fitted upon arecoline with the conjugated ester group in a *Z* conformation.



(15)



(16)



(17)

In producing the above-described molecular matches, we have assumed a rigid receptor model, consisting of a group interacting with the electron-rich ester part of the molecule (or another electron-rich group), and a negatively charged receptor site interacting with the amino and onium groups. The negatively charged receptor site was actually included in some of the molecular matches produced. It should be stressed that the receptor does not necessarily have to be rigid, *i.e.* the receptor sites can adopt different positions relative to each other. In fact, the receptor is assumed to change conformation upon activation. However, when two molecules can be matched upon each other, with both of them in a favourable interaction geometry with respect to the postulated receptor sites, it is a logical conclusion to state that both can be active.

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