Conformation of a *trans*-Diaxial Decalin Analog of Acetylcholine

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Abstract □ The three-dimensional structure of 3(a)-dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide was determined by X-ray crystallographic analysis. The spatial disposition of the N^+—C—C—O grouping of atoms was found to be anticlinal (147°). A conformation of $+150 \pm 15°$ for this linkage is felt to be one of a number of factors important in influencing the muscarinic potency and hydrolytic activity of a cholinergic ligand.

Keyphrases □ 3(a)-Dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide—conformational X-ray analysis, muscarinic potency □ X-ray crystallography—conformation, 3(a)-dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide □ Muscarinic potency—acetylcholine-decalin analog conformation

The use of conformationally constrained analogs of a drug entity for the elucidation of structure–activity relationships is a popular technique. Smissman *et al.* (1) synthesized and pharmacologically tested a series of *trans*-decalin analogs of acetylcholine to develop a relationship with regard to the muscarinic receptor. Of the four diastereoisomers studied (I, II, III, and IV), the *trans*-diaxial molecule (I) proved to be the most potent agonist. This isomer was the only one to be hydrolyzed by acetylcholinesterase at a substantial rate when compared to acetylcholine. The observations led these investigators to conclude that muscarinic response and hydrolytic activity require a *trans*-arrangement of the quaternary nitrogen relative to the acetoxy oxygen. Chothia (2) recently proposed that the *trans*-conformation of I makes it a relatively inactive cholinergic molecule. A question arises as to the validity of either idea, because structural data on these molecules were not utilized in the arguments. An X-ray structure analysis of I was undertaken to clear the record on the conformation of this molecule; the results are reported in this article.

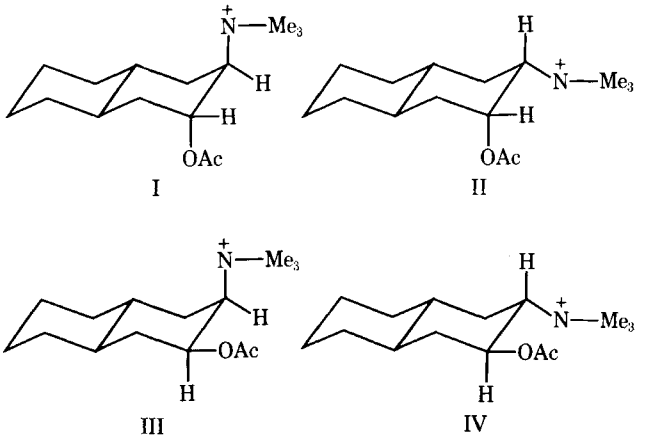
EXPERIMENTAL

Crystals of 3(a)-dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide (I) have the following measured parameters:

$a = 15.976\ (6)$ Å	density (calculated) $= 1.459$ g. cm.$^{-3}$
$b = 9.047\ (4)$ Å	density (measured) $= 1.457$ g. cm.$^{-3}$
$c = 12.752\ (5)$ Å	$Z = 4$
$\beta = 109.66\ (3)°$	space group $P2_1/c$

Intensity data were collected by the stationary crystal–stationary counter technique using Ni filter Cu$K\alpha$ radiation. The reflections between 0 and 100° in 2θ were measured: a total of 1854 independent reflections. Corrections to these measurements were made for absorption (approximately), α_1–α_2 splitting, and the Lorentz-polarization phenomena.

A trial structure was obtained using the "heavy atom" technique. The positional and thermal parameters of all the nonhydrogen atoms were refined by least squares to an R index of 0.12. With the exception of iodine, the atoms were assigned isotropic thermal parameters in the refinement. No attempt was made to locate the hydrogens in the structure[1].



RESULTS

The final atomic parameters from the least-squares refinement are given in Table I. The intramolecular bond distances and angles obtained with these parameters are shown in Fig. 1. The estimated standard deviations in the lengths and angles denoted in this figure are on the average 0.04 Å and 2°. Within the limits of error, the lengths and angles found agree with expected values (3).

The spatial disposition of atoms or groups of atoms about a particular bond is best discussed in terms of torsion angles. The Klyne and Prelog (4) recommendations for describing such angles are adhered to in this paper. The bulky trimethylammonium group attached to C(3) causes a significant degree of distortion in a portion of the decalin system. The optimum conformation angle about a ring bond is 60°; with the exception of those about the C(2)—C(3) and C(3)—C(4) bonds, there is a reasonable agreement. The torsion angles about these two bonds are (+) 31 and (−) 39°, respectively. In parentheses are the signs of these angles for the 2(S)—3(S) enantiomer.

The acetylcholine portion of the molecule can be spatially specified (5) by the torsion angles for the N^+—C(3)—C(2)—O(1), C(3)—C(2)—O(1)—C(11), and C(2)—O(1)—C(11)—C(12) groupings of atoms. The values for these angles in the 2(S)—3(S) enantiomer are 147, −89, and 179°, respectively. The 2(R)—3(R) isomer has torsion angles that are opposite in sign. A drawing of the 2(R)—3(R) enantiomer, as seen down the C(2)—C(3) bond, is shown in Fig. 2. The displacement of the N—C(3)—C(2)—O(1) torsion angle from 180° results from the repulsion between the C(1) and C(10) *axial* hydrogens and the hydrogen attached to C(13). This can be verified by molecular models[2], which show that an antiperiplanar (180°) model cannot be constructed without great strain.

DISCUSSION

Scientists interested in structurally describing the active site(s) of the cholinergic receptors have principally resorted to studying the comparative activities of ligands having constrained conformations. Numerous studies along these lines have been reported, but one of their principal drawbacks has been the syntheses of conformation-

[1] A tabulation of the observed and calculated structure factors will be supplied on requests directed to E. Shefter.

[2] CPK Space Filling, Ealing Corp., Cambridge, MA 02140

Table I—Positional and Thermal Parameters[a]

Atom	$x/a \cdot 10^4$	$y/b \cdot 10^4$	$z/c \cdot 10^4$	B^b, Å^2
I	1555(1)	1123(2)	−313(1)	
C(1)	3028(15)	7461(26)	4812(19)	5.4(0.4)
C(2)	2344(17)	8525(27)	4957(21)	6.3(0.5)
C(3)	1992(14)	9698(25)	3919(18)	5.1(0.4)
C(4)	2729(15)	10048(24)	3427(19)	5.4(0.5)
C(5)	4080(19)	9413(33)	2933(24)	7.5(0.6)
C(6)	4732(22)	8323(38)	2860(28)	8.9(0.8)
C(7)	5146(20)	7536(36)	4035(25)	8.1(0.6)
C(8)	4462(21)	6922(37)	4444(27)	8.6(0.7)
C(9)	3778(16)	8105(27)	4538(20)	6.0(0.5)
C(10)	3331(15)	8820(23)	3324(19)	5.1(0.4)
C(11)	2301(14)	10124(23)	6443(17)	4.7(0.4)
C(12)	2834(24)	11133(35)	7340(30)	8.8(0.8)
C(13)	1148(16)	7870(28)	2438(21)	6.4(0.6)
C(14)	436(22)	8998(32)	3686(28)	8.3(0.7)
C(15)	806(21)	10469(37)	2264(27)	8.6(0.7)
N	1096(13)	9205(20)	3057(17)	5.8(0.4)
O(1)	2786(10)	9518(17)	5868(12)	5.5(0.3)
O(2)	1534(11)	9804(19)	6223(14)	6.6(0.4)

[a] Their estimated standard deviations are in parentheses. [b] Iodine refined anisotropically; final values of the coefficients ($\times 10^4$) are: B_{11} = 88(1), B_{22} = 203(3), B_{33} = 100(1), B_{12} = 2(3), B_{13} = 83(2), and B_{23} = −5(3).

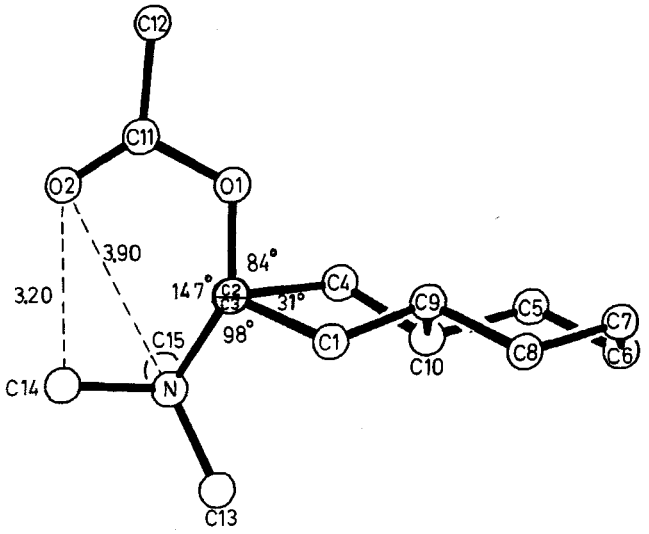


Figure 2—*A view of I showing conformation about the C(2)—C(3) bond.*

ally "rigid" analogs which are similar in "binding" and "efficacy" to acetylcholine. Two other important factors, which have not been considered to any great extent in such studies, concern the differences in electronic features and the thermodynamic properties of these ligands when the acetylcholine framework is chemically altered.

The "structure–activity relationship" formulator usually works under the premise that there is a simple "lock-and-key" mechanism operative for the various cholinergic receptors. The studies of Moran and Triggle (33) on the muscarinic "receptor" indicate a dual mode of agonist binding, thus questioning the validity of a single active site for this receptor. Multiple modes of ligand binding at the catalytic site of acetylcholinesterase also were demonstrated (34, 35). One should, therefore, measure "binding" and "efficacy" of a ligand to make more sense out of comparative pharmacological testing of constrained molecules.

Any consideration of the architecture of the various cholinergic receptors must be concerned with the spatial disposition of the pertinent chemical entities necessary to produce a significant pharmacological response. With regard to the muscarinic activity of acetylcholine and its hydrolysis by acetylcholinesterase, the spatial arrangement of the quaternary nitrogen relative to the acetate group appears to be important. Two conformation angles are sufficient in describing their spatial relationship in acetylcholine and most cholinergic ligands (5); these are the twist angles about the C(2)—C(3) and C(2)—O(1) bonds. Comparison of the pertinent torsion angles obtained from crystallographic studies on a number of cholinergic ligands are presented in Table II and Fig. 3, along with data on their overall rates of acetylcholinesterase hydrolysis and muscarinic potency (on concentration level). Since "binding" data and detailed kinetic data are essentially nonexistent for the compounds tabulated (a few exceptions do exist), a crude activity scale was devised to show the variability in the observed biological activities of the compounds. One must not consider these biological data as directly correlateable with conformation; in general, they are a function of a complex series of events dependent on many other chemical factors.

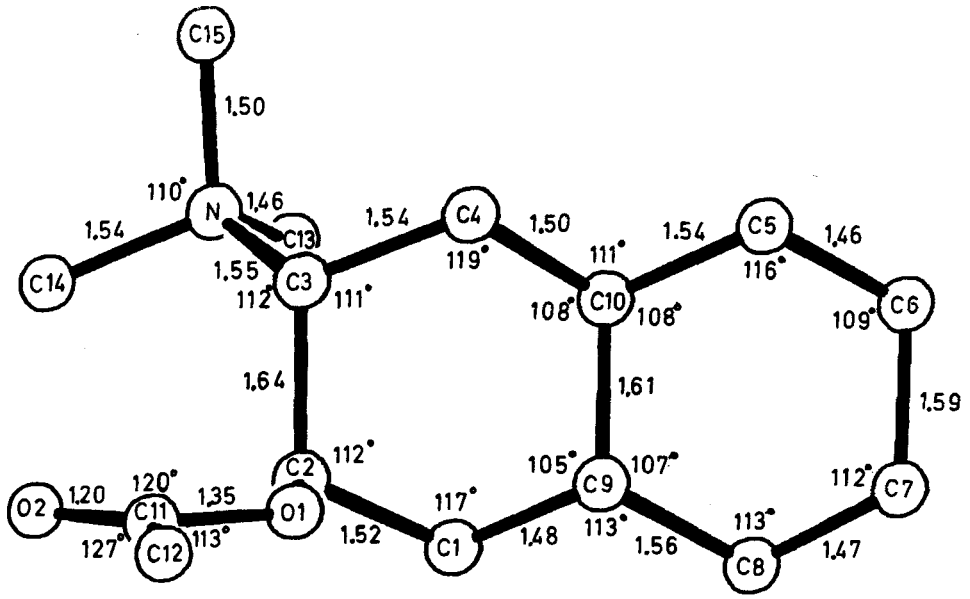


Figure 1—*Bond lengths and angles.*

C12-C11	1.49	C2-O1-C11	118°
C2-O1	1.45	C1-C2-O1	108
		C3-C2-O1	101
		C4-C3-N	115
		C3-N-C13	115
		C3-N-C15	105°
		C13-N-C15	109
		C13-N-C14	111
		C3-N-C14	107

Table II—Comparison of Conformation and Relative Pharmacological Data for Some Cholinergic Ligands

Compound	Torsion Angles: N^+—C(3)—C(2)—O(1)	Torsion Angles: C(3)—C(2)—O(1)—C(11)	Muscarinic Activity[a]	Acetylcholinesterase Hydrolytic Rate[a]	Reference
3(a)-Dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide [3(S)—2(S)] (1)	147°	−89°	+[b]	++[b]	1[d]
Acetylcholine bromide (chloride) (1)	77(85)°	79(167)°	++++	++++	8[c], 9[c]
Acetylthiolcholine bromide (2)	171°	129°	++	+++	10[c], 11[d]
Acetylselenolcholine iodide (3)	175°	123°	+	+++	12[c]
Acetylthionocholine bromide (4)	92°	76°	[g]	[g]	—
erythro-α,β-Dimethylacetylcholine iodide (5) α(R)-β(S)	76°	−156°	++[b]	0[b]	13[c], 1[d]
threo-α,β-Dimethylacetylcholine iodide (6) α(S)-β(S)	143°	−95°	+[b]	++[b]	13[c], 1[d]
(+)-*trans*-2-Acetoxycyclopropyl-trimethylammonium iodide (7)	137°	−151°	++++	++++	14[c], 15[d]
L(−)*S*-α-Methylacetylcholine iodide					
Molecule A [8*a*-α(R)]	90°	170°	—	—	—
Molecule B (8*b*)	148°	−176°	—	—	—
L(+)*S*-β-Methylacetylcholine iodide (9)	85°	−147°	++++(+++[b])	++(++[b])	17[c], 6[d]
Carbamoylcholine bromide (10)	178°	−174°	+++	+[e]	18[c], 19[d]
L(+)-Muscarinic iodide (11)	73°	144°	+++++	—	20[c], 7[d]
Lactoylcholine iodide, L(+)-isomer (12)	85°	157°	+(+[b])	+++++(+++[b])	21[c], 22[d]
L(+)-*cis*-2(S)-Methyl-4(R)-trimethyl-ammonium methyl-1,3-dioxolan iodide (13)	68°	100°	+++++	—	23[c], 24[d]
1-Methyl-3(a)-acetoxy-*trans*-decahydro-quinoline methiodide [3(R)] (14)	74°	153°	+	0[f]	25[d]
Propionylthiolcholine iodide (15)	176°	108°	—	—	10[c]
2(*N*,*N*-Diethyl-*N*-benzylammonium)-ethyl carbamate bromide (16)	81°	163°	0	—	26[c], 19[d]
2(*N*,*N*-Dimethyl-*N*-benzylammonium)-ethyl carbamate bromide (17)	162°	−107°	0	—	27[c], 19[d]
Choline chloride (18)	84°	—	0–+	—	28[c], 29[d]

[a] Relative scale: ++++++, greater than acetylcholine; ++++, equivalent to acetylcholine; +++, one-third to two-thirds that of acetylcholine; ++, a tenth to a third the activity; +, very weak response; and 0, inactive. [b] Measured for racemic mixture. [c] Structural reference. [d] Biological reference. [e] Hydrolysis of carbamylated enzyme takes place much more slowly than the acetylated enzyme. [f] Inhibitor. [g] Chu and Mautner (30) reported that the depolarizing ability (measured on the electroplax) of this compound is 1.7 times less potent than acetylcholine on a molar basis. The hydrolysis rate in the presence of acetylcholinesterase was reported to be significantly lower than that for acetylcholine, but their K_m values were similar.

The two receptors of concern here (nicotinic receptor neglected) were clearly demonstrated to be stereoselective (6, 7, 15). The conformational parameters given in Table II and Fig. 3 are for the enantiomer of a compound that corresponds to the highly active compounds: L(+)-muscarine and (+)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide. Molecular models show that steric factors influence the chirality attainable by a particular enantiomorph (5, 32).

A great deal of variability is found for the C(3)—C(2)—O(1)—C(11) arrangements of the cholinergic ligands tabulated. It is sterically feasible for primary and secondary esters to adopt a wide gamut of conformations about the C(2)—O(1) bond, with the exception of the *synplanar* angle (0 ± 30°). This is evident in Fig. 3. The rotational energy barrier about this bond is, however, greater for secondary esters than for primary esters. Steric factors also restrict the torsion angle range that secondary esters are able to achieve (5). L(+)-Muscarine and *cis*-2(S)-methyl-4(R)-trimethylammonium methyl-1,3-dioxolan are much more constrained about the C—O bond than in the ester acetylcholine analogs. Whether the +144 and +103° values in these two crystal structures represent the boundary condition on this conformation that will produce a muscarinic response remains to be answered. A suggestion that the relevant C(2)—O(1) conformations with regard to muscarinic activity (2) and acetylcholinesterase hydrolysis (32) is antiperiplanar (180 ± 30°) is based on inadequate evidence.

Molecular models of a large variety of cholinergic ligands show that if either of the two α-carbon hydrogens, *i.e.*, C(3), is replaced by bulky substituents, the conformations about C(2)—O(1) are further restricted. Wijngaarden *et al.* (31) postulated that a potent muscarinic agent should have an α-carbon hydrogen 2.3 Å away from the quaternary nitrogen which may bind to the receptor surface (based on the low potency of α-methyl acetylcholine). Specific receptor binding of an α-hydrogen is unlikely; it is more reasonable to consider that the substitution by other groups on the α-carbon influences the important C(2)—O(1) conformation. This ester conformation angle directly affects the position of the methyl group of the acetyl moiety in relation to the cationic head of the molecule. The importance of the corresponding methyl group in muscarine relative to muscarinic activity was shown by Waser (7). In such a saturated system, the C(2)—O(1) conformation is not enough to fix the position of the methyl group. In such cases, the conformation of the C(12)—C(11)—O(1)—C(2) atoms should be defined. This conformation in the case of esters is *antiperiplanar* (180°) but varies for the other muscarinic agents. Pauling and Petcher (39) suggested a value of −137° for nonester cholinergics.

The acetylcholine–decalin analogs were synthesized with the idea of spatially constraining the acidic (N^+) and basic (ester) portions of the cholinergic ligand acetylcholine. With the exception of the *trans*-diaxial isomer (I), space-filling molecular models show that their sterically preferred N^+—C—C—O— conformations are between 70 and 90°. The racemate of I, although substantially less potent a muscarinic agent compared to acetylcholine (on concentration basis), is much more active than the other isomers. Similarly, its hydrolytic behavior is greater.

Much more "rigid" N^+—C—C—O— conformations are found in the *cis*- and *trans*-cyclopropane analogs of acetylcholine (15). Only the *trans*-isomer was found to be a potent muscarinic agent. It was also hydrolyzed by acetylcholinesterase at an overall rate similar to acetylcholine. Its N—C—C—O torsion angle is quite similar to that of the *trans*-decalin analog. None of the other highly active cholinergic ligands has such a constrained conformation, but models show that they are all capable of attaining this spatial arrangement. Studies on conformationally constrained ligands other than those tabulated clearly suggest that conformations between 135 and 165° for the N^+—C—C—O atoms will impart higher activity, whether it be muscarinic or hydrolytic (36–38).

It is felt that there is good indication that a N^+—C—C—O— conformation of 150 ± 15° gives the optimal separation between the acid and basic portions of a cholinergic ligand leading to a substantial biological response at the muscarinic receptor or at the active site of acetylcholinesterase. There is also a preference by these receptors for the (+)-chiral conformation (5, 32, 36, 39) over the (−)-chiral. The ester conformation at the receptor sites remains to be disclosed, but without a doubt it is of equal importance with the N^+—C—C—O— conformation. Other factors, such as the basicity of the ester (10), hydrophobic nature of the ligand, and general size of the ligand, can

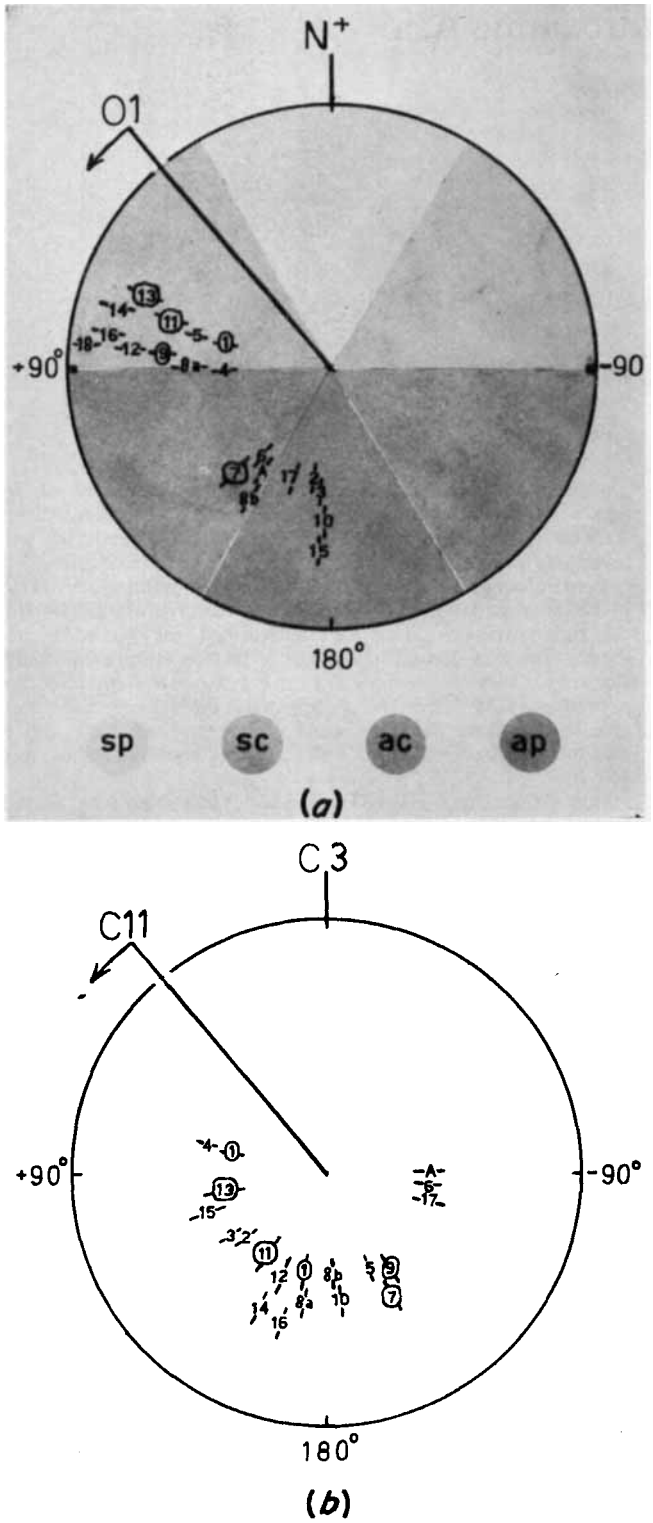


Figure 3—*A plot of the torsion angles of compounds listed in Table II. Key: (a), N+—C(3)—C(2)—O(1); and (b), C(3)—C(2)—O(1)—C(11). Circled numbers refer to most potent agonists listed.*

be considered to influence significantly the responses observed (Table II).

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