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Muscarinic Agonists. Crystal and Molecular Structure of 2-Methyl-4-trimethylammoniummethyl-1,3-dioxolan Iodide and Similar Substances

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The crystal and molecular structure of the muscarinic agonist, 2-methyl-4-trimethylammoniummethyl-1,3-dioxolan iodide, is described and compared with those of other muscarinic agonists. With one uncertain exception, the absolute configuration of all known active muscarinic agonists the enantiomers of which have been separated are the same and their observed conformations are similar.

Triggle and Belleau[1] have prepared and determined the absolute configuration of the separate enantiomers of the isomers of 2-methyl-4-trimethylammoniummethyl-1,3-dioxolan,[2] and Belleau and Puranen[3] have tested their pharmacological properties as muscarinic cholinergic agonists on guinea pig ileum. The DL-cis isomer is about 5 times as potent a muscarinic agonist as the DL-trans isomer, and the L(+)-cis enantiomer is approximately 100 times as potent as the D(−)-cis enantiomer.[3] We have determined the crystal structure of the L(+)-cis enantiomer by three-dimensional X-ray diffraction analysis of crystals of the iodide supplied by Professor Belleau, and compared the molecular structure with those of other muscarinic agonists.

Experimental Section

Crystals of L(+)-*cis*-2(*S*)-methyl-4(*R*)-trimethylammoniummethyl-1,3-dioxolan iodide are orthorhombic, space group D_2^4-$P2_12_12_1$, a = 1214 (1), b = 1413 (2), c = 719 (1) pm, Z = 4 molecules per unit cell. Three-dimensional diffraction data (3723 observations covering 2 octants of reciprocal space) were measured on a computer-controlled[4] diffractometer employing Zr-filtered Mo Kα radiation within the range $2\theta \leq 45°$. The data were corrected for Lorentz and polarization effects and averaged to provide 395 unique observed diffraction maxima greater than 3 times the respective statistical standard deviations. The solution of the structure by Patterson and Fourier methods was difficult due to the pseudocentric positions of the I atoms, which impose false mirror planes in the heavy atom phased Fourier syntheses at z = 0.25 and z = 0.75. The structure was refined by iterative full matrix least-squares methods, with anisotropic thermal parameters for the I atom and isotropic thermal parameters for all other atoms, H excluded, to a value of the unweighted reliability index R = 0.087 over the 395 independent observed maxima. Refined coordinates and thermal parameters of the structure are given in Table I with statistical standard deviations of the parameters.

Structure.—The structure of L(+)-*cis*-2(*S*)-methyl-4(*R*)-trimethylammoniummethyl-1,3-dioxolan is shown in Figure 1, with atoms numbered[5a] to correspond with previous publications.[5b,6] Interatomic distances and angles of the molecule as determined in this analysis correspond to within experimental error with those normally found for similar atomic contacts. Excluding any puckering of the ring, the molecular structure of this dioxolan is essentially a one-parameter problem, as is the structure of muscarine (Figure 2). That one parameter is the O1–C5–C4–N torsion angle.[7] In principle there are several other torsion angles as parameters but all of these have threefold symmetry, and all are expected to have the staggered conformation, $\tau = \pm 60°$ or 180°. These other torsion angles are C5–C4–N–C1 (or the equivalents C5–C4–N–C2 and C5–C4–N–C3) and the 4 Me orientations C4–N–C1–H, C4–N–C2–H, C4–N–C3–H, and O1–C6–C7–H where H is any hydrogen in the appropriate Me. The torsion angles of this 1,3-dioxolan and muscarine[8] are given in Table II. It is seen that τ O1–C5–C4–N is +94° in this dioxolan and +73° in L(+)-muscarine, nearly the same. The other torsion angle parameter τ C5–C4–N–C1 (C2, C3) is −88°, (32, 150°) in this dioxolan and −61° (68, −175°) in muscarine. In all cases this compares with −60° (60, 180°) expected in a staggered bond. The positions of the hydrogens have not been determined in this analysis, but the Me groups are all expected to have a staggered conformation, as shown in the structure analysis of glycerylphosphorylcholine[9] where some H atom positions were determined.

The tetrahydrofuran ring of muscarine is not flat, C9 is in the plane of C5–C4–N–C3 and out of the plane of the ring on the same side as the Me and trimethylammoniummethyl groups, whereas in this dioxolan, C8, while part of the planar extended chain C8–C5–C4–N–C3, is out of the plane of the ring on the side opposite to the substituent groups. This difference causes

TABLE I

REFINED COORDINATES AND THERMAL PARAMETERS WITH STANDARD DEVIATIONS IN PARENTHESES IN TERMS OF THE LEAST SIGNIFICANT DIGIT GIVEN

Atom	x/a	y/b	z/c	B_{iso}
I	0.2525(4)	0.1852(1)	0.2535(4)	*a*
N	0.546(2)	0.643(2)	0.731(10)	3.78
C(1)	0.634(3)	0.565(3)	0.712(7)	4.70
C(2)	0.478(4)	0.644(5)	0.564(8)	4.70
C(3)	0.598(4)	0.737(3)	0.731(10)	3.14
C(4)	0.478(6)	0.619(5)	0.906(9)	4.80
C(5)	0.389(3)	0.562(3)	0.917(6)	3.60
O(1)	0.434(3)	0.477(2)	0.965(6)	6.88
C(6)	0.434(7)	0.432(6)	1.152(10)	10.66
C(7)	0.526(6)	0.401(5)	1.205(10)	6.89
O(2)	0.376(2)	0.508(2)	1.269(8)	6.87
C(8)	0.326(5)	0.573(5)	1.100(8)	4.84

[a] Anisotropic thermal parameters of the I atom in the form: $\exp-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + 2b_{12}hk + 2b_{13}hl + 2b_{23}kl)$

b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
0.0079	0.0081	0.0266	0.0004	−0.0006	−0.0062

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(5) (a) The numbering system in this paper is not consistent. Conventional numbering is used only in the names of compounds. In comparing individual atoms of different molecules, the numbering of the figures is used. (b) F. G. Canepa, P. J. Pauling, and H. Sörum, *Nature (London)*, **210**, 907 (1966).

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(7) The torsion angle τ of the atomic bond A–B in the bonded group X–A–B–Y is the angle between the planes XAB and ABY. It is positive in the clockwise sense viewed down A–B, and negative in the counterclockwise direction. Values of the torsion angle τ = 0, 60, 120, and 180° are termed synplanar, synclinal, anticlinal, and antiplanar, respectively.

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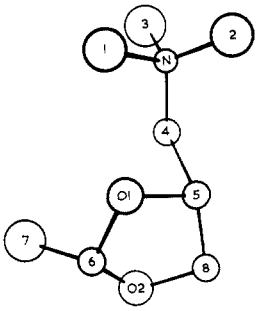

Figure 1.—L(+)-*cis*-2(*S*)-Methyl-4(*R*)-trimethylammoniummethyl-1,3-dioxolan, as observed in crystals of the iodide.

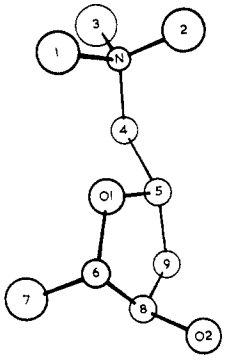

Figure 2.—L(+)-Muscarine observed in crystals of the iodide.[8]

the torsion angle C6–O1–C5–C4 to be different in the two structures. We consider this difference to be a crystalline artefact; both conformations satisfy the preferred conformation of 5-membered heterocyclic rings and the energy difference between the two must be very small. In solution, the rate of interconversion is probably high. We think the muscarine conformation with τ C6–O1–C5–C4 equal to about +145° is that relevant to interaction with the muscarinic receptor because of a closer similarity to other more rigid muscarinic agonsits (see later). Other than C9 and O2, this 1,3-dioxolan and muscarine have nearly identical structures, with O2 in dioxolan occupying the position of C8 in muscarine and about 140 pm distant from the position of O2 in muscarine. This conformation is consistent with one stable conformation of ACh as observed in crystals of several derivatives,[6,10] in solution[11] and as calculated theoretically.[12]

It is also consistent with a theoretical conformational analysis of muscarine based on van der Waals energy,[13] in which the conformation with τ O1–C5–C4–N = 78.7° was calculated to be stable. In the structures of this 1,3-dioxolan, muscarine, and L(+)-acetyl-β-methylcholine,[10] (I) the N–O1 distances are all about 320 pm, and the N–C7 distances vary from 484 to 540 pm. The N–O2 distances vary, but as several molecules, such as 2-methyl-5-trimethylammoniummethyl furan (II), which do not have an O atom O2, are potent muscarinic agonists,[14] we feel that this must be unimportant.

Other Substances.—With these consistent values of the conformational parameters available, it is possible to predict the conformation of many muscarine-like molecules. This has already been done for muscarone and this 1,3-dioxolan[15] on the basis of the structure of muscarine. The stable conformations of all the muscarines, muscarones, 1,3-dioxolans, and other ring structures can be predicted with some certainty. Many non-

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(14) H. L. Friedman, in "Drugs Affecting the Peripheral Nervous System", A. Burger, Ed., Martin Dekker, New York, N. Y., 1967, Table 2.16, p 100.
(15) P. J. Pauling, in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson- Ed, W. H. Freeman and Co., San Francisco, Calif., 1968, p 555.

TABLE II

TORSION ANGLES AND SOME DISTANCES IN THIS 1,3-DIOXOLAN AND L(+)-MUSCARINE

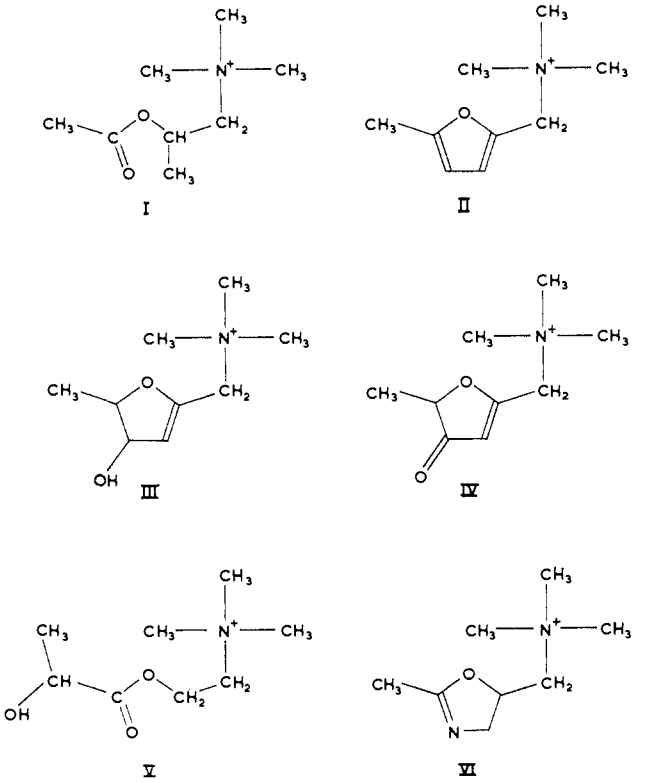
	Dioxolan	Muscarine
O1–C5–C4–N	+94°	+73°
C5–C4–N–C3	+150	−175
C7–C6–O1–C5	−132	−136
C6–O1–C5–C4	+103	+144
C6–O1–C5–C8	−12	+21
C8–C5–C4–N	−166	−168
N^+–O1	320 pm	307 pm
N^+–C6	446	450
N^+–O2	479	563
N^+–C7	484	540

TABLE III

OBSERVED TORSION ANGLES OF THE MORE POTENT ENANTIOMERS OF MUSCARINIC AGONISTS (DEG)

Compound	τ O1–C5–C4–N	τ C6–O1–C5–C4	τ C7–C6–O1–C5
Title compound	+94	+103	−132
L(+)-Muscarine	+73	+144	−136
D(+)-Acetyl-α-methylcholine	+89	+167	−177
L(+)-Acetyl-β-methylcholine	+87	−143	+177
(+)-*trans*-Acetoxy-cyclopropyltrimethylammonium	+137	−151	+177
(−)-3-Acetoxyquinuclidine	+120[a]		

[a] Unobserved but required by the rigidity of the quinuclidine ring system.



ring structures, such as ACh itself, can adopt a conformation similar to those presented here. Some of the active ring structures cannot adopt the exact conformation of this 1,3-dioxolan and muscarine, but predicted conformations of these compounds are similar to those observed. The unsaturated ring compounds sometimes are required to have a conformation with τ C6–O1–C5–C4 = 180°, due to the planarity of the double bond. Such

compounds include 4,5-dehydromuscarine (III) and -muscarone, (IV) and 2-methyl-5-trimethylammoniummethylfuran (II). This antiplanar conformation is, however, nearly that found in acetyl-β-methylcholine[10] and in lactoylcholine[6] (V). In the furan and in 2-methyl-4-trimethylammoniummethyl-1,3-oxazoline (VI), the Me group is in the plane of the ring, as it is in acetylcholine or acetyl-β-methylcholine; τ C7–C6–O1–C5 is always 180° in esters due to the partial double bond character of the C6–O1 bond.[5,6,10,15]

Absolute Configuration.—Much information is available on the absolute configuration of potent muscarinic agonists. In addition to this 1,3-dioxolan[1,3] and muscarine,[16] evidence of potency and absolute configuration are available for muscarone,[16] acetyl-α-methylcholine,[10,17] acetyl-β-methylcholine,[10,17] acetoxycyclopropyltrimethylammonium,[18] and 3-acetoxyquinuclidine.[19] With the exception of muscarone (for which we can offer at this stage no explanation of the more or less equal activities of the two enantiomers), all the muscarinically more active enantiomers of these compounds have an absolute configuration consistent with those of this 1,3-dioxolan and muscarine. In Table III are listed three observed torsion angles of the more potent enantiomers of these substances. It is seen that τ O1–C5–C4–N varies from +73 to +137°, τ C6–O1–C5–C4 = 180 ± 37°, and τ C7–C6–O1–C5 equals either 180 or −137°.

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Solid Phase Synthesis and Antibacterial Activity of N-Terminal Sequences of Melittin

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N-Terminal peptides representing the 1–8, 7–17, 1–17, and 14–17 sequences of melittin were synthesized by the solid phase method and shown to be homogeneous by elemental and amino acid analyses and by tlc and tl electrophoresis. The yields were 40–66% starting from BOC-amino acid resin esters prepared from dimethyl(arylmethylene)sulfonium bicarbonate resins. Some interesting observations were made during the deblocking of BOC-Ile-O-resin reflecting steric and environmental considerations. Whereas melittin exhibited general antibacterial activity, none of the synthetic peptides showed any significant activity, thus demonstrating that the antibacterial activity of melittin does not reside in the N-terminal portion of the molecule.

The basic polypeptide melittin (**1**) is the main component of the venom of the honey bee, *Apis mellifica*.[1] Crude, dry bee venom contains 40–50% (by weight) **1** and about 10% of **1** is formylated at the N-terminus.[2]

H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Try-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH$_2$

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

1

The primary structure of **1** is rather unique. The N-terminal end, *i.e.*, 1–20 sequence, consists mainly of amino acids having hydrophobic side chains, exceptions being 7-lysine and 10,11-threonine.[3] The C-terminal end, on the other hand, consists mainly of amino acids containing basic and/or hydrophilic side chains. Hence, the molecule is a natural surfactant and many of its toxic reactions have been attributed to this property. For example, the hemolytic and inflammatory properties of **1** may be explained by its ability to disrupt lipid biomembrane structures.[4] The interaction of **1** with artificial lipid membranes has also been demonstrated.[5] Not all of the biological properties of **1** are untoward. Jentsch[6] reported that **1** at 2.5×10^{-6} *M* suppressed the uptake of thymidine, uridine, and leucine in DNA, RNA, and protein biosynthesis of cancer (ascities) cells. Melittin has been shown to protect mice against lethal doses of X radiation when administered sc.[7] Finally, Fennell, *et al.*,[8] showed that **1** was the antibacterial component of bee venom. These workers found that **1** was more effective against Gram-positive than Gram-negative bacteria. They raised the point of whether the antibacterial property of **1** was associated with the entire peptide molecule. In other words, might a smaller peptide sequence of **1** also exhibit the observed antibacterial activity, but without having the toxicity of **1**?

This question has been a principal concern of ours. In this connection we wish to report herein the synthesis and antibacterial activity of peptide sequences of the N-terminal portion of **1**, namely the 1–8, 1–17, 7–17, and 14–17 sequences. These peptides were synthesized by the Merrifield[9] solid phase method. As an adjunct

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