Recognition of Cholinergic Agonists by the Muscarinic Receptor. 2. Pilocarpine

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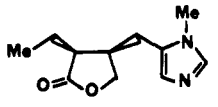
The conformational potential energy surface of the muscarinic agonist pilocarpine is studied by molecular mechanics (MMP2) and by semiempirical (AM1) and ab initio self-consistent-field (SCF) calculations. Six minima were located, two of which correspond to the known X-ray structures of pilocarpine. Possible active conformations of pilocarpine are inferred from the potential energy surfaces used in conjunction with the proposed active conformation of muscarine previously obtained from a theoretical model.

The alkaloid pilocarpine (1), derived from South American plants of genus *Pilocarpus*, is a well-known muscarinic cholinergic agonist having diaphoretic, miotic, and central nervous system effects.[1] Its pharmacological study includes the classic work of Langley[2] and his notion of a "receptive substance." Pilocarpine is currently used clinically to treat glaucoma.[1,3] The structure of pilocarpine was proposed by Jowett in 1900[4] and subsequently confirmed by chemical degradation,[5] syntheses,[6] and X-ray analyses.[7] It is noteworthy that the pilocarpine ethyl and imidazolylmethyl groups on C_7 and C_{11} of the dihydrofuranone ring (Figure 1) are cis, making the configurations at these carbons *R* and *S*, respectively.[5] Thus, pilocarpine is thermodynamically less stable, but more active as a miotic,[8] than its *R,R* trans epimer isopilocarpine (2).



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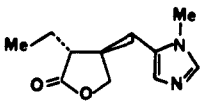

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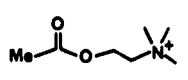
The conformation of pilocarpine is largely defined by the values of three torsional angles ($\alpha = C_4$–C_5–C_6–C_7; $\beta = C_5$–C_6–C_7–C_8; $\gamma = C_7$–C_{11}–C_{13}–C_{14}, Figure 1) and the extent of pucker in the lactone ring. The X-ray structure of the trichlorogerminate salt of pilocarpine has been reported by Fregerslev and Rasmussen (FR).[7a] Two nearly equivalent forms of pilocarpine are found in each asymmetric unit; the values of the three torsional angles are $\alpha = 7.4°$, $\beta = 71.7°$, $\gamma = -64.4°$, and $\alpha = 18.2°$, $\beta = 72.3°$, $\gamma = -58.4°$. The imidazole ring is nearly planar and essentially perpendicular to the lactone ring, which has an envelope form with C_7 in the flap position; the C_7–C_8–O_9–C_{10} torsional angle is ca. 22.0°. These trichlorogerminate structures differ from that of the hydrochloride salt studied by Codding and James (CJ),[7b] in which $\alpha = -4.2°$, $\beta = 168.4°$, $\gamma = -171.4°$ and the lactone envelope has its C_7 flap bent in the opposite sense, the C_7–C_8–O_9–C_{10} torsional angle being -20.4°. The existence of two very different structures in the solid state implies either at least two minima or a flat region of the conformational potential energy surface with respect to the torsional angles. Structures found in the solid state need not of course be biologically active forms, as in the case of acetylcholine itself.[9]

While a number of studies have probed structure-activity relationships in pilocarpine and its analogues[10] no clear picture has emerged of the manner in which 1 might be recognized as an agonist by muscarinic receptors. This is due in part to the absence of any obvious resemblance of pilocarpine to acetylcholine (3), muscarine (4), or other



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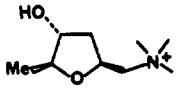

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well-known muscarinic agonists. Because of the recent resurgence of interest in compounds related to pilocarpine,[11] we have undertaken a theoretical study of its conformational potential energy surface, including an attempt to infer its biologically active form.

Methods

The conformational energy of pilocarpine was obtained by semiempirical (AM1[12]) and ab initio (STO-3G[13a] and 6-31G*[13b]) molecular orbital calculations and by the MMP2 molecular mechanics method.[14a] Some MMP2 parameters

(1) Goodman, L. S.; Gilman, A. *The Pharmacological Basis of Therapeutics*, 7th ed.; MacMillan: New York, 1985; p 100.
(2) Langley, J. *J. Physiol.* **1878**, *1*, 339.
(3) (a) Paton, W. D. M.; Payne, J. P. *Pharmacological Principles and Practice*; Churchill: London, 1968; p 147. (b) Watson, P. G. *Br. J. Opthalmol.* **1972**, *56*, 145.
(4) Jowett, H. A. D. *J. Chem. Soc.* **1900**, *77*, 473; J. Chem. Soc. **1900**, *77*, 851; *J. Chem. Soc.* **1901**, *79*, 1331; *J. Chem. Soc.* **1901**, *79*, 580.
(5) Hill, R. K.; Barcza, S. *Tetrahedron* **1966**, *22*, 2889.
(6) (a) Jowett, H. A. D. *J. Chem. Soc., Abstr.* **1905**, *87*, 794. (b) Preobrashenski, N. A.; Poljakowa, A. M.; Preobrashenski, W. A. *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 1835. (c) Dey, A. N. *J. Chem. Soc.* **1937**, 1057. (d) Noordam, A.; Maat, L.; Beyerman, H. C. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 441. (e) Compagnone, R. S.; Rapoport, H. *J. Org. Chem.* **1986**, *51*, 1713.
(7) (a) Fregerslev, S.; Rasmussen, S. E. *Acta Chem. Scand.* **1968**, *22*, 2541. (b) Codding, P. W.; James, M. N. G. *Acta Crystallogr.* **1984**, *B40*, 429.
(8) Anderson, R. A.; Cowle, J. B. *Br. J. Opthalmol.* **1968**, *52*, 607.
(9) Schulman, J. M.; Sabio, M. L.; Disch, R. L. *J. Med. Chem.* **1983**, *26*, 817.
(10) For reviews, see: (a) Aboul-Enein, H. Y. In *Trends In Medicinal Chemistry*; van der Goot, H., Domány, G.; Pallos, L., Timmerman, H., Eds.; Elsevier: Amsterdam, 1988. (b) Aboul-Enein, H. Y.; Al-Badr, A. A. *Methods Find. Exp. Clin. Pharmacol.* **1982**, *4*, 321.
(11) (a) Aboul-Enein, H. Y.; Al-Badr, A. A.; Rashed, M. S.; Ismail, M. *Toxicol. Environ. Chem.* **1986**, *11*, 183. (b) Aboul-Enein, H. Y.; Ibrahim, S. E.; Al-Badr, A. A.; Ismail, M. *Toxicol. Environ. Chem.* **1986**, *11*, 253. (c) Gonzalez, F. B.; Baz, J. P.; Espina, M. I. R. *Tetrahedron Lett.* **1989**, *30*, 2145. (d) Sauerberg, P.; Chen, J.; WoldeMussie, R. E.; Rapoport, H. *J. Med. Chem.* **1989**, *32*, 1322. (e) Jones, R. C. F.; Hirst, S. C. *Tetrahedron Lett.* **1989**, *30*, 5361, 5365.
(12) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
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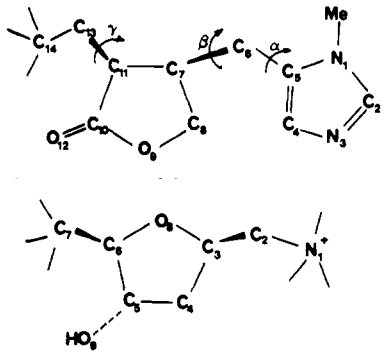

Figure 1. Torsional angles for pilocarpine and atomic numberings for pilocarpine and muscarine.

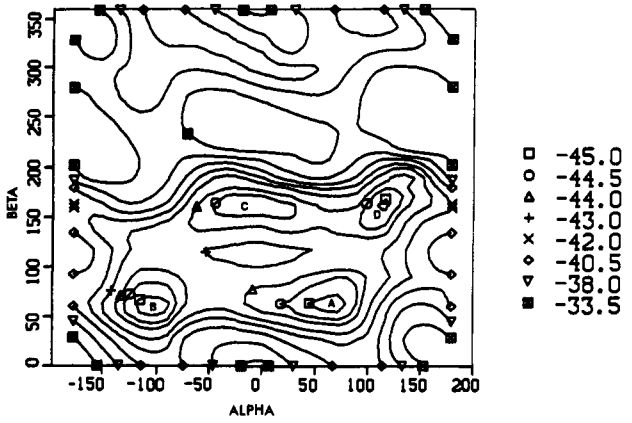

Figure 2. AM1 potential energy contours of pilocarpine.

which involve the pyrrole and imine nitrogens were obtained from Tai and Allinger.[14b] Other parameters involving a pyrrole-type and/or imine-type nitrogen and an sp^3 carbon were not available and these were taken to be identical with certain known MMP2 parameters under the assumption that an imine- or pyrrole-type nitrogen can be approximated by an sp^2 carbon. Their values are contained in the supplementary material.

The AM1 and MMP2 calculations were performed on a 30° grid of α–β values. For every α–β pair, the remaining $3N - 8 = 85$ parameters were optimized with each method.[15] The calculations are reported for the neutral base, since the cationic head group would be largely neutralized by the anionic receptor site. Moreover, proton NMR evidence suggests that the conformations of protonated and unprotonated pilocarpine are similar, at least in water.[16]

The fitting of pilocarpine to muscarine and generation of the perspective pictures were carried out with the Chem-X molecular modeling package, developed and distributed by Chemical Design, Ltd., Oxford, England.

Energetics of Pilocarpine

Figures 2 and 3 depict the potential energy contours of the AM1 and MMP2 conformational surfaces of pilocarpine as the torsional angles α and β are varied. Figure 4 shows a three-dimensional view of the MMP2 potential energy surface. For most points on the energy surfaces, γ is ca. 70°, although for higher values of β, γ values closer to 160° are found. Also, for $\beta > 210°$, there are large steric repulsions between the imidazole and lactone rings and therefore higher conformational energies.

The AM1 and MMP2 surfaces contain four nearly equienergetic minima, labeled A, B, C, and D. There are

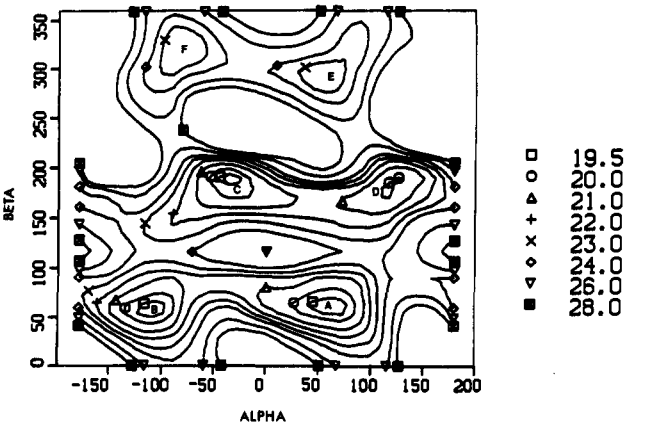

Figure 3. MMP2 potential energy contours of pilocarpine.

Table I. Torsional Angles of Pilocarpine Structures (deg)

description	α	β	γ
	A		
AM1	64.2	68.0	81.2
MMP2	60.1	60.1	64.9
STO-3G SCF	18.2	71.2	61.9
FR X-ray (1)	7.4	71.7	64.4
FR X-ray (2)	18.2	72.3	58.4
	B		
AM1	-107.0	66.2	81.0
MMP2	-113.5	61.5	64.4
STO-3G SCF	-102.2	65.2	70.2
	C		
AM1	3.9	162.4	171.3
MMP2	-32.3	-177.2	-178.3
STO-3G SCF	-4.2	169.2	-172.0
CJ X-ray	-4.2	168.4	-171.4
	D		
AM1	117.2	163.7	172.9
MMP2	119.0	179.8	-177.4
STO-3G SCF	99.8	169.1	-177.9
	E		
AM1[a]	-8.8	310.8	86.2
MMP2	54.9	292.4	63.8
STO-3G SCF	44.0	297.3	71.7
	F		
AM1[a]	-74.2	325.4	82.9
MMP2	-74.8	318.7	63.8
STO-3G SCF	-73.4	317.7	70.8

[a] These minima are not well-characterized and appear as broad regions on the AM1 surface shown in Figure 2.

Table II. Relative Energies of Pilocarpine Structures (kcal/mol)[a,b]

	energy			
description	AM1	MMP2	STO-3G	6-31G*[c]
A	0.1	0.1	0.0	0.0
B	0.0	0.0	0.2	0.6
C	0.7	0.4	0.1	1.3
D	0.3	0.3	0.4	2.2
E	3.7	2.8	3.4	5.3
F	4.6	2.5	3.8	5.8

[a] Energies of the conformations whose torsional angles are given in Table I. The AM1 $\Delta H_f°$ and the MMP2 steric energy at the global minimum B are -45.5 and 19.1 kcal/mol, respectively. [b] The STO-3G and 6-31G* energies for minimum A are -675.9924 and -684.4858 au (1 au = 627.5 kcal/mol). [c] Computed at the STO-3G geometry.

(15) An INDO semiempirical study of the potential energy surface of pilocarpine, without relaxation of parameters other than α and β, was reported: Kang, S. *Int. J. Quant. Chem.* 1974, *S1*, 109. His results are qualitatively similar to those found in the present work.

(16) Maat, L.; Peters, J. A.; Aboul-Enein, H. Y. *Org. Magn. Reson.* 1984, *22*, 455.

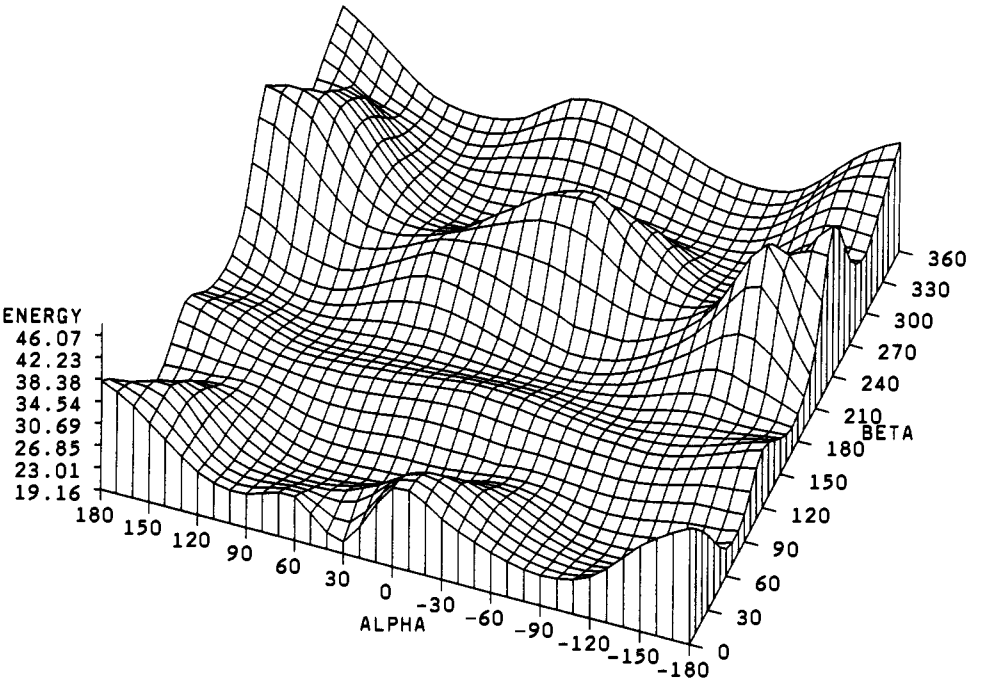

Figure 4. Three-dimensional depiction of the MMP2 potential energy surface of pilocarpine.

two additional minima, E and F, somewhat higher in energy on the MMP2 surface. These minima are poorly characterized on the AM1 surface. Torsional angles and energies of the six minima are given in Tables I and II, respectively.

Minimum A corresponds to the FR trichlorogerminate X-ray structures,[7a] although the AM1 and MMP2 α values of 64.2° and 60.1° are considerably larger than those in the two FR structures, 7.4° and 18.2°. Ab initio STO-3G calculations suggest that the region around minimum A is more elongated than that predicted by AM1 and MMP2: the conformational energy for $\beta = 72°$ remains constant to within 1 kcal/mol for α values ranging from 7° to 60°. The torsional angle C_7–C_8–O_9–C_{10} for minimum A has MMP2 and STO-3G values of 17.7° and 23.5°, respectively, in good agreement with the FR X-ray values, ca. 22.0°. The energies of minimum A and the FR structures are very similar. Minimum A is the lowest of the six minima according to the STO-3G calculations; in the AM1 and MMP2 calculations, minimum B is the lowest-energy structure.

Energy minimum C corresponds to the CJ X-ray structure.[7b] The STO-3G and MMP2 values of the torsional angle C_7–C_8–O_9–C_{10} are −19.8° and −16.1°, in good agreement with the CJ X-ray value, −20.4°. The ab initio STO-3G and 6-31G* SCF calculations furnish nearly equal energies for minima A and C, A being slightly lower. This is in agreement with the AM1 and MMP2 results. No X-ray structures corresponding to minima B, D, E, and F have been observed. Minima E and F are higher in energy by at least 2.5 kcal/mol, perhaps considerably more, than minima A, B, C, and D. They are compact structures with the two rings close together.

For $\beta < 180°$, maxima on each surface are found at (α,β) values of (0°,120°) and (180°,120°), where the lactone hydrogen on C_7 is close to an imidazole hydrogen on C_4 or an N_1-methyl hydrogen, respectively. In addition, $\beta = 120°$ corresponds to an eclipsed conformation about C_6–C_7. It may be noted that the AM1 surface is flatter than the MMP2 surface, due in part to the tendency of the AM1 method to underestimate ring-puckering effects. For example, energies of the maxima relative to the global minimum, B, on each surface, are 7.0 and 10.1 kcal/mol in MMP2 and 2.7 and 6.7 kcal/mol in AM1 for (0°,120°) and (180°,120°), respectively. (The ab initio STO-3G relative energy of the (0°,120°) maximum has an intermediate value, 4.9 kcal/mol.) The lactone rings produced by AM1 are also flatter than the envelope forms observed in the X-ray structures or calculated by the MMP2 and ab initio methods. Thus, the torsional angle C_7–C_8–O_9–C_{10} of minimum A is only 7.6° compared with MMP2, STO-3G, and X-ray values of ca. 20°.

On the Active Conformation of Pilocarpine

As seen from its potential energy surface, pilocarpine is a flexible molecule. Since the surface alone cannot rule out sufficient numbers of conformers to enable an inference of the biologically active conformation(s), additional evidence is needed. One approach is to compare the energetically accessible conformations of pilocarpine to the active and X-ray conformations of muscarine **4**. We therefore turn to the question of the structural relationship between pilocarpine and muscarine.

Pilocarpine and several other muscarinic agonists contain an imidazole group. For example, Schunack[17] has found some muscarinic potency on the guinea pig ileum for short-chain esters of 4-imidazoleacetic acid. Schulman and Goyal[18] have reported a class of muscarinic agonists in which an imidazole ring is coupled to a second heterocyclic moiety. Studies of both the pilocarpine proton NMR spectrum[7b] and pilocarpine-induced contractions of the guinea pig ileum[19] as a function of pH infer a pK_a of 7.0; ca. 30% of the molecules would be N_3-protonated at a physiological pH of 7.4. The positively charged ring undoubtedly interacts electrostatically with an anionic receptor site. Since the π electrons of the two imidazole nitrogens are electronically conjugated, both nitrogens carry a formal positive charge and therefore either the N_1-methyl group or N_3H may interact with the anionic receptor site. Recent data derived from the receptor sequences[20] and proteolysis of covalently bound propyl-

(17) Schunack, W. *Arch. Pharm.* (*Weinheim*) **1974**, *307*, 46.
(18) Schulman, J. M.; Goyal, R. K. U. S. Patent 4,871,758, Oct. 3, 1989; U. S. Patent 4,992,457, Feb. 12, 1991.
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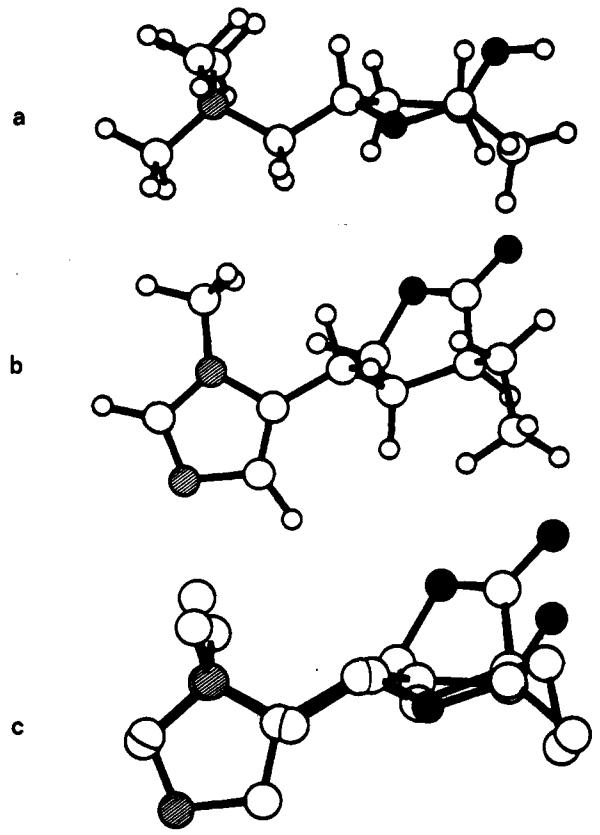


Figure 5. (a) The active form of muscarine (ref 9). (b) The active conformer of pilocarpine derived from fitting it to the active form of muscarine, as shown in c, where hydrogens are deleted for clarity. Solid atoms are oxygen, hatched atoms nitrogen.

benzilylcholine mustard[21] suggest that this site corresponds to the carboxylate oxygen(s) on one of two aspartates in (putative) helix 3 of the receptor.

It seems reasonable to assume that the terminal methyl of the ethyl group in 1 corresponds to the terminal methyl on the tetrahydrofuran ring of muscarine. This is supported by the well-known "five-atom rule", which requires the muscarine methyl group for potency[22] and the *S* configuration at C_{11} in pilocarpine, which fixes the apparently critical configuration of its ethyl group.

Muscarine and pilocarpine each have two oxygens. However, their different intramolecular separations imply that at most one oxygen of each molecule corresponds. While it is difficult to determine rigorously which of the two pilocarpine nitrogens and oxygens are the elements of its pharmacophore, a computer search suggests an interesting possibility: if N_1 and the carbonyl oxygen of pilocarpine are assumed to correspond to the muscarine

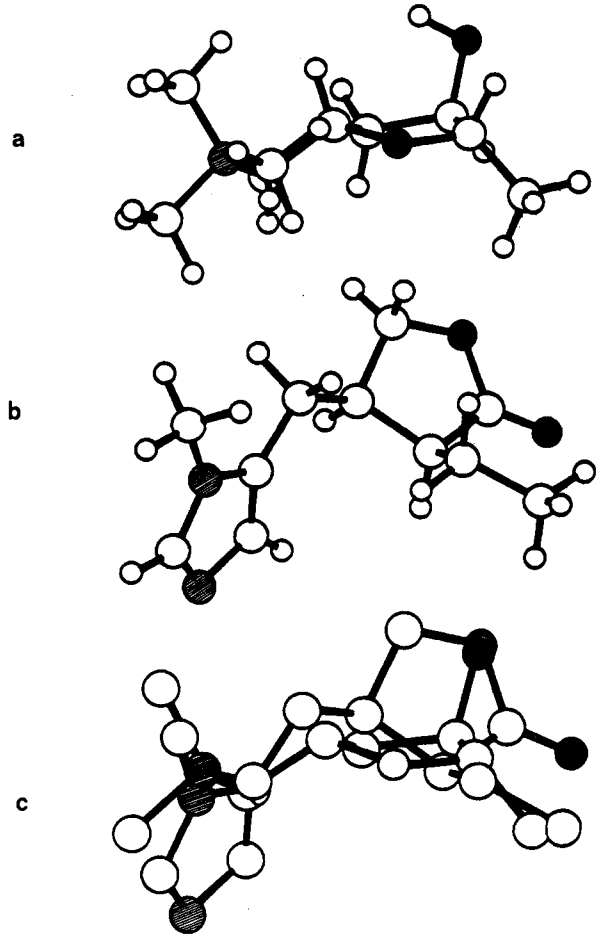


Figure 6. (a) The crystal form of muscarine (ref 23). (b) An alternate active conformer of pilocarpine derived from fitting it to the crystal form of muscarine, as shown in c, where hydrogens are deleted for clarity. Solid atoms are oxygen, hatched atoms nitrogen.

nitrogen and hydroxyl oxygen, respectively, a considerable number of coincidences between the atoms of each molecule can be found. This is confirmed by fitting pilocarpine to the muscarine active conformer proposed in a previous theoretical study (Figure 5a).[9] When the following atom pairs of each molecule are juxtaposed, the resulting interatomic deviations (the pilocarpine atom is given first) in angstroms are as follows: N_1 and N_1, 0.16; C_5 and C_2, 0.20; C_6 and C_3, 0.22; C_7 and C_4, 0.47; C_{11} and C_5, 0.27; C_{13} and C_6, 0.67; C_{14} and C_7, 0.30. The average deviation between corresponding atoms of 1 and 4 (Figure 5b) is 0.33 Å, the largest deviation being found for the methylene of the ethyl group of 1. (When the carbonyl oxygen, O_{12}, and the hydroxyl oxygen, O_9, are included in the fit, the average deviation becomes 0.42 Å; the distance between these oxygens is 0.89 Å.) The fitted conformation, which resembles minimum A, is shown in Figure 5c. Its torsional angles are as follows: $\alpha = 56°$, $\beta = 84°$, and $\gamma = 70°$. The AM1 and MMP2 energies of the structure are each only 0.7 kcal/mol higher than the global minimum, B, on their respective potential energy surfaces (Figures 2 and 3). Its STO-3G and 6-31G* energies are, respectively, 1.4 and 1.8 kcal/mol higher than those of the lowest energy ab initio structure, A. Thus, this conformation of pilocarpine, derived from its juxtaposition with muscarine as a template, is energetically accessible.

The active conformer of muscarine differs from the crystal structure of muscarine iodide[23] in having a 143°

(20) (a) Kubo, T.; Fukuda, K.; Mikami, A.; Maeda, A.; Takahashi, H.; Mishina, M.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Kojima, M.; Matsuo, H.; Hirose, T.; Numa, S. *Nature* **1986**, *323*, 411. (b) Kubo, T.; Maeda, A.; Sugimoto, K.; Akiba, I.; Mikami, M.; Takahashi, H.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Matsuo, H.; Hirose, T.; Numa, S. *FEBS Lett.* **1986**, *209*, 367. (c) Peralta, E. G.; Winslow, J. W.; Peterson, G. L.; Smith, D. H.; Ashkenazi, A.; Ramachandran, J.; Schimerlik, M. I.; Capon, D. J. *Science* **1987**, *236*, 600. (d) Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. *Science* **1987**, *237*, 527, 1556, 1628. (e) Akiba, I.; Kubo, T.; Maeda, A.; Bujo, H.; Nakai, J.; Mishina, M.; Numa, S. *FEBS Lett.* **1988**, *235*, 257.

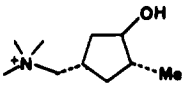
(21) (a) Curtis, C. A. M.; Wheatley, M.; Bansal, S.; Birdsall, N. J. M.; Eveleigh, P.; Pedder, E. K.; Poyner, D.; Hulme, E. C. *J. Biol. Chem.* **1989**, *264*, 489. (b) Fraser, C. M.; Wang, C.-D.; Robinson, D. A.; Gocayne, J. D.; Venter, J. C. *Mol. Pharm.* **1989**, *36*, 840.

(22) Ing, H. R. *Science* **1949**, *109*, 264.

(23) Jellinek, F. *Acta Crystallogr.* **1957**, *10*, 277.

NCCO angle vs 73° in the crystal structure. A reasonable fit of pilocarpine to the muscarine crystal form (Figure 6a) is obtained with the former in a conformation similar to its own (CJ) crystal form, i.e. near minimum C. This is an alternate possibility for the active conformer of 1. The interatomic deviations (the pilocarpine atom is given first) in angstroms are as follows: N_1 and N_1, 0.48; C_5 and C_2, 0.11; C_6 and C_3, 0.60; C_7 and C_4, 0.63; C_{11} and C_5, 0.64; C_{13} and C_6, 0.44; C_{14} and C_7, 0.49; O_9 and O_9, 0.66 (the lactone and hydroxyl oxygens have been juxtaposed). The average deviation between corresponding atoms of 1 and 4 (Figure 6b) is 0.50 Å, higher than in the previous fit. The fitted conformation, shown in Figure 6c, has torsional angles of $\alpha = -6°$, $\beta = 171°$, and $\gamma = -172°$.

We have not identified either pilocarpine oxygen with the tetrahydrofuran oxygen of muscarine: a computer search failed to find low-energy conformers of 1 which successfully juxtaposed the pilocarpine lactone or carbonyl oxygen with the ether oxygen of 4, while simultaneously superimposing nitrogens and terminal methyls of the two molecules. Although the muscarine ether oxygen has been assumed to be important for high efficacy,[9] weaker muscarinic agonists lacking this oxygen or its equivalent (such as the triple bond of oxotremorine) are known. For example, desethermuscarine (5)[24] has moderate muscarinic



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potency. Also, Raventós and Clark[25] and Stephenson[26] studied alkyltrimethylammonium cations which, although they have no oxygen, are full or partial agonists, depending upon the chain length. Pilocarpine itself is a partial agonist on some systems.[27]

The proposed structures of pilocarpine can be compared with those of classical muscarinic agonists which possess an NCCOCC backbone.[9] In the case of 1, the intermediate oxygen atom is absent, but the $N_1C_5C_6$–$C_{13}C_{14}$ spacing has been maintained, in part, by the cis fusion of C_6 and C_{13} on the lactone ring.

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