bearing on crystal interactions, the associated atoms would be statistically disordered. The resolution of the data and the small displacements involved to avoid these short contacts do not allow that disorder to be detected on maps or by inspection of temperature factors.

Crystal contacts are another cause for a deviation of the trimer from threefold symmetry. No major main-chain movement was found necessary to avoid unfavorable van der Waals interactions between trimers in a crystal (r.m.s. deviation of main-chain atoms for residues involved in a contact after superposition by the trimer symmetry: 0·24 Å). However, Fig. 4 gives an example of a difference of conformation due to a crystal contact, of side chains of residues which are otherwise related by the trimer symmetry. Finally, another consequence of crystal contacts is a difference of the temperature factors of otherwise threefold equivalent atoms: *e.g.* residues 127–129 and 156–164 of HA1 have average temperature factors of 19 and 25 $Å^2$ when they are at a crystal contact and of 25 and 32 $Å^2$ otherwise (the r.m.s. deviation of temperature factors between atoms of these residues in the two equivalent monomers which are not involved in a contact is 0·6 $Å^2$).

MK acknowledges support from the MRT (IPB/78), the CNRS (ATP France-USA 3/84), the Centre de Calcul Vectoriel pour le Recherche for Cray 1/S time, and the use of the LURE graphics facility. DCW acknowledges support from NIH grant No. AI 13654.

References

AGARWAL, R. C. (1978). *Acta Cryst.* A**34**, 791–809.
BLUNDELL, T. L., CURFIELD, J. S., CUTFIELD, S. M., DODSON, E. J., DODSON, G. G., HODGKIN, D. C. & MERCOLA, D. A. (1972). *Diabetes*, **21**, 492–505.
BOOTH, A. D. (1946). *Trans. Faraday Soc.* **42**, 444–448.
BRICOGNE, G. (1976). *Acta Cryst.* A**32**, 832–847.
COCHRAN, W. (1948). *Acta Cryst.* **1**, 138–142.
COCHRAN, W. (1951). *Acta Cryst.* **4**, 408–411.
DANIELS, R. J., DOWNIE, J. C., HAY, A. J., KNOSSOW, M., SKEHEL, J. J., WANG, M. L. & WILEY, D. C. (1985). *Cell*, **40**, 431–439.
DEISENHOFER, J. (1981). *Biochemistry*, **20**, 2361.
FREER, S. T., ALDEN, R. A., CARTER, C. W. & KRAUT, J. (1975). *J. Biol. Chem.* **250**, 46–54.
HENDRICKSON, W. A. (1981). In *Refinement of Protein Structures*, edited by P. A. MACHIN, J. W. CAMPBELL & M. ELDER, pp. 1–7. SRC Daresbury Laboratory.
HENDRICKSON, W. A. & KONNERT, J. H. (1980). In *Computing in Crystallography*, edited by R. DIAMOND, S. RAMASESHAN & K. VENKATESAN. Bangalore: Indian Academy of Sciences.
JACK, A. & LEVITT, M. (1978). *Acta Cryst.* A**34**, 931–935.
JONES, A. (1978). *J. Appl. Cryst.* **11**, 268–272.
KNOSSOW, M., DANIELS, R. S., DOUGLAS, A. R., SKEHEL, J. J. & WILEY, D. C. (1984). *Nature (London)*, **311**, 678–680.
LUZZATI, V. (1952). *Acta Cryst.* **5**, 802–810.
MOEWS, P. C. & KRETSINGER, R. M. (1975). *J. Mol. Biol.* **91**, 201–228.
SKEHEL, J. J., STEVENS, D. J., DANIELS, R. S., DOUGLAS, A. R., KNOSSOW, M., WILSON, I. A. & WILEY, D. C. (1984). *Proc. Natl Acad. Sci. USA*, **81**, 1779–1783.
TEN EYCK, L. F. (1977). *Acta Cryst.* A**33**, 486–492.
WILEY, D. C., WILSON, I. A. & SKEHEL, J. J. (1984). In *Biological Macromolecules and Assemblies*, Vol. 1, edited by A. MCPHERSON & F. W. JURNAK, pp. 299–336. New York: Wiley.
WILSON, I. A., LADNER, R. C., SKEHEL, J. J. & WILEY, D. C. (1983). *Biochem. Soc. Trans.* **11**, 145–147.
WILSON, I. A., SKEHEL, J. J. & WILEY, D. C. (1981). *Nature (London)*, **289**, 366–373.

Acta Cryst. (1986). B**42**, 632–638

The Structure and Absolute Configuration of (+)-Biperiden: a Chiral Ligand for the Pirenzepine Binding Site

BY PENELOPE W. CODDING

Departments of Chemistry and Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta T2N 1N4, Canada

(*Received* 6 *January* 1986; *accepted* 30 *June* 1986)

Abstract

The crystal structures of (+)-biperiden (*A*) and (+)-biperidenium L-(+)-mandelate (*B*) have been determined by X-ray crystallography. Crystal data: (*A*): $C_{21}H_{29}NO$, $M_r = 311·5$, orthorhombic, $P2_12_12_1$, $a = 5·8116\,(8)$, $b = 19·476\,(4)$, $c = 16·411\,(2)$ Å, $V = 1857·5\,(5)$ $Å^3$, $Z = 4$, $D_x = 1·11$ g cm^{-3}, λ(Mo $K\alpha$) = 0·071069 Å, $\mu = 0·66$ cm^{-1}, $F(000) = 680$, $T = 295$ K, $R = 0·041$, $wR = 0·042$ for 1812 contributing reflections; (*B*): $C_{21}H_{29}NO.C_8H_8O_3$, $M_r = 463·6$, monoclinic, $P2_1$, $a = 10·653\,(5)$, $b = 10·981\,(2)$, $c = 11·150\,(6)$ Å, $\beta = 94·09\,(2)°$, $V = 1301\,(1)$ $Å^3$, $Z = 2$, $D_x = 1·18$ g cm^{-3}, λ(Mo $K\alpha$) = 0·71069 Å, $\mu = 0·084$ cm^{-1}, $F(000) = 500$, $T = 295$ K, $R = 0·036$, $wR = 0·042$ for 1766 contributing reflections. The absolute configuration of (+)-biperiden is *S* as determined by correlation with the known configuration of L-(+)-mandelic acid. The (+)-biperiden ion conformation is superimposable on that of pirenzepine

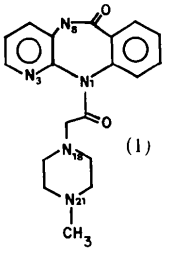
0108-7681/86/060632-07$01.50

and other chiral antimuscarinic drugs. These superpositions show that the two pirenzepine sites in smooth muscle and in brain are structurally similar and that the previous model, based on torsion angles, for the conformation of anticholinergic agents is insufficient to describe the shape of these ligands. The free-base conformation differs from that of the ion because of a difference in the H bonds formed in the two crystalline environments.

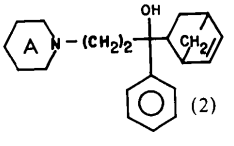
Introduction

Recent pharmacological studies of muscarinic agents have identified heterogeneous receptor subclasses. In particular, the anti-ulcer drug pirenzepine (1) has been used to classify the heterogeneity of muscarinic receptors since it has high affinity for receptors in the brain and low affinity for heart and smooth-muscle receptors (Hammer, Berrie, Birdsall, Burgen & Hulme, 1980). The presence of subclasses of muscarinic receptors requires a re-examination of the current models that describe the structural features of muscarinic receptor ligands to learn whether these models discriminate between the receptor subclasses or merely recognize the general features of muscarinic agents.

Stereoselectivity is a property of the muscarinic receptor and may be part of the discrimination between the receptor subclasses. Yamamura, Watson & Roeske (1983) demonstrated that the rat cerebral cortex, a high-affinity site for pirenzepine, is stereoselective and that pirenzepine binding to that site can be inhibited by muscarinic antagonists, but not by agonists. The smooth-muscle binding site for pirenzepine is also stereoselective. Using the chiral muscarinic agent, biperiden (2), Eltze & Figala (1984) found, in two *in vitro* tests, that (+)-biperiden is more effective than the (−)-enantiomer: the (+)-enantiomer was 280 times more effective as an antagonist than the (−)-enantiomer in guinea-pig ileum and 57 times more effective in the rat left atrium.



(1)



(2)

Prior to the identification of receptor subclasses, structural models for the receptor have been developed from the crystal structures and the potential-energy profiles of muscarinic ligands. Structure-function studies have identified the chemical groups necessary for activity as: a quaternary or tertiary N atom, a phenyl ring, an O atom (usually a hydroxyl group) and some other lipophilic group (usually bulky). Guy & Hamor (1975) identified a particular shape for these features which required a separation between the N atom and phenyl ring of 6·0 Å and a 'claw' shape for the fragment containing the N atom and the phenyl ring. Trummlitz, Schmidt, Wagner & Luger (1984) found that the pyridine and piperidine rings of the active form of pirenzepine, the monocation, have the shape and separation to fit this model. This preliminary model was extended to a single consistent conformation for anticholinergic agents by Pauling & Datta (1980). Their consistent conformation is based on the calculated minimum-energy conformations of 24 anticholinergic agents and is defined in terms of six torsion angles that define the relative orientations of the N atom, a chiral C atom, the ether O atom that connects the chiral center and the N atom, the crucial phenyl ring, and the additional hydrophobic group required for activity. The compounds included in this study were mostly of the same structural type: alkyl amine-substituted chiral esters; only the active enantiomer of each compound was studied. This model is difficult to apply to antagonists with chemical structures that do not contain esters. Trummlitz *et al.* (1984) conclude that pirenzepine has a shape similar to the consister conformation identified by Pauling & Datta; however, the fit is difficult to quantify because the six torsion angles are undefined in an achiral compound.

The stereoselectivity of ligand recognition is not adequately described by the two models presented above. The 'claw' model is two dimensional and hence achiral. Unfortunately, in the three-dimensional model, the six torsion angles that determine the consistent conformation do not define the relative orientation of *all four* substituents on the chiral C atom; thus, the active enantiomer is not specified. Even though the muscarinic receptor is stereoselective, neither of these models provides a method for determining which enantiomer of a new agent will be more effective.

The structures of (+)-biperiden free base and its mandelic acid salt were determined to establish the absolute configuration of (+)-biperiden and to probe the structural similarities and chirality of the two receptor subclasses in smooth muscle and in cerebral cortex. The structures of biperiden and pirenzepine are compared to establish the features that are common and the three-dimensional shapes of chiral ligands for the two sites are compared. These comparisons provide a test of the discriminatory power of the current models for muscarinic agents and can lead to a fuller description of the receptor site.

Experimental

The samples of (+)-biperiden free base (*A*) and the L-(+)-mandelic acid salt (*B*) of biperiden were provided by Dr V. Figala of Byk Gulden Pharmazeutika,

Konstanz, West Germany. Both compounds were recrystallized by slow evaporation from ethanol. Unit-cell parameters and orientation angles for each crystal were obtained by least squares from positional parameters of 25 reflections [(*A*): $17<\theta<21{\cdot}5°$; (*B*): $11{\cdot}4<\theta<20{\cdot}2°$] individually centered on an Enraf-Nonius CAD-4 diffractometer. Both crystals were clear rectangular solids; crystal sizes: biperiden $0{\cdot}2\times0{\cdot}3\times0{\cdot}4$ mm; biperidenium mandelate $0{\cdot}2\times0{\cdot}4\times0{\cdot}6$ mm. Diffracted intensities were collected by ω-2θ scan to max. θ of 27·5° in a manner previously described in detail (Codding, 1983). Three standard reflections, no intensity variation. 5100 reflections were collected from two octants (h 0–7, k 0–25, l 21–21) for (*A*); they were averaged ($R_{int}=0{\cdot}03$) to give 2445 reflections of which 1812 had $I>2{\cdot}5\sigma(I)$ and were taken as observed. 3372 reflections were collected from one quadrant (h 13–13, k 0–14, l 0–14) for (*B*); of these, 3126 were unique and 1766 had $I>2{\cdot}0\sigma(I)$ and were taken as observed. Intensities were corrected for Lorentz and polarization effects, but not for absorption.

The structures were solved using *MULTAN* (Germain, Main & Woolfson, 1971). For each compound, H atoms were identified in difference Fourier syntheses and included in the model. In the free-base determination, the positional parameters and isotropic thermal parameters for the H atoms were refined alternately with cycles that varied the positional parameters and anisotropic thermal parameters of the non-H atoms. In the (+)-biperiden salt structure determination, the H atom of the hydroxy O atom in mandelic acid was not identified. The H atoms were assigned isotropic thermal parameters equal to 1·2 times the thermal parameter of the atom to which they were bonded and were not refined. Because of the low ratio of observations to parameters, the non-H atoms of the mandelic acid salt were refined in cycles that alternately varied the anisotropic thermal parameters and positional parameters of the mandelic acid moiety in one cycle and those of the biperiden ion in the other cycle. In both structures, only the observed reflections were used in the refinement. The weights were defined as $w^{-1}=\sigma^2(F_o)$ for biperiden and $w^{-1}=[\sigma^2(F_o)+0{\cdot}001(F_o)^2]$ for the mandelate. The shift/e.s.d. ratios in the final cycle of least squares were 0·0245 and 0·0123 for free base and cation, respectively. The goodness of fit was 1·65 for biperiden and 1·71 for the mandelate; the errors in the final Fourier syntheses were 0·15 and 0·30 e Å^{-3}, respectively. The scattering factors used in the refinement were those of Cromer & Mann (1968) except for the H atoms (Stewart, Davidson & Simpson, 1965). Unless otherwise stated, the programs used were those of the *XRAY*76 system (Stewart, Machin, Dickinson, Ammon, Heck & Flack, 1976).

The absolute configuration of the (+)-biperiden ion was obtained by correlation of the mandelic acid fragment with the known absolute configuration of L-(+)-mandelic acid (Klyne & Buckingham, 1978). The configuration about C(1) is *S*. The configuration of (+)-biperiden free base was obtained by correlation with the ion.

The atomic coordinates for (+)-biperiden and (+)-biperiden L-(+)-mandelate are given in Tables 1 and 2* with the atoms labeled as in Figs. 1 and 2.

* The bond distances and angles, torsional angles, anisotropic thermal parameters, H-atom parameters and lists of structure factors for both structures have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 43074 (35 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

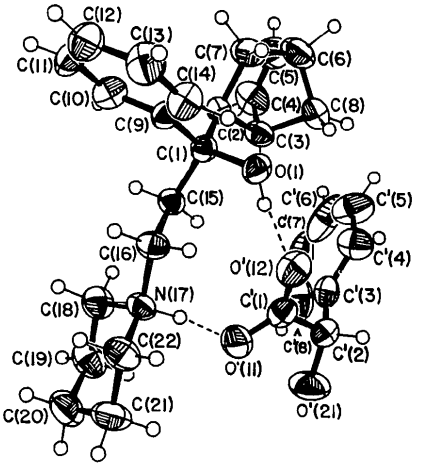


Fig. 1. The molecular conformation and atomic labeling scheme for (+)-bipiridenium mandelate. The H bonds are shown as dashed lines. The drawing was made with *ORTEP* (Johnson, 1970). Thermal ellipsoids here and in Fig. 2 are drawn at the 50% probability level.

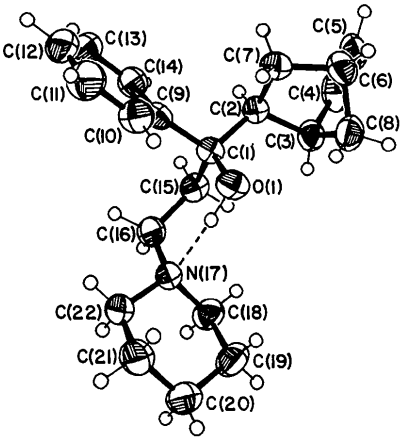


Fig. 2. The molecular conformation and atomic labeling scheme for (+)-biperiden free base. The H bond is shown as a dashed line. The drawing was made with *ORTEP* (Johnson, 1970).

Table 1. *Fractional coordinates* ($\times 10^4$) *and* B_{eq} *values* ($Å^2 \times 10$) *for the non-*H *atoms of* (+)*-biperiden free base*

E.s.d.'s are given in parentheses. B_{eq} is defined as one-third the trace of the U_{ij} matrix.

	x	y	z	B_{eq}
C(1)	−1500 (4)	232 (1)	9534 (1)	32 (1)
O(1)	537 (3)	389 (1)	9986 (1)	39 (1)
C(2)	−1448 (4)	−543 (1)	9321 (1)	33 (1)
C(3)	−1238 (5)	−1056 (1)	10056 (1)	44 (1)
C(4)	−1961 (5)	−1738 (1)	9698 (2)	57 (1)
C(5)	−296 (6)	−1942 (1)	9197 (2)	57 (1)
C(6)	1576 (5)	−1407 (1)	9221 (2)	49 (1)
C(7)	634 (4)	−760 (1)	8786 (1)	41 (1)
C(8)	1373 (5)	−1170 (1)	10112 (2)	52 (1)
C(9)	−1545 (4)	636 (1)	8727 (1)	34 (1)
C(10)	250 (5)	1064 (1)	8506 (2)	46 (1)
C(11)	208 (6)	1410 (1)	7766 (2)	60 (1)
C(12)	−1598 (7)	1330 (1)	7232 (2)	59 (1)
C(13)	−3385 (6)	912 (1)	7444 (1)	52 (1)
C(14)	−3361 (5)	568 (1)	8185 (1)	43 (1)
C(15)	−3647 (4)	404 (1)	10046 (1)	37 (1)
C(16)	−3771 (4)	1155 (1)	10308 (1)	42 (1)
N(17)	−1873 (3)	1359 (1)	10846 (1)	36 (1)
C(18)	−2167 (5)	1093 (1)	11678 (1)	48 (1)
C(19)	−90 (6)	1254 (1)	12195 (2)	56 (1)
C(20)	359 (6)	2020 (1)	12233 (2)	56 (1)
C(21)	465 (6)	2313 (1)	11371 (2)	54 (1)
C(22)	−1608 (6)	2109 (1)	10873 (1)	47 (1)

Table 2. *Fractional atomic coordinates* ($\times 10^4$) *and* B_{eq} ($Å^2 \times 10$) *for the non-*H *atoms of* (+)*-biperiden* L-(+)*-mandelic acid salt*

The *y* coordinate of C(1) was held invariant to define the cell origin. B_{eq} is as defined for the free base.

	x	y	z	B_{eq}
C(1)	−9914 (5)	−2819	−175 (5)	31 (3)
O(1)	−9452 (3)	−1612 (4)	75 (3)	39 (2)
C(2)	−9011 (5)	−3533 (6)	−920 (5)	35 (3)
C(3)	−7610 (5)	−3601 (7)	−429 (5)	40 (3)
C(4)	−7033 (6)	−4607 (8)	−1142 (7)	56 (4)
C(5)	−6947 (7)	−4204 (10)	−2227 (8)	70 (5)
C(6)	−7421 (7)	−2920 (9)	−2286 (6)	57 (4)
C(7)	−8873 (6)	−2991 (8)	−2205 (5)	51 (4)
C(8)	−7030 (6)	−2482 (7)	−1008 (6)	50 (4)
C(9)	−11185 (5)	−2686 (6)	−854 (5)	34 (3)
C(10)	−11657 (6)	−1579 (7)	−1220 (6)	45 (3)
C(11)	−12826 (7)	−1481 (8)	−1870 (7)	53 (4)
C(12)	−13520 (6)	−2499 (8)	−2158 (6)	50 (4)
C(13)	−13064 (6)	−3620 (8)	−1801 (6)	48 (4)
C(14)	−11909 (6)	−3719 (7)	−1170 (6)	43 (3)
C(15)	−10054 (5)	−3477 (6)	1030 (5)	33 (3)
C(16)	−10951 (5)	−2804 (6)	1796 (5)	37 (3)
N(17)	−10756 (4)	−3129 (5)	3106 (4)	36 (2)
C(18)	−10886 (7)	−4476 (7)	3334 (6)	47 (4)
C(19)	−10694 (6)	−4757 (7)	4655 (6)	53 (4)
C(20)	−11635 (7)	−4069 (9)	5361 (7)	61 (4)
C(21)	−11485 (7)	−2723 (9)	5134 (6)	60 (4)
C(22)	−11661 (6)	−2418 (7)	3799 (6)	50 (4)
C′(1)	−7685 (4)	−1980 (5)	3208 (4)	40 (2)
O′(11)	−8431 (3)	−2501 (4)	3889 (3)	59 (2)
O′(12)	−7941 (3)	−1585 (3)	2188 (3)	50 (2)
C′(2)	−6311 (4)	−1872 (5)	3742 (4)	44 (2)
O′(21)	−6278 (3)	−2134 (4)	4988 (3)	73 (2)
C′(3)	−5501 (4)	−2723 (5)	3084 (4)	38 (2)
C′(4)	−5124 (4)	−2397 (5)	1956 (4)	55 (3)
C′(5)	−4476 (5)	−3189 (8)	1290 (6)	75 (4)
C′(6)	−4164 (7)	−4308 (9)	1721 (10)	98 (6)
C′(7)	−4506 (6)	−4665 (7)	2822 (10)	94 (5)
C′(8)	−5169 (5)	−3850 (6)	3511 (6)	64 (3)

Results

The conformation of (+)-biperiden is different in the ion and in the free base, as shown in Figs. 1 and 2. In the free base, the alkyl chain connecting the chiral C(1) atom and the piperidinyl ring is folded. The torsion angle along this chain [C(1)–C(15)–C(16)–N(17)] is −63·2(2)°. In contrast, the ionized form of (+)-biperiden is in an extended conformation: the same torsion angle is −158·8(3)°. This difference in orientation is achieved by a rotation about the C(15)–C(16) bond in the free base to bring the N atom close to the hydroxyl group. The positions of the other substituents on C(1), relative to the C(15)–C(16) bond, are the same in the two structures: the torsion angles of the type C(16)–C(15)–C(1)–*X*, where X is O(1), C(9) or C(2), differ by less than 6° in the two structures.

The molecular conformation is dependent upon the formation of H bonds in the two crystalline environments. In the free base, the H bond is formed by intramolecular donation of the hydroxyl H atom to the N atom of the piperidine ring. In this conformation N(17) is 2·744 (2) Å from O(1) and 1·89(2) Å from H(1) with an angle N(17)···H(1)–O(1) of 154(3)°. The extended conformation of the ionized form allows the formation of two H bonds to the counterion, L-(+)-mandelate. Both the protonated N atom and the hydroxyl O atom form H bonds to the carboxylate group of the counterion. The distances are N(17)···O′(11) 2·658(5) and H(17)···O′(11) 1·61 Å for one bond and O(1)···O′(12) 2·758(5) and H(1)···O′(12) 1·80 Å for the other. The angles are N(17)–H(17)···O′(11) 171 and O(1)–H(1)···O′(12) 164°. Evidently, the two conformations, extended and folded, differ in energy by less than the stabilization provided by one H bond, *ca.* 21 kJ mol^{-1}.

There are differences in the dimensions and the conformation of the piperidine ring [ring *A* in (2)] in the two forms of (+)-biperiden. The average N(17)–C bond distance in the protonated form is 1·502(3) Å and in the free base 1·469(2) Å. This difference reflects the redistribution of electron density about the N atom when a fourth bond is formed. In the free base the conformation of the piperidine ring is flattened around C(20): the torsion angles are C(18)–C(19)–C(20)–C(21) −52·4(3) and C(19)–C(20)–C(21)–C(22) 51·5(3)°. Such flattening decreases the 1,3 diaxial interaction of H atoms with the lone pair on N(17). The protonated piperidine ring in the mandelate structure has a nearly ideal chair conformation.

Discussion

H-bond formation is an important contributor to the stabilization of any particular conformation of either biperiden or pirenzepine, the potent muscarinic

antagonist. Depending on the H-bonding pattern, biperiden has two conformations; similarly, pirenzepine has three conformations in its three ionization states which are: folded conformations with the piperazine ring in close proximity to the pyridine ring of the tricyclic system for the free base of pirenzepine (Ruzic-Toros & Kojic-Prodic, 1983) and the monoprotonated form (Trummlitz *et al.*, 1984) and an extended conformation which facilitates the formation of N–H···Cl H bonds to both protonated N atoms for the diprotonated pirenzepine structure (Trummlitz *et al.*, 1984). The two folded conformations of pirenzepine differ in the relative orientation of the two heterocyclic rings. Unlike (+)-biperiden, neither of the folded conformers is stabilized by an intramolecular H bond. Instead, the conformations are due to intermolecular H bonding to either a solvent molecule or to a counterion.

The conformations of the monocationic forms of (+)-biperiden and pirenzepine are similar despite the differences in crystalline environments. Since the usual pK_a range for a piperidine N atom is 10·38–11·12 (Albert & Serjeant, 1984), (+)-biperiden will be ionized at physiological pH and the extended form will be the relevant conformation pharmacologically. Fig. 3 shows the superposition of the crystal structures of (+)-biperiden and pirenzepine, both as monocations, as calculated (Smith, 1980) by the minimization of the differences in the coordinates of the centers of rings and the positions of N(17) to N(21) and O(1) to O(16), where the second atom in each pair is from pirenzepine. The phenyl ring of pirenzepine overlaps with the aromatic ring of biperiden; in addition, the protonated N atoms and the neighboring O atoms are placed so that each molecule could bond to the same receptor site features. The H bonds found in the biperidenium mandelate structure provide a model of the type of interaction possible with these groups and suggest that these muscarinic antagonists interact with an acidic group in the receptor.

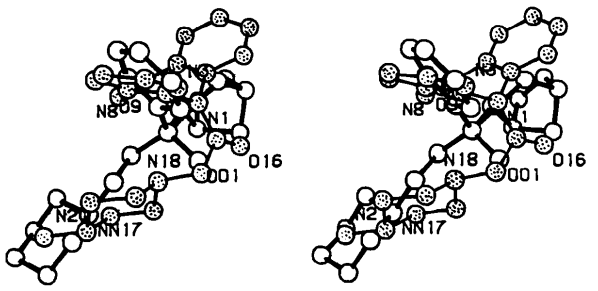
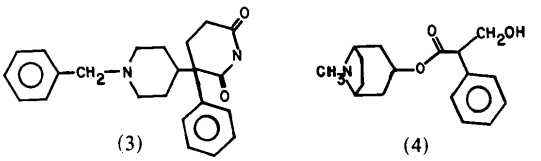


Fig. 3. A stereoscopic drawing of the superposition of the structures of (+)-biperiden (2) ion and pirenzepine (1) monocation. The pirenzepine atoms are dotted and the heteroatoms of biperiden are double lettered. There are three points of overlap: 1. the piperidine/piperazine ring N atoms, NN(17) and N(21); 2. the hydroxyl, OO(1), or carbonyl, O(16), O atoms; 3. the aromatic rings in the upper left corner. This drawing and those in Figs. 4 and 5 were made with *PLUTO* (Motherwell, 1979).

The enantiomer of (+)-biperiden does not overlap with pirenzepine with the same correspondence.

The structure of the active enantiomer of biperiden is the same as the structures of chiral ligands for the pirenzepine binding site in rat brain even though biperiden has higher affinity for the smooth-muscle site. Yamamura, Watson & Roeske (1983) have shown that the high-affinity pirenzepine site can be inhibited by dexetimide (3) and atropine (4) but not by levetimide, the enantiomer of dexetimide. The stereoselectivity of the binding site is exemplified by these two enantiomers: levetimide requires 1000 times the concentration of dexetimide needed to achieve 50% antagonism of the muscarinic response. Fig. 4 shows the overlap of the (+)-biperiden ion with the crystal structure of dexetimide (3) (Spek, 1976). The same structural homology is observed between these structures as was found between pirenzepine and (+)-biperiden. The phenyl ring in dexetimide overlaps with that of biperiden, as do the piperidine ring N atoms and neighboring O atoms. A small rotation of the piperidine ring in the dexetimide structure would direct the H atom on the protonated N atom to the same point as in the (+)-biperiden ion. The correspondence between (+)-biperiden and atropine (4) (Kussather & Haase, 1972) shown in Fig. 5 is not as good as in the other cases; nevertheless, the N^+ atoms, the O atoms, and the aromatic rings superimpose. Single-bond rotations of the atropine structure could improve the superpositions.

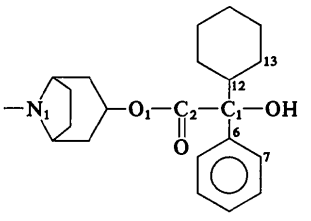


(3) (4)

Even though these four ligands for the muscarinic receptor can be superimposed with a good fit, most of the torsion angles that define their conformation do not fit the model developed by Pauling & Datta (1980). The single consistent conformation identified by Pauling & Datta (see Table 3) arises from simplified energy calculations that identify energetically favorable conformations that may not be observed in the crystalline state. The torsion angles of the solid-state conformations of the compounds examined in Figs. 3–5 vary from the consistent conformation by as much as 120°; this wide variance either indicates that these torsion angles are not sufficiently discriminatory or that the energy difference between conformers, is minimal. What is particularly disturbing about the torsion-angle description of molecular conformation is that similar molecular shapes (*cf.* Figs. 3, 4 and 5) cannot be detected by the six torsion angles defined in this model (*cf.* Table 3). It would appear that the torsion-angle descriptors can only be useful when applied within a given chemical structure class. This problem of insufficient discriminatory

Table 3. *Comparisons of the torsion angles in chiral muscarinic ligands with the single consistent conformation of Pauling & Data* (1980)

Angles defined with respect to this typical muscarinic ligand:



In the four chiral ligands, angles equivalent to those of the Pauling-Datta model were defined by assuming, in each case, that the N atom, the chiral C atom and the phenyl ring correspond to these same atoms in the model.

			Angles (°)		
	Model	(+)Bip*	Atrop	Dext	Dext
τ(N1) N(1)-O(1)-C(2)-C(1)†	−132	−159	125	−146	−154
τ(N2) N(1)-C(2)-C(1)-C(6)†	35	−76	−49	129	99
τ(N3) N(1)-C(1)-C(6)-C(7)†	−85	−83	−86	−78	−84
τ_2 O(1)-C(2)-C(1)-O(6)	54	−58	−77	69	36
τ_3 O(2)-C(1)-O(6)-C(7)	−91	−59	−63	−97	−101
τ_4 C(2)-C(1)-C(12)-C(13)	−58	−66	‡	−178	−173

* Abbreviations: (+)Bip = (+)-biperiden cation, Atrop = atropine, Dext = dexetimide (there were two unique molecules).
† Improper torsion angles involving nonbonded atoms.
‡ Undefined in this structure.

power in torsion-angle descriptions of active conformations has been observed in other structure-activity studies (Codding & James, 1984).

The improper torsion angle, τ(N3), that describes the orientation of the crucial phenyl ring to the line between the N atom and the chiral C atom is consistent in these four ligands and does fit the model proposed by Pauling & Datta. Concomittantly, the separation between the N atom and the center of this phenyl ring is nearly the same in these four ligands: the average separation is 5·9 Å and compares favorably with the value of 6·0 Å for the 'claw' model of Guy & Hamor (1975). These comparisons indicate that the relative orientation and separation of the N atom and a phenyl ring are the important determinants of muscarinic receptor affinity; but this one parameter does not describe the chiral nature of the receptor site.

The superpositions of the chiral ligands for the pirenzepine binding site indicate that the brain and smooth-muscle sites recognize the same features: a specific arrangement of an aromatic ring, an H-bond donor (OH group in biperiden), a lipophilic group, and an N atom. It is the addition of the fourth site, the hydroxyl O atom in biperiden, that produces stereoselectivity. The low affinity of both (−)-biperiden and levetimide indicates that the binding site recognizes a counterclockwise grouping of N^+

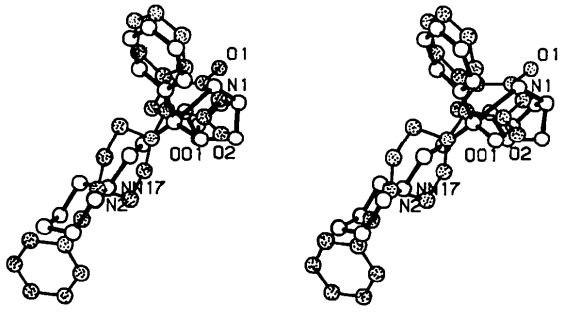


Fig. 4. A stereoscopic drawing of the superposition of the structures of (+)-biperiden (2) ion and dexetimide (3). The dexetimide atoms are dotted and the heteroatoms of biperiden are double lettered. There are three points of overlap as in Fig. 3: 1. the N atoms, NN(17) and N(2); 2. the O atoms OO(1) and O(2); 3. the phenyl rings at upper left.

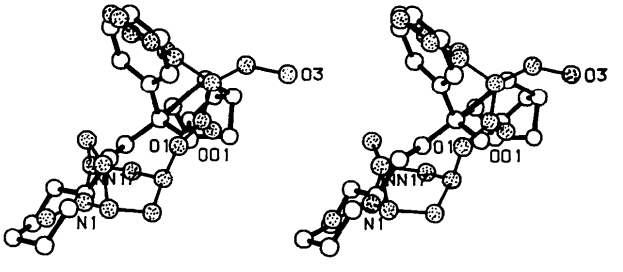


Fig. 5. A stereoscopic drawing of the superposition of the structures of (+)-biperiden (2) ion and atropine (4). The atropine atoms are dotted and the heteroatoms of biperiden are double lettered. There are three points of overlap as in Fig. 3: 1. the N atoms, NN(17) and N(1); 2. the O atoms, OO(1) and O(1); 3. the aromatic rings at upper left.

atom, O atom and aromatic ring. This model suggests that higher-affinity ligands for the pirenzepine site could be developed if the relative orientation of the N^+-containing ring were restricted to the common position found in the four structures: pirenzepine, (+)-biperiden, dexetimide and atropine.

The structural comparisons of chiral ligands for the pirenzepine site show both that structural differences in the two types of binding sites are not evident and that structural similarities in the ligands are not detected by the torsion angles of the consistent conformation for anticholinergics. Since the chiral ligands for both of the pirenzepine binding sites are structurally superimposable, the difference between these sites must arise from factors other than simple shape recognition; these factors may be related to induced conformational changes in either the receptor or the ligand. The ligands compared in this study have a close structural match which belies the wide variation in the values of the torsion angles of the consistent anticholinergic conformation. This discrepancy suggests that more detailed models that compare overall shapes and juxtapose similar heteroatoms are required so that a range of chemical types of ligands for a common receptor can be compared.

This work was supported by the Medical Research Council of Canada (grant MA 8087). The author thanks Dr V. Figala and Dr M. Elitze of Byk Gulden Pharmazeutika for providing the samples and the results of their pharmacological tests and Norma E. Duke for collecting the (+)-biperiden data.

References

ALBERT, A. & SERJEANT, E. P. (1984). *The Determination of Ionization Constants*, p. 151. London: Chapman and Hall.
CODDING, P. W. (1983). *Can. J. Chem.* **61**, 529-532.
CODDING, P. W. & JAMES, M. N. G. (1984). *Acta Cryst.* B**40**, 429-434.
CROMER, D. T. & MANN, J. B. (1968). *Acta Cryst.* A**24**, 321-324.
ELTZE, M. & FIGALA, V. (1984). Private communication.
GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368-376.
GUY, J. J. & HAMOR, T. A. (1975). *J. Chem. Soc. Perkin Trans.* 2, pp. 1074-1077.
HAMMER, R., BERRIE, C. P., BIRDSALL, N. J. M., BURGEN, A. B. V. & HULME, E. C. (1980). *Nature (London)*, **283**, 90-92.
JOHNSON, C. K. (1970). *ORTEP.* Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.
KLYNE, W. & BUCKINGHAM, J. (1978). *Atlas of Stereochemistry*, Vol. 1, 2nd ed. New York: Oxford Univ. Press.
KUSSATHER, E. & HAASE, J. (1972). *Acta Cryst.* B**28**, 2896-2899.
MOTHERWELL, S. (1979). *PLUTO.* Program for plotting molecular and crystal structures. Univ. of Cambridge, England.
PAULING, P. & DATTA, N. (1980). *Proc. Natl Acad. Sci. USA*, **77**, 708-712.
RUZIC-TOROS, Z. & KOJIC-PRODIC, B. (1983). *Acta Cryst.* C**39**, 93-95.
SMITH, G. D. (1980). *PROFIT.* A molecular superposition program. Medical Foundation of Buffalo, Buffalo, New York.
SPEK, A. L. (1976). *Acta Cryst.* B**32**, 870-877.
STEWART, J. M., MACHIN, P. A., DICKINSON, C. W., AMMON, H. L., HECK, H. & FLACK, H. (1976). The *XRAY*76 system. Tech. Rep. TR-446. Computer Science Center, Univ. of Maryland, College Park, Maryland.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175-3187.
TRUMMLITZ, G., SCHMIDT, G., WAGNER, H.-U. & LUGER, P. (1984). *Arzneim. Forsch.* **34**, 849-859.
YAMAMURA, H. I., WATSON, M. & ROESKE, W. R. (1983). *CNS Receptors-From Molecular Pharmacology to Behavior*, edited by P. MANDEL & F. V. DEFEUDIS, pp. 331-336. New York: Raven Press.

International Union of Crystallography

Acta Cryst. (1986). B**42**, 638

Deposition of Data with Brookhaven Protein Data Bank

Acta Crystallographica policy provides authors of biological-macromolecule papers with the option of requesting that their list of structure factors deposited with the Brookhaven Protein Data Bank be granted privileged status for a period of no longer than four years from the date of publication, see *Notes for Authors* [*Acta Cryst.* (1983), A**39**, 174-186]. In a recent paper by Comarmond, Giegé, Thierry, Moras & Fischer [*Acta Cryst.* (1986), B**42**, 272-280] the atomic coordinates were wrongly assigned this status also. The atomic coordinates are available for immediate distribution.

Currently acceptable formats for the machine-readable media are: magnetic tape with fixed line length and fixed block size in 9 track, ASCII or EBCDIC, 800, 1600 or 6250 CPI; or punched cards with IBM 026 or IBM 029 codes.

Copies of data in machine-readable form are available from the Protein Data Bank at Brookhaven or one of the affiliated centers at Melbourne or Osaka (but no longer at Cambridge).