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Conformational studies on the four stereoisomers of the novel anticholinergic 4-(dimethylamino)-2-phenyl-2-(2-pyridyl)pentanamide

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Summary

To interpret differences in the anticholinergic activity among the four stereoisomers of 4-(dimethylamino)-2-phenyl-2-(2-pyridyl)pentanamide (**1**–**4**), we performed conformational studies using the semiempirical molecular orbital method. The structures of the global minimum-energy conformations obtained for **1**–**4**, however, could not explain the different activities, particularly in terms of distances between the essential pharmacophores. We thus implemented superimposition studies, using the energetically stable conformations of the most active stereoisomer, **1**(2*S*,4*R*), as a template. The energy penalties for a conformation change of the less active stereoisomers **2**–**4** from their global minimum-energy structure to a new conformation, fitting onto the global minimum-energy conformation of **1**, appear to account for the differences in the pharmacological potency better than using the other conformations of **1** as a template. We thus presume that the global minimum-energy conformation of **1** is closely related to the bioactive conformation for these anticholinergics, and also that the pharmacological potency is linked to how readily these substances can change their conformations to fit the muscarinic receptor.

Introduction

Previously we have described the synthesis and anticholinergic activity of the four stereoisomers **1**–**4** of 4-(dimethylamino)-2-phenyl-2-(2-pyridyl)pentanamide [1]. Among these stereoisomers, large differences in potency were seen from the in vitro assay, acetylcholine-induced contraction of isolated guinea pig ileum.

Anticholinergic agents which act on the muscarinic receptor have been demonstrated to be useful for treating, e.g., spasm of internal organs and overactive detrusor syndrome, associated with bladder muscle instability [2,3]. Regarding the muscarinic receptor, three distinct subtypes, M_1, M_2 and M_3, have been identified pharmacologically [4], and molecular cloning studies have identified five unique sequences for muscarinic receptors m_1–m_5 [5]. Although the relationships between pharmacologically and genetically defined muscarinic receptor subtypes have not been established unequivocally, it is likely that the m_1 sequence corresponds to that of the M_1 receptor, m_2 to the M_2 receptor and m_3 to the M_3 receptor [6]. On the basis of the amino acid sequences, these muscarinic receptors have been shown to belong to the G-protein-coupled (GPC) receptor family and consist of seven transmembrane segments, presumably adopting an α-helical conformation [7]. However, highly refined 3D structures of GPC receptors are unknown, although several 3D models of the transmembrane regions have been reported through homology-based modeling by using bacteriorhodopsin which has been determined by high-resolution electron cryomicroscopy [8].

Numerous studies on the structure–activity relationships of anticholinergic substances have been performed to elucidate the prerequisite pharmacophores [9,10] and the stereochemistry [11] required for effective binding to the muscarinic receptor. Despite these studies the bioactive conformation remains unsolved, although recent molecular modeling studies, particularly on conformationally rigid analogues, have provided some pharmacophore models [12–19].

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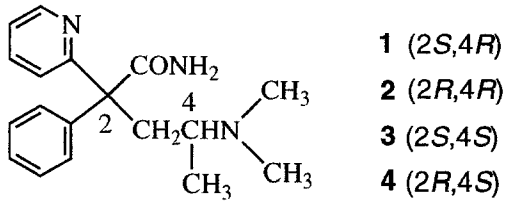

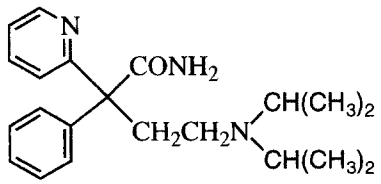

Scheme 1. Structures of the four stereoisomers **1**–**4** (left) and structure of disopyramide (right).

The subject compounds in this paper, the four stereoisomers of 4-(dimethylamino)-2-phenyl-2-(2-pyridyl)pentanamide, **1**–**4**, definitely act on the M_3 receptor with a variety of binding affinities, because contraction of guinea pig ileum induced by acetylcholine is ascribed to the action of the M_3 receptor. In addition, these compounds bear exactly the same elements for receptor binding, albeit with diverse spatial orientations. Therefore, it is reasonable to expect the spatial orientations of the essential pharmacophores to reflect the potency of their anticholinergic activities. As mentioned above, there are no high-resolution 3D structures of the muscarinic receptors located in the membrane; therefore, docking studies to explore likely conformations of these substances in the binding site are impossible. Hence, we performed conformational studies on **1**–**4** to gain further insight into their structural forms bound to the muscarinic receptor.

We describe herein conformational studies on the four stereoisomers of 4-(dimethylamino)-2-phenyl-2-(2-pyridyl)-pentanamide using the semiempirical molecular orbital method, resulting in an interpretation of their stereochemistry–activity relationship and also in a proposal for the likely bioactive conformation on the receptor.

Methods

We previously reported the crystal structures of stereoisomers **2** and **4** [1], showing that an intramolecular hydrogen bond (H-bond) does not exist between the pyridine and amide moieties in the solid state. However, we did not employ these crystal structures for the conformational studies on **1**–**4**; rather, we used the molecular structure (Fig. 1) with an intramolecular H-bond and the protonated form of the tertiary amine. There is considerable evidence to suggest that such an intramolecular H-bond exists in aqueous solution. Czeisler et al. [20] have concluded from studies on the pK_a values of disopyramide that an intramolecular H-bond between the pyridine and the amide exists in aqueous solution. Although no actual pK_a value for the pyridine nitrogen atom in disopyramide was determined, they suspected that it would be below 2. We measured the pK_a values for the racemic mixture of **2** and **3** [21,22]. The tertiary amine (pK_{a1}, measured according to Ref. 21) and the pyridine (pK_{a2}, measured according to Ref. 22) had pK_a values of 10.4 and 1.6, respectively. The value of pK_{a2} is abnormally low in comparison with simple pyridine moieties (pK_a ~3–5), indicating that an intramolecular H-bond between the pyridine and amide groups exists in this type of compound in aqueous solution. Therefore, it is likely that the intramolecular H-bond also exists under more lipophilic conditions or in the gas phase, which is usually considered to represent the lipophilic binding pocket in the receptor.

To find the global and local minimum-energy conformations of **1**(2*S*,4*R*) and **2**(2*R*,4*R*), we first generated energy maps around the C2-C3 (τ1) and C3-C4 (τ2) bonds (Fig. 1) using the semiempirical molecular orbital method AM1 (MOPAC, Version 5) [23]. Keeping the two aromatic rings as well as the intramolecular H-bond moiety flat, the other structural parameters except the torsion angles τ1 and τ2 were optimized in 15° increment steps to provide the grid points of the map. The energetically stable conformations in these maps (Fig. 2) were then optimized in terms of all the structural parameters, including τ1 and τ2, using AM1, while still keeping the intramolecular H-bond moiety flat. In addition to the heat of formation obtained using AM1, the total energy of each finally optimized conformation was provided by a single-point calculation using the 3-21G (GAUSSIAN-92) basis set [24]. All the conformations within approximately 10 kcal mol^{-1} of the global minimum-energy conformation were selected as local minimum-energy conformations. The global and local minimum-energy conformations for **4**(2*R*,4*S*) and **3**(2*S*,4*S*) were also found as the mirror images of the corresponding enantiomers **1**(2*S*,4*R*) and **2**(2*R*,4*R*), respectively.

Supposing that the bioactive conformation of these anticholinergics is represented by an energetically stable conformation of the most active stereoisomer, **1**, superimposition studies and calculation of an energy penalty for a conformation change could be achieved by the following method. The global and four local minimum-energy conformations of **1** were used as a template, which

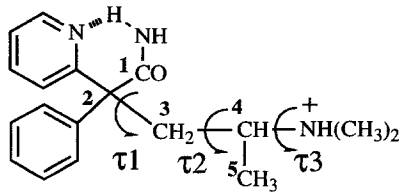

Fig. 1. Structural form adopted in the conformational studies and definition of torsion angles: τ1 = C1-C2-C3-C4, τ2 = C2-C3-C4-N and τ3 = C3-C4-N-H.

was treated as a rigid conformation throughout the superimposition studies. Four points in the molecule, i.e., the center of the phenyl ring, the carbon atom in the 2-position, the carbon atom of the amide carbonyl group and the tertiary amino nitrogen atom, were chosen to superimpose **3** onto one of the templates of **1**. For **2** and **4**, the center of the pyridine nucleus was used instead of the phenyl ring (see Results and Discussion). The former three points in **2**–**4**, i.e., the center of the phenyl or pyridyl ring, the carbon atom in the 2-position, and the carbon atom of the amide carbonyl group, were first overlayed onto the template by the least-squares method and they were geometrically fixed during the subsequent superimposition procedure. The spatial position of the nitrogen atom in tertiary amino groups could be treated as the next target for superimposition, indicating that only torsion angles τ1, τ2 and τ3 (Fig. 1) have to be varied. In order not to overlook a possible well-fitted conformation, the SYBYL systematic conformation search method [25] was employed. Varying τ1, τ2 and τ3 in 5°, 5° and 30° increments, respectively, the conformations having the nitrogen atom within 0.5 Å of the corresponding template were searched using a scale factor of 0.5 for all the van der Waals radii. Subsequently, the conformations satisfying these conditions were classified into colonies. In each colony, the conformation with the nitrogen atom in the tertiary amino group closest to the template was selected as a representative of the colony. If conformations were present having the same value of the distance of nitrogen atoms between each representative and the template (deviation of the nitrogen atom), the one with lowest energy was defined as the representative. Then, the representative conformation was optimized using the simplex minimization technique [26,27] as a function of τ1 and τ2 in terms of deviation of the nitrogen atom. The newly constructed conformations of stereoisomers **2**–**4** (prototype conformations) were then energy optimized using AM1, while τ1 and τ2 in these conformations were kept fixed and the intramolecular H-bond moiety was kept flat. If the optimization with AM1 resulted in more than 0.1 Å dissociation of the nitrogen atom from the prototype conformation, the cycle procedure(s) of the simplex minimization technique and subsequent AM1 optimization was repeated to reach values lower than 0.1 Å. In addition to the heat of formation obtained using AM1, the total energy of the finally optimized new conformation was obtained by a single-point calculation using the 3-21G basis set. The difference between the heat of formation or the total energy of each global minimum-energy conformation and that of the newly constructed conformation was defined as an energy penalty for a conformation change.

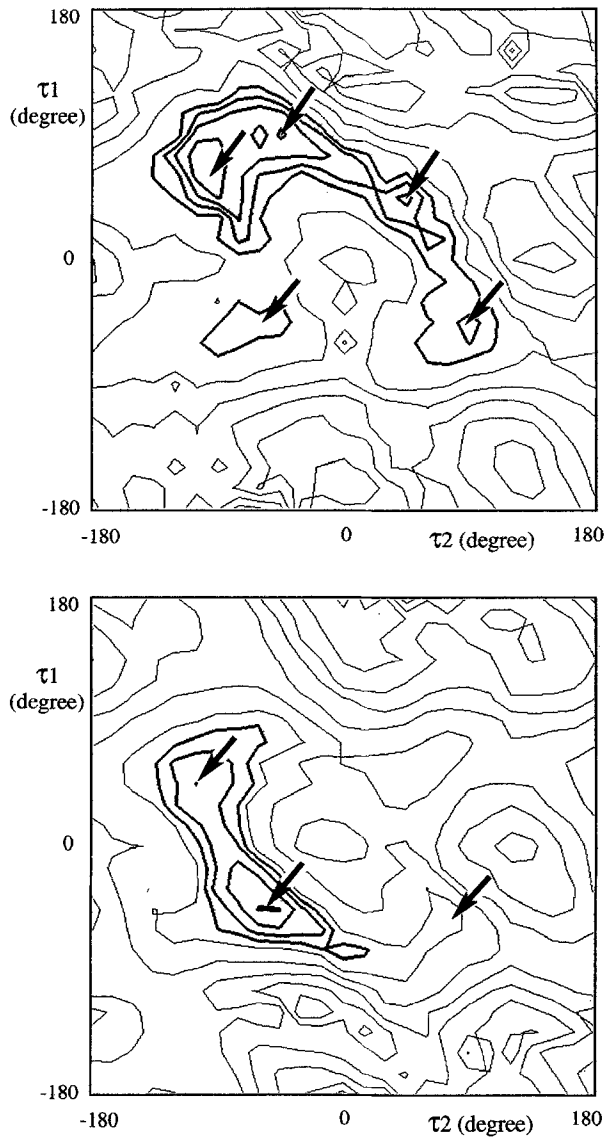

Fig. 2. Energy maps of **1** (top) and **2** (bottom) generated by 15° increments of rotation around torsion angles τ1 and τ2. The contour levels are drawn at intervals of 2.5 kcal mol^{-1} (thick line) and 5.0 kcal mol^{-1} (thin line). Arrows indicate energetically stable conformations.

Results and Discussion

Anticholinergic activity of the four stereoisomers and the essential pharmacophores

The pharmacological results of **1**–**4** demonstrate that **1**(2*S*,4*R*) is most potent for anticholinergic activity, whereas **2**(2*R*,4*R*), **3**(2*S*,4*S*) and **4**(2*R*,4*S*) are approximately 3-, 240- and 1300-fold less potent, respectively (Table 1). From these results it is likely that the configuration of the 4-position, rather than that of the 2-position, plays a critical role in exerting biological activity, although the preferred configurations are *R* at the 4-position and *S* at the 2-position.

Many studies [9,10,12,16] investigating the pharmacophores of anticholinergic agents belonging to the same structural category have found that several functional groups are required for a molecule to elicit maximum

TABLE 1
INHIBITORY EFFECT OF FOUR STEREOISOMERS ON ACETYLCHOLINE-INDUCED CONTRACTION OF GUINEA PIG ILEUM

Compound	IC_{50} (μM)[a]
1(2*S*,4*R*)	0.13 ± 0.01
2(2*R*,4*R*)	0.40 ± 0.03
3(2*S*,4*S*)	31 ± 7
4(2*R*,4*S*)	170 ± 20

[a] IC_{50} values are expressed as mean ± SEM (n = 3). See Ref. 1.

activity: a quaternary or protonated tertiary amine (cation head), a hydroxyl group or an ester (hydrogen-bond acceptor), a phenyl or a similar group (main hydrophobic group) and in some cases an additional lipophilic group (subsidiary hydrophobic group). In compounds **1–4,** these groups may correspond to the dimethylamino, the amide carbonyl, and the phenyl or pyridyl groups. In other words, these groups may be defined as the essential pharmacophores. The configuration of the 2-position, i.e., the relative spatial orientations of the amide, phenyl and pyridyl groups, appears not necessarily important for exerting the biological activity as described above, suggesting that the phenyl and pyridyl rings are practically interchangeable for binding to the receptor if these stereoisomers act on the same site in the receptor, and if the receptor requires strict spatial orientations for the dimethylamino moiety and the amide carbonyl group. It seems probable that a phenyl group is more favorable than a pyridyl group in binding to the lipophilic pocket of the receptor. Therefore, in the most potent stereoisomer, **1**, the phenyl group appears to play the role of a main hydrophobic group, and the pyridyl group is a subsidiary hydrophobic group. Extending this postulate to the second most active stereoisomer, **2**, the pyridyl group would play the leading role for binding to the lipophilic pocket of the receptor.

Global and local minimum-energy conformations

According to the procedures described in the Methods, five energetically stable conformations for the stereoisomer **1**(2*S*,4*R*) and three conformations for **2**(2*R*,4*R*) were determined through the conformational search and the subsequent optimization using AM1. As summarized in Table 2 along with their corresponding enantiomers **4**(2*R*,4*S*) and **3**(2*S*,4*S*), however, some inconsistent results of energy calculations in AM1 and with the 3-21G basis set were obtained for the optimized conformations. The ranking of two of the local minimum-energy conformations, 1-LMb and 1-LMc (4-LMb and 4-LMc as well), is different for the two methods. Furthermore, different global minimum-energy conformations were defined in the stereoisomers **2** and **3**. These discrepancies in the AM1 and the 3-21G basis set calculations could not be theoretically explained, but it seems probable that evaluation of the intramolecular H-bond and the protonated tertiary amine is insufficient or inconsistent. We thus present the following discussion taking these discrepancies into consideration. Energy differences from the global level and torsion angles τ1 and τ2 in each conformation are listed in Table 2 and the global minimum-energy conformations are depicted in Fig. 3.

TABLE 2
TORSION ANGLES OF GLOBAL AND LOCAL MINIMUM-ENERGY CONFORMATIONS, AND ENERGY DIFFERENCES

Compound	Minimum-energy conformation	τ1 (°)	τ2 (°)	Energy difference (kcal mol^{-1})	
				AM1[a]	3-21G[b]
1(2*S*,4*R*)	1-GM[c]	68.1	−98.4	–	–
	1-LMa[d]	93.6	−48.0	1.4	2.7
	1-LMb	−47.5	82.0	5.5	6.3
	1-LMc	44.0	42.3	3.6	6.9
	1-LMd	−37.9	−56.4	8.0	9.3
2(2*R*,4*R*)	2-GM(AM1)	−39.8	−56.4	–	0.4
	2-GM(3-21G)	45.1	−104.9	3.3	–
	2-LMa	−49.8	80.2	8.9	10.8
3(2*S*,4*S*)	3-GM(AM1)	39.8	56.4	–	0.4
	3-GM(3-21G)	−45.1	104.9	3.3	–
	3-LMa	49.8	−80.2	8.9	10.8
4(2*R*,4*S*)	4-GM	−68.1	98.4	–	–
	4-LMa	−93.6	48.0	1.4	2.7
	4-LMb	47.5	−82.0	5.5	6.3
	4-LMc	−44.0	−42.3	3.6	6.9
	4-LMd	37.9	56.4	8.0	9.3

[a] Difference in the heat of formation obtained in AM1.
[b] Difference in the total energy obtained by a single-point calculation using the 3-21G basis set.
[c] Global minimum-energy conformation.
[d] Local minimum-energy conformation.

We first attempted to explain differences in the anticholinergic potency of the four stereoisomers **1**–**4**, based on the structures of their global minimum-energy conformations which were defined through both AM1 and 3-21G basis set calculations. As mentioned in the Methods, the structure used for the conformational search and energy calculations, which has an intramolecular H-bond and a protonated tertiary amine, can be extended to the

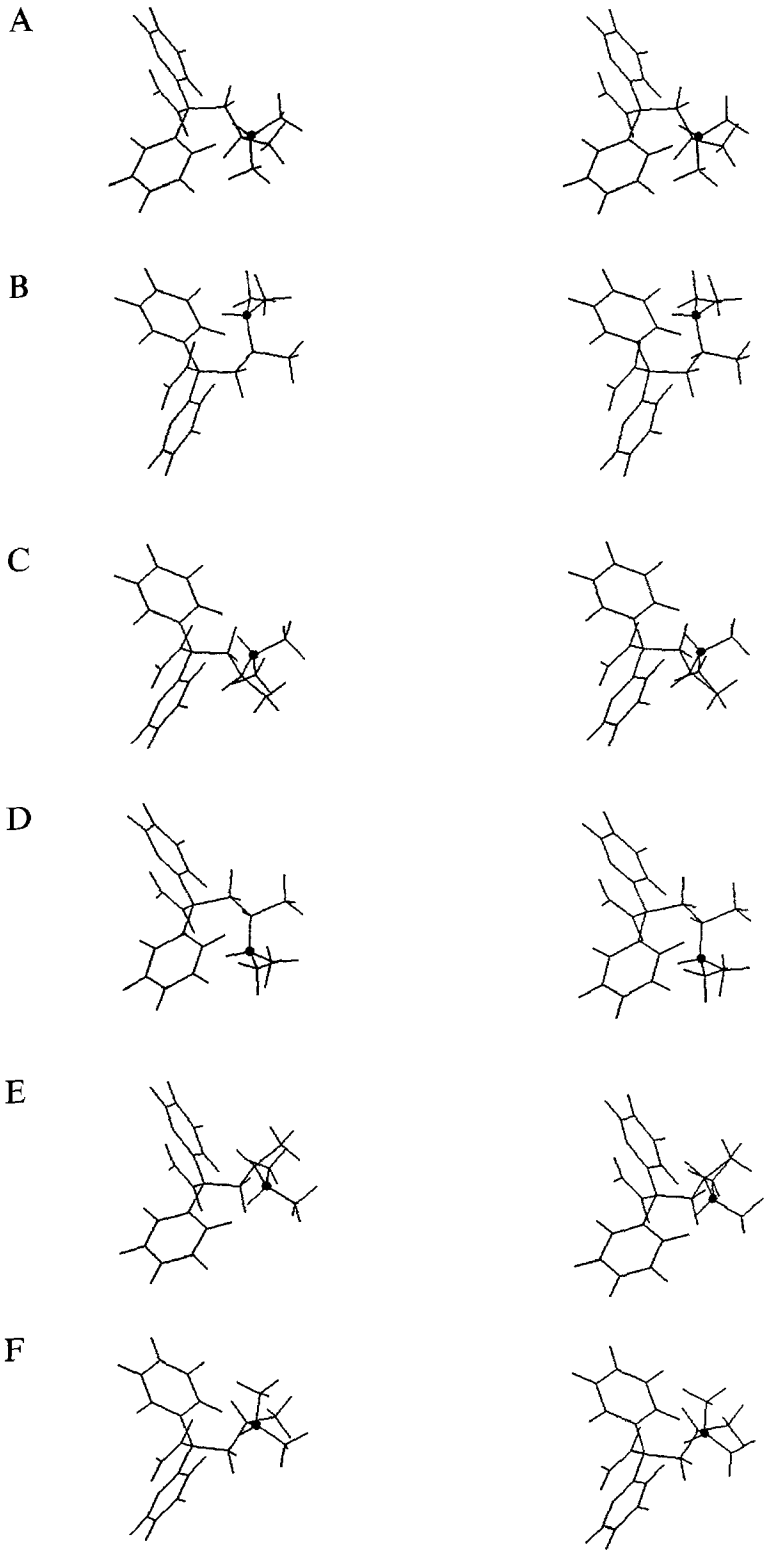

Fig. 3. Stereoviews of global minimum-energy conformations obtained by conformational analyses: (A) 1-GM; (B) 2-GM(AM1); (C) 2-GM(3-21G); (D) 3-GM(AM1); (E) 3-GM(3-21G); and (F) 4-GM.

form bound to the receptor. If these stereoisomers act on the same site of the receptor in the same manner, the pharmacological activity may be adequately correlated with the spatial orientations of the essential pharmacophores. We thus investigated the relationship between the activity and distances of the essential pharmacophores in the global minimum-energy conformations (Table 3). It is difficult, however, to provide a reasonable explanation for these results. There seems to be no significant relationship for the distances between the amino nitrogen atom and

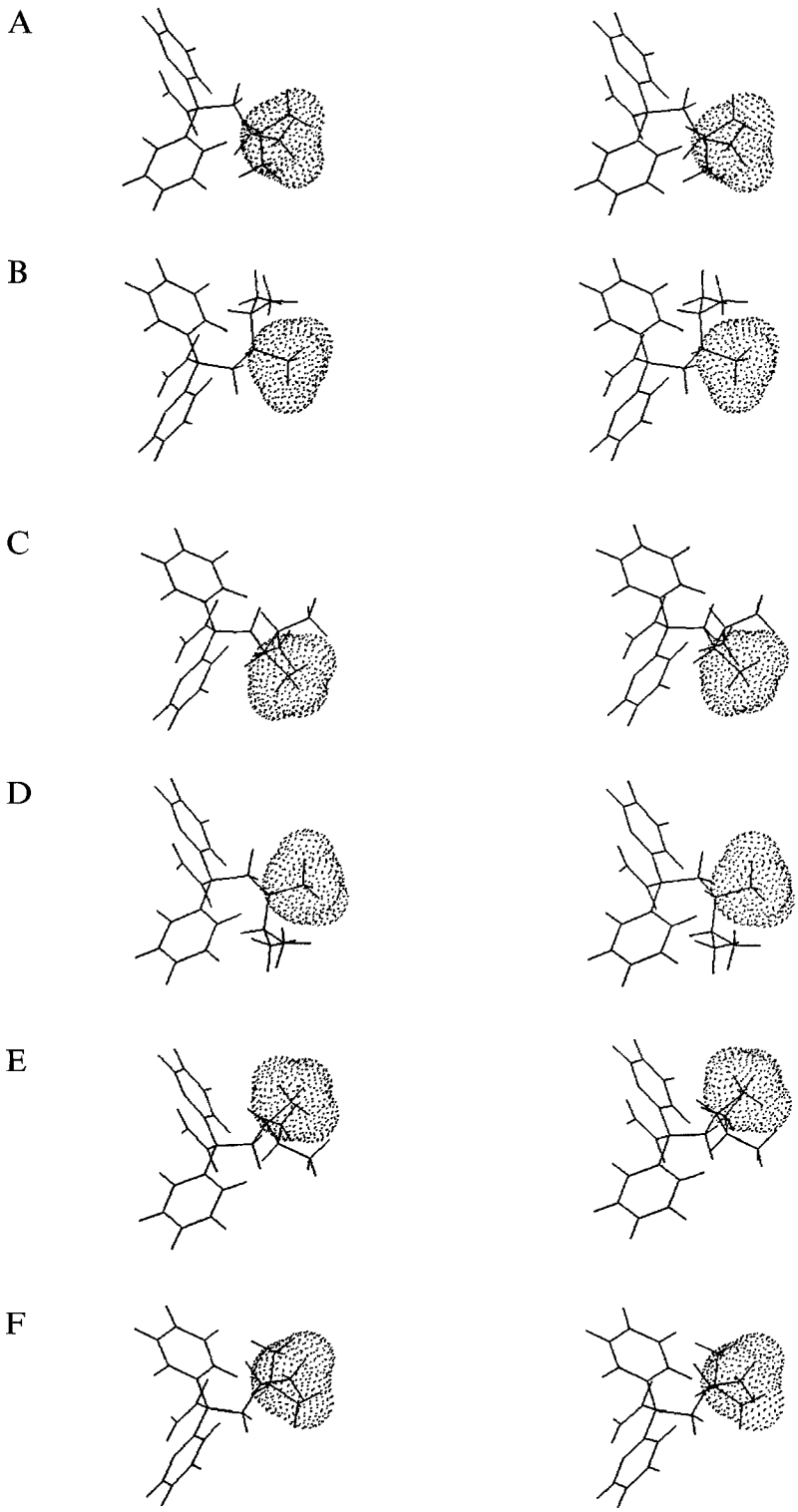

Fig. 4. Van der Waals surfaces of a methyl group at the 4-position in the global minimum-energy conformation: (A) 1-GM; (B) 2-GM(AM1); (C) 2-GM(3-21G); (D) 3-GM(AM1); (E) 3-GM(3-21G); and (F) 4-GM.

TABLE 3
DISTANCES BETWEEN THE ESSENTIAL PHARMACOPHORES IN THE GLOBAL MINIMUM-ENERGY CONFORMATIONS OF **1–4**

	1-GM	2-GM(AM1)	2-GM(3-21G)	3-GM(AM1)	3-GM(3-21G)	4-GM
Main aromatic[a]	5.2	5.9	5.8	3.9	5.7	6.1
Subsidiary aromatic[b]	6.1	3.9	5.7	5.9	5.8	5.2
Carbonyl group[c]	3.3	3.4	3.2	3.4	3.2	3.3

Distances (Å) between the nitrogen atom of the tertiary amine and
[a] the center of the main aromatic group.
[b] the center of the subsidiary aromatic group.
[c] the carbon atom of the carbonyl group.

the carbonyl, the essential aromatic or the subsidiary aromatic group in each set of the global minimum-energy conformations obtained through AM1 or 3-21G basis set calculations.

We have emphasized that the configuration of the 4-position in **1–4**, rather than that of the 2-position, plays a critical role. We next investigated the effect of the methyl group in the 4-position upon the pharmacological activity, particularly with regard to bulk tolerance to the receptor. As can be seen in Fig. 4, the methyl groups in the global minimum-energy conformations, 1-GM, 2-GM(AM1) and 2-GM(3-21G), of the potent stereoisomers **1** and **2** are located close to the main aromatic group, whereas the less active stereoisomers **3** and **4** bear the

TABLE 4
NEW CONFORMATIONS CONSTRUCTED BY SUPERIMPOSITION STUDIES AND ENERGY PENALTIES FOR THE CONFORMATION CHANGE

New conformation	τ1 (°)	τ2 (°)	Energy penalty (kcal mol^{-1})		Deviation of nitrogen atom (Å)[a]
			AM1	3-21G	
2(1-GM)	70.9	−85.7	6.3	5.0	0.25
3(1-GM)-1[b]	−11.1	93.8	4.8	6.3	0.04
3(1-GM)-2	66.5	−94.1	10.3	11.9	0.50
4(1-GM)-1	75.9	−77.2	9.9	10.7	0.28
4(1-GM)-2	−3.5	72.8	10.4	16.0	0.22
2(1-LMa)-1	106.9	−65.0	13.7	16.2	0.09
2(1-LMa)-2	39.4	60.0	14.6	19.5	0.10
3(1-LMa)	40.9	54.2	0.1	0.4	0.05
4(1-LMa)-1	39.2	67.3	8.7	10.4	0.11
4(1-LMa)-2	104.7	−52.0	18.3	26.4	0.15
2(1-LMb)-1	−32.9	66.2	11.3	13.8	0.54
2(1-LMb)-2	31.6	−65.2	9.5	11.1	0.41
3(1-LMb)-1	−46.3	88.4	4.2	0.7	0.05
3(1-LMb)-2	30.2	−77.9	13.6	17.0	0.04
4(1-LMb)-1	−30.5	73.2	4.7	7.2	0.57
4(1-LMb)-2	39.0	−79.0	7.7	6.5	0.54
2(1-LMc)-1	106.1	−68.8	13.6	15.9	0.03
2(1-LMc)-2	34.7	62.5	15.6	21.4	0.14
3(1-LMc)	38.2	56.8	0.1	0.2	0.03
4(1-LMc)-1	104.1	−53.8	18.2	26.3	0.10
4(1-LMc)-2	36.2	70.1	8.9	11.2	0.06
2(1-LMd)-1	−55.1	−1.6	10.4	11.5	0.64
2(1-LMd)-2	−39.0	−27.2	8.3	7.4	0.67
2(1-LMd)-3	−63.8	13.5	11.1	14.5	0.62
3(1-LMd)-1	−99.0	60.1	10.2	9.1	0.09
3(1-LMd)-2	−36.2	−46.1	10.1	16.5	0.35
4(1-LMd)-1	−75.3	37.3	4.1	3.6	0.63
4(1-LMd)-2	−32.2	−47.2	4.9	7.1	0.76

[a] Distance (Å) between the tertiary amino nitrogen atoms in a template and in a new conformation.
[b] One of the two conformations of **3**, constructed by fitting onto the global minimum-energy conformation of **1** (1-GM).

methyl group around the subsidiary aromatic group. If the receptor would require a vacant space around the subsidiary aromatic group (or a subsidiary hydrophobic group), the potency order of **1**–**4** might be reasonable. However, this assumption seems unlikely, because tropine analogs such as atropine fully occupy the space around a cation head, which may spread over the main and subsidiary hydrophobic groups in a manner similar to that of the less active stereoisomers **3** and **4**.

Superimposition studies and energy penalties for conformation changes

To find an explanation for the differences in pharmacological potency of **1**–**4**, we compared the energy penalty for a conformation change of each stereoisomer from the most energetically stable structure to the preferred conformation on the receptor. As a matter of course, the latter (bioactive) conformation of the compounds discussed here has not yet been defined. Therefore we adopted the energetically stable (the global and four local minimum-energy) conformations of the most active stereoisomer **1** as a tentative bioactive conformation, and then implemented superimposition studies to examine the relationship between energy penalty and anticholinergic activity. In these superimposition studies, we mainly focused on the relative spatial orientations of the essential pharmacophores mentioned above, in particular on both the distance and the direction of the cation head from the main

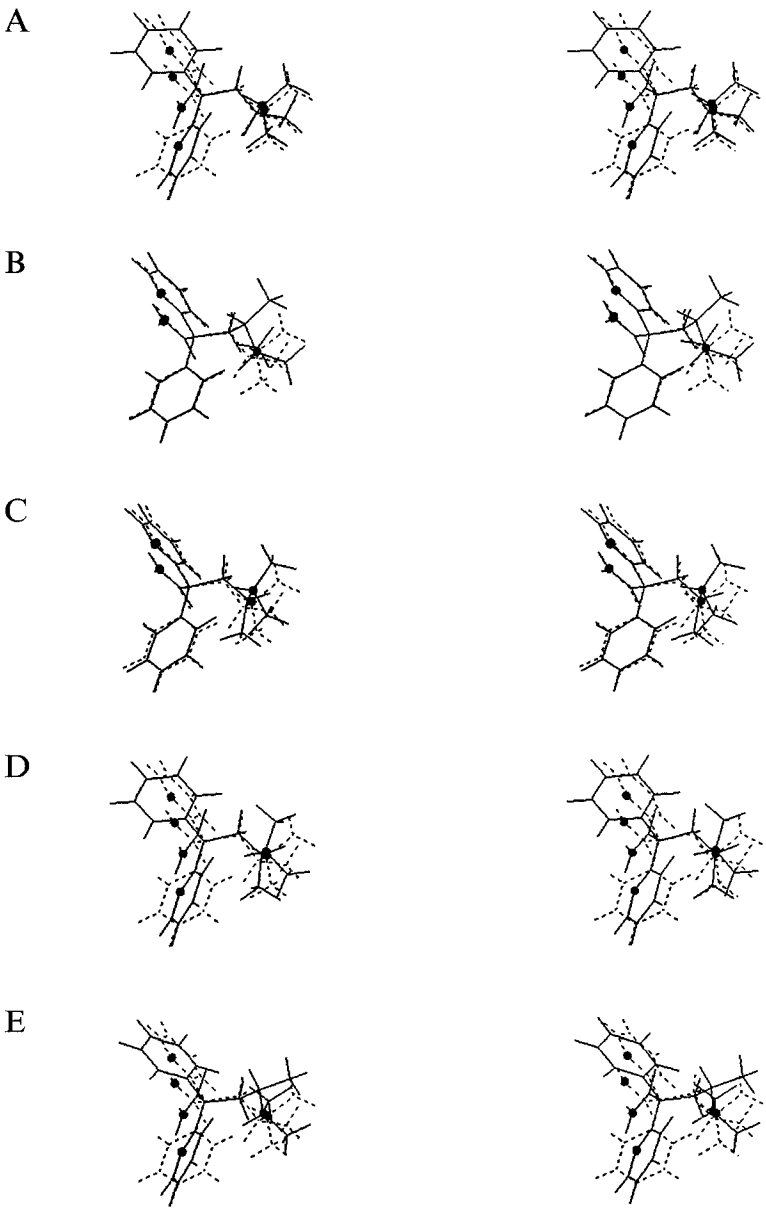

Fig. 5. New conformations constructed by a superimposition study using the global minimum-energy conformation of **1** (dotted line) as a template: (A) 2(1-GM); (B) 3(1-GM)-1; (C) 3(1-GM)-2; (D) 4(1-GM)-1; and (E) 4(1-GM)-2.

aromatic group, i.e., the phenyl group for **3** and the pyridyl group for **2** and **4**. However, the direction of the carbonyl group, in other words the spatial orientation of the oxygen atom, was not taken into consideration, because it is apparently impossible to superimpose this particular part in **2** or **4** onto that in **1** under the conditions where the intramolecular H-bond exists between the amide and pyridine moieties.

The results of the superimposition studies are summarized in Table 4, which contains the torsion angles $\tau 1$ and $\tau 2$ of a new conformation, energy penalties, and deviations of the amino nitrogen atom with respect to a template. When the global minimum-energy conformation of **1** (1-GM) was used as a template (Fig. 5), only one new conformation, 2(1-GM), was constructed for **2**. In the case of **3**, 3(1-GM)-1 and 3(1-GM)-2 were obtained as possible conformations, and two conformations, 4(1-GM)-1 and 4(1-GM)-2, were also found for **4**. The energy penalty for a conformation change from the global minimum-energy structure to a new conformation was calculated in AM1 and in the 3-21G basis set. The energy penalty of 2(1-GM) is 6.3 kcal mol^{-1} in AM1, whereas it is 5.0 kcal mol^{-1} in the 3-21G basis set. For 3(1-GM)-1 it is 4.8 kcal mol^{-1} in AM1 and 6.3 kcal mol^{-1} in 3-21G, while for 3(1-GM)-2 these values are 10.3 and 11.9 kcal mol^{-1} in AM1 and 3-21G, respectively. Furthermore, the values of 4(1-GM)-1 and 4(1-GM)-2 were found to be 9.9 and 10.4 kcal mol^{-1} in AM1 and 10.7 and 16.0 kcal mol^{-1} in 3-21G. In this study, the energy penalty for **1** is 0 kcal mol^{-1} by definition.

Similar superimposition studies were performed using the other stable conformations of **1** (1-LMa,b,c,d) as a template. The results are also shown in Table 4.

The energy penalty values do not necessarily coincide in AM1 and in the 3-21G basis set. However, the results obtained using the global minimum-energy conformation of **1** (1-GM) as a template could better explain the tendency in the pharmacological potency order (**1** > **2** > **3** > **4**) than could the results from the other stable conformations of **1** (1-LMa,b,c,d).

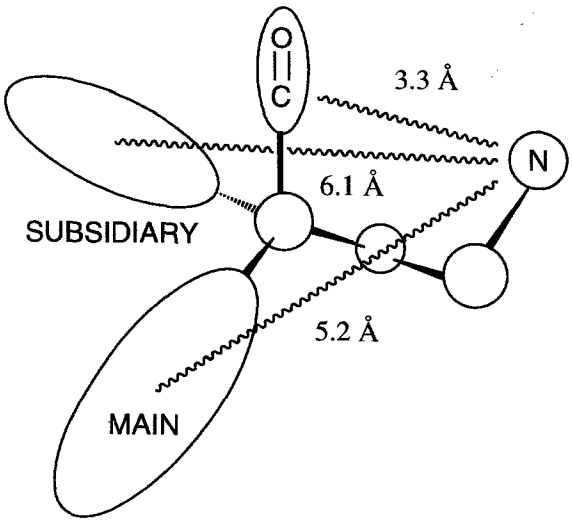

Fig. 6. The proposed pharmacophore model of anticholinergic agents, based on the global minimum-energy conformation of the most active stereoisomer **1**.

Likely bioactive conformation of the four stereoisomers and a pharmacophore model of anticholinergic agents

The results described above suggest that the global minimum-energy conformation of the most active compound, **1**, is closely related to the bioactive conformation of the four stereoisomers **1**–**4** on the muscarinic receptor, and also that the pharmacological potency is linked to how readily these substances can change their conformation to fit the receptor. We thus propose a pharmacophore model for anticholinergic agents (Fig. 6), based on spatial orientations of the essential pharmacophores in the most active stereoisomer **1**. We emphasize that in this model both the distance and the direction of the cation head from the essential aromatic group are tightly restricted, for maximum interaction with the receptor. The spatial orientation of the carbonyl group and the direction of the main aromatic ring plane are not defined. Our proposed model appears to be consistent with the conformation model proposed by Pauling et al. [12] in terms of the spatial orientations of the pharmacophores, except for the distance between the cation head and the aromatic ring (5.83 Å from Pauling, 5.2 Å as proposed in this report).

Conclusions

The differences in anticholinergic activity among the four stereoisomers **1**–**4** could not be explained on the basis of the distances between the essential pharmacophores in their global minimum-energy conformations. It seems unlikely, but still remains unproven, however, that in the global minimum-energy conformations of **3** and **4** occupation of the necessary space (presumably around a subsidiary hydrophobic group) by a methyl group at the 4-position results in dramatic attenuation of the activity. From the superimposition studies, a more probable explanation has been obtained. The energy penalties for a conformation change of these stereoisomers from the global minimum-energy structure to a new conformation (the tentative bioactive conformation) account for the differences in pharmacological activities. The superimposition study, using the global minimum-energy conformation of the most active stereoisomer **1** as a template, could better explain the pharmacological potency order than could the studies with the other stable conformations of **1**. We thus presume that the global minimum-energy conformation of **1** is closely related to the bioactive conformation for the anticholinergics described here, and also that the pharmacological potency is linked

to how readily these substances can change their conformation to fit the muscarinic receptor.

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