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Conformational studies on (+)-anatoxin-a and derivatives

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SUMMARY

Anatoxin-a (AnTX) is a highly potent agonist acting at the nicotinic acetylcholine receptor (nAChR) and represents a valuable tool in the study of this receptor. Molecular mechanics, semi-empirical and ab initio molecular orbital energy minimization procedures were conducted to investigate the conformation of AnTX. For each minimization procedure, the *s-trans* enone isomer of protonated AnTX was the energetically favoured conformer due to intramolecular electrostatic interactions. Our studies are discussed in the light of previous experimental observations and conformational studies, in addition to their importance in the development of future pharmacophore models for nAChR agonist binding.

INTRODUCTION

Since 1970, the activity of ligands acting at the nicotinic acetylcholine receptor has been explained in terms of a pharmacophore postulated by Beers and Reich [1]. This pharmacophore identified the geometric arrangement of a cationic centre (usually an ammonium nitrogen atom) and an atom bearing unshared electrons (usually a carbonyl oxygen). The emergence of new potent and selective ligands and the development of computerized techniques for studying molecular conformation have recently been used to test the validity of this pharmacophore model. For example, Sheridan et al. [2] developed a nicotinic receptor pharmacophore using a modification of Marshall's '*Active Analogue Approach*' [3] and these studies served to corroborate and extend the pharmacophore model of Beers and Reich [1].

Although these models appear adequate to explain the 3D structural requirements of many nicotinic agonists, they conflict with the results of a recent ^{1}H NMR investigation of the conformation adopted by acetylcholine when bound to the nicotinic receptor [4]. This study suggested that

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receptor-bound acetylcholine adopts a conformation that is quite distinct from that found in either the solid state or in solution. This is significant since the Beers–Reich model was defined on the basis of the conformational preferences of the unbound ligand. In addition, the Beers–Reich pharmacophore [1] has been unable to explain the activity of a number of structurally related compounds derived from known nicotinic agonists [5,6]. In an attempt to improve the above pharmacophore models, Barlow and Johnson suggested that agonist activity may be associated with a positively charged atom and an area of planarity [7]. This idea was supported by studies investigating the proposed agonist binding site of the receptor which suggested that a phenylalanine residue was a key site of interaction for agonist compounds [8] and this proposal is in accord with a generalized pharmacophore model for central nervous system drugs and their receptors [9]. A more recent model developed by Hacksell and Mellin [10] has re-examined the 'Active Analogue Approach' [3] to propose a pharmacophore which includes newly-identified potent nicotinic agonists. In particular, these authors examined agonist conformations that can be used to explain stereoselective interactions of agonists with the nicotinic receptor [10]. Central to the model proposed by Hacksell and Mellin was (+)-anatoxin-a (AnTX), a toxin found in the blue green alga *Anabaena flos-aquae* [11] which has been shown to be a highly potent and selective agonist that acts stereospecifically at nicotinic acetylcholine receptors [12–14]. AnTX has a semirigid bicyclic structure and may adopt one of four distinct conformations [15] comprised of two possible ring conformations (twist chair and twist boat) and two α,β-unsaturated ketone (enone) conformations (*s-cis* and *s-trans*) (Fig. 1).

While some controversy exists over the identity of the likely bioactive conformer of AnTX [10,15,16], both the *s-cis* conformers (Figs. 1B,D) meet the requirements of the Beers–Reich pharmacophore and have, as a result, been suggested as likely bioactive conformers [15,16]. In contrast, Hacksell and Mellin argued that the *s-cis* conformer, which was not consistent with their pharmacophore model, also did not explain the activity profile of other AnTX analogues [10]. Consequently, these authors have proposed that the twist chair *s-trans* conformer (Fig. 1A) of AnTX can be used to explain these features and suggested it as the bioactive conformation.

Previous molecular modelling studies examining the conformation of AnTX have been reported [15,16]. However, these investigations were limited to using molecular mechanics techniques. The current study was initiated in order to investigate the conformational flexibility of AnTX using molecular mechanics, but in addition, semi-empirical and ab initio molecular orbital (MO) calculations have also been performed. Our object has been to provide information that will aid in the refinement of future models of the nicotinic acetylcholine receptor (nAChR) and direct the rational design of new and potent ligands.

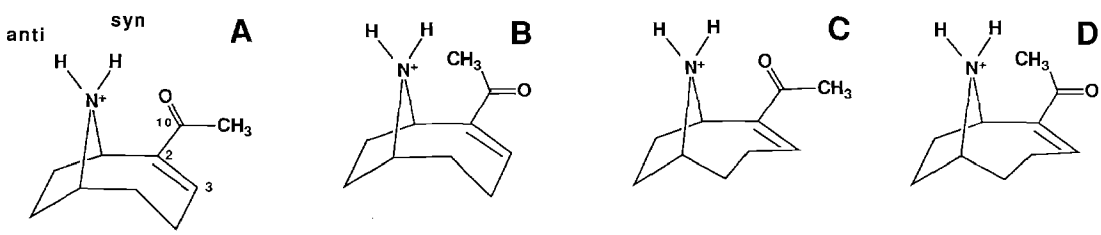


Fig. 1. Structures of the four conformations of AnTX (ring conformation, enone configuration). A, chair, *s-trans* (CT) (the *syn* and *anti* hydrogen atoms have been indicated); B, chair, *s-cis* (CC); C, boat, *s-trans* (BT); D, boat, *s-cis* (BC).

MATERIALS AND METHODS

The molecular modelling package COSMIC [17,18] was employed to construct the twist chair and twist boat conformers of AnTX in both protonated and unprotonated forms. In the uncharged species the hydrogen atom bound to the nitrogen atom was oriented in both configurations (i.e. *syn* and *anti*, see Fig. 1A). Each structure was then minimized using the molecular mechanics force fields within COSMIC [18]. In addition, the *N*-methyl and *N,N*-dimethyl analogues of AnTX were minimized using the same procedures. Partial charges were employed in these calculations and were obtained using the semi-empirical MO program CNDO [19]. Further for the calculation of molecular geometries and energies for the above AnTX configurations, simulation of the rotation of the acetyl group relative to the bicyclic ring system of AnTX was performed using the SPIN01 program within COSMIC [17]. A limited study was also conducted on the protonated form of AnTX using the MMX force field contained in the PCMODEL molecular modelling package [20].

Semi-empirical PM3 MO calculations were carried out using the MOPAC program (QCPE 455) [21]. The low-energy conformations determined using COSMIC were employed as the starting structures for full geometry optimization, by implementing the PRECISE option within MOPAC [21]. In addition, the barrier to rotation of the acetyl side chain was examined by calculating the PM3 conformational energies for the rotation about the bond C2–C10 (Fig. 1A) incremented by 20-degree steps. The bond order of the C2–C10 bond was calculated with the 'BONDS' keyword using the method described by Armstrong et al. [22].

Ab initio SCF MO calculations were performed using the Gaussian STO 3-21G basis set implemented in Pople's GAUSSIAN 86 program [23] on an IBM 3081 computer. As used previously, the starting geometries for the chair *s-cis*, *s-trans* conformers of the protonated and unprotonated (N–H *anti*) forms of AnTX were obtained using COSMIC and 3–4 days of computer time were required for full geometry optimization. The GAUSSIAN 90 program [24] was used to calculate the degree of bonding in the C2–C10 (Fig. 1A) bond for each conformer of AnTX. The theory used to calculate the bond orders within MOPAC cannot be applied in this case because it assumes that the orbitals are orthonormal. The off-diagonal elements of the full Mulliken population analysis (condensed to atoms) were therefore used as a measure of the degree of bonding in the C2–C10 bond. This method is much more sensitive to subtle changes in the structure.

RESULTS AND DISCUSSION

A search of the Cambridge crystallographic data base [25] for compounds containing a 1-acetyl-1-cyclohexene substructure was conducted to compare the geometry of the α,β-unsaturated ketone system with that of AnTX. From this search, seven compounds were extracted possessing the relevant structure suitable for comparative purposes [26–31]. Of these seven compounds, six possessed an *s-trans* configuration [26–30] while the seventh had an *s-cis* configuration [31]. The latter enone which was the enol form of a 1,3-diketone, was locked in the *s-cis* conformation by an internal hydrogen bond and was thus excluded from this analysis. Of the remaining six *s-trans* compounds the average length of the C–C bond separating the C=C and C=O groups was 1.48 Å (range 1.46 to 1.49 Å) and the average dihedral angle between the groups was 175.9° (range −156.7° to +151.1°). Thus the crystal structure of AnTX·HCl, which has a C–C bond length of

TABLE 1
SUMMARY OF PREVIOUS STUDIES RELATING TO THE ENONE CONFIGURATION OF AnTX·HCl, AnTX AND *N*-ACETYL-AnTX

Compound	Experimental method	Ring conformation	Enone configuration (dihedral angle)[a]	Reference
AnTX·HCl	X-ray	twist chair	*s-trans* (−163°)	[15]
	UV	-	nonplanar	[15,33]
	IR	-	both populated	[33]
	NMR	twist chair	both populated	[15]
AnTX	NMR	twist chair	both populated	[15]
N-Acetyl-AnTX	X-ray	twist chair	*s-trans* (175.4°)	[32]

[a]The dihedral angle is defined as τ(C3,C2,C10,Oxygen), see Fig. 1A.

1.495 Å and a dihedral angle of −162.7° [15], compares well with the crystal structures surveyed above. Interestingly, the C–C bond length observed for *N*-acetyl-AnTX was 1.469 Å, implying that there is a reduction in the conjugation of the α,β-unsaturated ketone system in the protonated form of AnTX [32]. A closer examination of the crystal structure of AnTX·HCl reveals a twist chair *s-trans* enone conformation with the enone distorted from planarity by 17.3° [15]. Such a deviation from planarity is in accord with the observed UV absorbance for AnTX·HCl in ethanol, which has a lower than expected extinction coefficient (Table 1) [33]. The IR spectrum in $CHCl_3$ shows a carbonyl absorption at 1670 cm^{-1} (strong) and alkene absorptions at 1645 cm^{-1} (weak) and 1588 cm^{-1} (medium) indicative of both *s-trans* and *s-cis* conformations being present (Table 1) [15,33]. A quantitative measure of these proportions has not been made, however, Spivak et al. [12] concluded that a 'considerable proportion' of AnTX exists in the *s-cis* conformation. Koskinen and Rapoport [15] assigned the 500 MHz NMR spectrum of both AnTX (in $CDCl_3$) and AnTX·HCl (in D_2O) and, using a combination of NOE (see below) and the magnitude of coupling constants, proposed a twist chair as the solution conformation (Table 1). Studies examining AnTX as its free base [15] and its *N*-acetyl derivative [32] have also been performed and the results have been summarized in Table 1. These results should be evaluated cautiously as variation in the electronic environment of the nitrogen centre has been suggested to have a significant effect on the overall conformation [15]. One result worth noting was the NOE difference spectrum of AnTX in $CDCl_3$ which showed a very small enhancement of both bridgehead and β-protons (< 5%). This suggested that both the *s-cis* and *s-trans* populations of AnTX were significantly populated in $CDCl_3$ solution [15] (Table 1).

Two molecular mechanics packages were employed to examine the structure of AnTX (COSMIC and MMX). Greater consideration was given to the protonated form of AnTX (pKa of AnTX 9.36) [15] as approximately 99% would be protonated at physiological pH. For both the protonated and unprotonated forms of AnTX, four low-energy conformations were isolated corresponding to the chair *s-cis* (CC), chair *s-trans* (CT), boat *s-cis* (BC) and boat *s-trans* (BT) conformations (Fig. 1). Briefly, the results obtained for unprotonated AnTX demonstrated that the

TABLE 2
COMPARISON OF THE ENONE GEOMETRY OF THE CHAIR FORMS OF *N*-ACETYL-AnTX AND AnTX·HCl BASED ON X-RAY CRYSTALLOGRAPHIC ANALYSIS AND VARIOUS GEOMETRY-OPTIMIZATION METHODS ON THE STRUCTURES OF AnTX AND PROTONATED AnTX

Method	Molecular state	Energy (kcal/mol)	Torsion angle[1]	Bond length[2] (Å)	Bond order[2]
X-ray	*N*-Acetyl-AnTX [17]	-	175.4	1.469	-
	AnTX·HCl [15]	-	−162.7	1.495	-
COSMIC	a	4.64	2.0	1.45	1.38
	b	5.02	179.4	1.45	1.38
	c	6.63	2.0	1.45	1.38
	d	6.51	179.9	1.45	1.38
	e	34.92	−3.0	1.45	1.38
	f	32.58	−174.9	1.45	1.38
MMX[3]	e i	20.5	−51.0	1.48	-
	ii	22.4	41.0	1.48	-
	f	18.4	165.0	1.48	-
MMP2 [15]	e	23.77	21.0	-	-
	f	24.52	−162.5	-	-
MOPAC	a i	−36.43	44.3	1.50	0.94
	ii	−35.94	−36.5	1.50	0.94
	b	−36.15	139.7	1.50	0.94
	c i	−37.93	43.4	1.50	0.94
	ii	−37.98	−38.1	1.50	0.94
	d	−37.11	138.7	1.50	0.94
	e i	115.56	−45.6	1.51	0.91
	ii	116.76	35.6	1.51	0.91
	f	113.55	−141.4	1.51	0.91
GAUSSIAN 86	a	2.13	4.5	1.50	0.304[g]
	b	0.00	177.8	1.49	0.322[g]
	e	6.21	−3.8	1.51	0.215[g]
	f	0.00	−174.9	1.50	0.242[g]

[1] The torsion angle is defined as τ(C3,C2,C10,Oxygen) (see Fig. 1A).

[2] Bond length and bond order refer to the bond C2–C10 (see Fig. 1A).

[3] The MMX force field was derived from the molecular mechanics program MM2 (QCPE 395) and uses the pi-VESCF routines contained in MMP1 (QCPE 318) developed by N.L. Allinger and co-workers. This force field is able to examine conjugated systems such as that within the structure of AnTX.

[a] Unprotonated, N–H *anti*, *s-cis* enone conformer.

[b] Unprotonated, N–H *anti*, *s-trans* enone conformer.

[c] Unprotonated, N–H *syn*, *s-cis* enone conformer.

[d] Unprotonated, N–H *syn*, *s-trans* enone conformer.

[e] Protonated, *s-cis* enone conformer.

[f] Protonated, *s-trans* enone conformer.

i and ii indicate two local minima were identified.

[g] Electron density within the C2–C10 bond calculated using the Mulliken analysis from 3–21G wave functions.

TABLE 3
MOLECULAR ENERGIES AND GEOMETRY OF THE ENONE MOIETY OF THE BOAT CONFORMER OF AnTX AND PROTONATED AnTX

Method	Molecular state	Energy (kcal/mol)	Torsion angle[1]	Bond length[2] (Å)	Bond order[2]
COSMIC	a	6.54	0.3	1.45	1.38
	b	6.61	178.4	1.45	1.38
	c	6.97	−0.2	1.45	1.38
	d	7.57	177.5	1.45	1.38
	e	37.04	−0.4	1.45	1.38
	f	35.93	178.5	1.45	1.38
MMP2 [15]	e	25.23	15.4	-	-
	f	25.76	172.7	-	-

[1] The torsion angle is defined as τ(C3,C2,C10,Oxygen) (see Fig. 1A).
[2] Bond length and bond order refer to the bond C2–C10 (see Fig. 1A).
[a] Unprotonated, N–H *anti*, *s-cis* enone conformer.
[b] Unprotonated, N–H *anti*, *s-trans* enone conformer.
[c] Unprotonated, N–H *syn*, *s-cis* enone conformer.
[d] Unprotonated, N–H *syn*, *s-trans* enone conformer.
[e] Protonated, *s-cis* enone conformer.
[f] Protonated, *s-trans* enone conformer.

lowest energy conformation was the CC (N–H *anti*) form (Tables 2 and 3). The difference in energy of the corresponding CT conformer was not large and presumably both forms would be well-populated. Both MMX and COSMIC calculated the CT conformer to be approximately 2–2.5 kcal/mol lower in energy than the CC form in protonated AnTX (Table 2). These results, however, disagree with those previously reported by Koskinen and Rapoport [15] who determined the CC conformer to be 0.75 kcal/mol lower in energy than the CT conformer (Table 2). Examination of energy contributions led these authors to suggest that this difference in energy was due to steric strain experienced by the methyl group. Furthermore, COSMIC calculated that the enone moiety adopts a planar arrangement while MMP2 [15,34] suggested a distortion of approximately 20° from planarity [15] (Table 2), the latter being in accord with X-ray and UV observations [15,33]. Unfortunately, COSMIC also did not adequately reproduce the bond length between atoms C2–C10 or the change in this bond length when protonated AnTX was examined.

To examine further the results obtained using the COSMIC force field, we investigated the individual energy contributions comprising the molecular energies quoted in Table 2 for the protonated form of AnTX. Comparison of the CC and CT conformers of protonated AnTX demonstrated only minor differences in the VDW, bond, angle and torsion energy components (Table 4). Interestingly, the Coulombic component explained approximately the total energy difference between these conformers which is probably due to the attractive interaction between the partial negative charge on the carbonyl oxygen atom and the positively charged N–H *syn* hydrogen atom. Indeed, the distance between these nuclei is greater in the *s-cis* conformer (Table 4), explaining the increase in the Coulombic energy component. The BT and BC conformers exhibit the same phe-

TABLE 4
SUMMARY OF THE ENERGY COMPONENTS OF PROTONATED LOW-ENERGY AnTX CONFORMERS DERIVED USING THE COSMIC MOLECULAR MECHANICS FORCE FIELD[a]

Configuration	Total (kcal/mol)	Coulombic (kcal/mol)	VDW (kcal/mol)	Bond (kcal/mol)	Angle (kcal/mol)	Torsion (kcal/mol)	Distance[b] N–H·O (Å)
CC	34.92	20.13	0.83	0.57	5.02	8.38	4.27
CT	32.58	17.71	0.90	0.56	5.14	8.28	3.68
BC	37.04	20.80	1.03	0.63	5.24	9.35	4.74
BT	35.93	19.18	1.28	0.61	5.54	9.34	4.40

[a] It should be noted that the COSMIC force field does not make a special case out of angles which deviate from normal hybridization states. Overall energies are therefore high, while relative energy differences may be compared reliably.
[b] Distance refers to the distance between the N–H *syn* hydrogen atom and the oxygen atom of AnTX.

nomenon. An important difference between the boat and chair conformers lies primarily in the higher energy of the torsional energy component for the boat configuration (Table 4). If we neglect intramolecular electrostatic interactions then the chair form is 1.5–1.9 kcal/mol lower in energy than the boat (*s-trans* and *s-cis*, respectively) (c.f. [15], Table 2). Using this same criterion, the CC and CT conformers differ by less than 0.1 kcal/mol and, as these calculations were performed in vacuo, conformational preferences may be influenced greatly by solvation effects. As Coulombic interactions may be attenuated by polar solvents, changes to conformational preferences would not be unexpected. This may explain the observations made from NMR experiments [15] which demonstrated that the *s-cis* and *s-trans* conformers are both populated in $CDCl_3$ solution. Moreover, intramolecular electrostatics may be negated to a large extent at the nicotinic receptor, as the nitrogen and carbonyl oxygen atoms of AnTX are regarded as key points of interaction [1]. Based on these results, it is not inconceivable that either the *s-cis* or *s-trans* form of AnTX could act as a bioactive conformation.

The stability of the possible chair conformations was further investigated using the semi-empirical MO program MOPAC (Table 2). For unprotonated AnTX the *s-cis* conformer (with a *syn* N–H configuration) was calculated to be more stable (0.87 kcal/mol) although the enone system was considerably distorted from planarity. Calculations carried out on protonated AnTX showed that the CT conformation was energetically favoured over the CC form by 2 kcal/mol (Table 2). MOPAC was able to demonstrate a change in the C2–C10 bond length in going from the unprotonated to the protonated forms of AnTX. However, this bond length appeared to be too long in the case of the free base.

As the results obtained from MOPAC revealed a distortion from planarity for the enone system, in addition to a low bond order and long bond length for the C2–C10 bond (Table 2), it was decided to compare the flexibility of the acetyl side chain using both COSMIC and MOPAC. The results obtained from COSMIC (Fig. 2) demonstrated that the low-energy conformations correspond to a planar enone system with a 5.3 kcal/mol barrier to rotation of the CT to the CC form in protonated AnTX. Contrasting this were the results obtained from MOPAC, where the energy of each conformation was related to the distance between the nitrogen and oxygen atoms of AnTX (Fig. 3). Conformational flexibility, therefore, appears to be dependent on the electrostatic

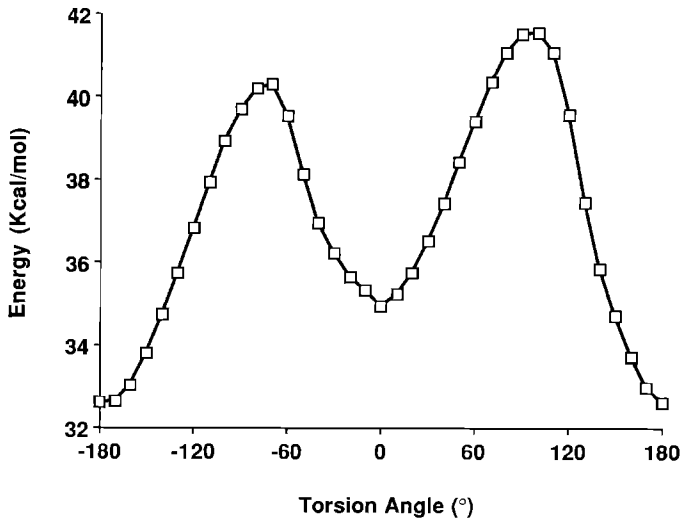


Fig. 2. Plot of energy vs. the torsion angle τ(C3,C2,C10,Oxygen) for the protonated chair conformation of AnTX using the molecular mechanics force field within COSMIC. The lowest energy conformer represents an *s-trans* enone configuration.

attraction of the partial negative charge on the oxygen atom to the positively charged NH_2 moiety. This implies that the MOPAC calculations consider the C2–C10 bond to have single bond characteristics as evidenced by the low bond order and long bond length of the C2–C10 bond. Clear-

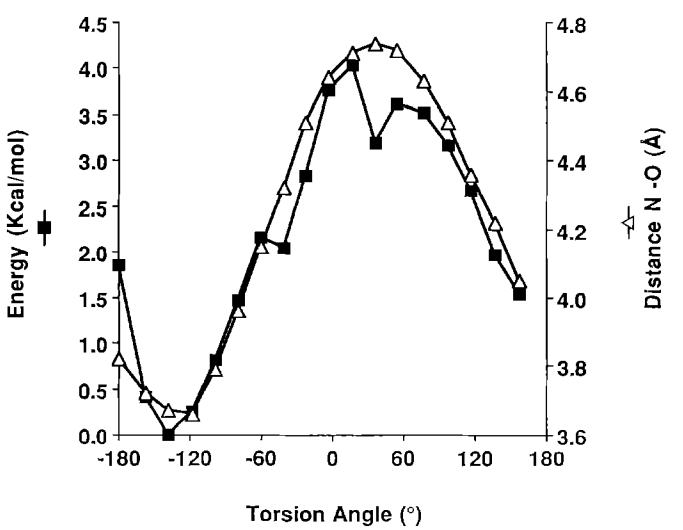
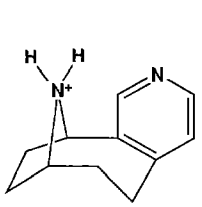


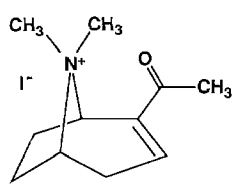
Fig. 3. Plot of energy (above global minumum) (■) and the distance between the nitrogen and oxygen atoms (△) vs. the torsion angle τ(C3,C2,C10,Oxygen) for the protonated chair conformation of AnTX using the semi-empirical MO program MOPAC. The lowest energy conformer coincides closely with the point where the distance between the nitrogen and oxygen atoms is at a minimum.

ly, MOPAC underestimates the contribution that conjugation makes to the C2–C10 bond and calculations involving the enone group, based on this package, must be treated with caution. A thorough investigation using the AM1 method has also demonstrated that calculated energy barriers to rotation for conjugated systems are not handled well [35], although PM3 is probably the best method in the MOPAC suite. To investigate this matter further, ab initio MO geometry-optimization calculations were performed and resulted in planar enone configurations. The CT conformer was favoured in both protonated and unprotonated AnTX (Table 2), however, the energy differences appeared to be somewhat large and were thus not compatible with experimental observations. The reasons for these large energy differences were not clear to us. The problem of not being able to adequately determine the bond order for the C2–C10 bond does not allow comparison of this result with those obtained from COSMIC and MOPAC. The bond length of the C2–C10 bond from the ab initio minimized structures was, however, comparable to those derived using MOPAC.

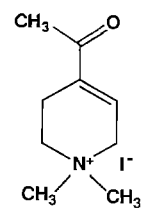
A key factor in determining the bioactive conformation of AnTX may come from evaluating structure–activity relationships of a range of analogues. Recent reports have described AnTX analogues which could provide useful information concerning the conformational requirements for binding to the nicotinic receptor [36–39]. Of these analogues, (+)-*N*-methyl-AnTX and (+)-*N*,*N*-dimethyl-AnTX have poor affinity for the nicotinic receptor [36–38]. Similarly, (±)-9-pyridohomotropane (9-PHT, Fig. 4) also loses affinity on *N*-methylation [40]. These observations are in contrast with the agonists, (−)-ferruginine methiodide and isoarecoline methiodide (Fig. 4), in which the *N*,*N*-dimethylammonium function is essential for activity [41]. Kofuji et al. have attempted to explain these differences in terms of the effect of *N*-methylation on the conformation of the AnTX skeleton [37]. These authors argued that *N*-methylation resulted in destabilization of the *s-cis* conformation (relative to *s-trans*) and suggested that these observations supported the view that it was the *s-cis* and not the *s-trans* form that was biologically significant. Unfortunately,



9-PHT



(-)-Ferruginine Methiodide



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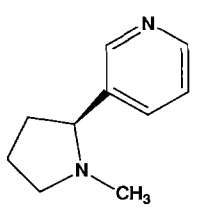

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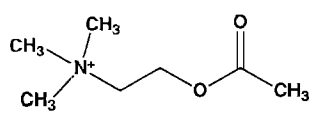
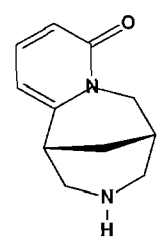


Acetylcholine



(-)-Cytisine

Fig. 4. Structures of various nAChR ligands.

this is not consistent with the lack of activity observed with *N*-methyl-9-PHT, which has the *s-cis* 'enone' rigidly contained within its structure. Hacksell and Mellin have subsequently used *N*-methyl-9-PHT to characterize an excess volume which defines the nicotinic 'receptor essential volume' [10] and *N*-methylated AnTX derivatives could conceivably reside within this region of excess volume. Interestingly, isoarecolone methiodide does not produce an excess volume in the model described by Hacksell and Mellin [10].

In view of these observations, we examined the conformational preferences of the *N*-methylated

TABLE 5
COMPARISON OF THE ENERGY AND GEOMETRY OF THE ENONE MOIETY OF *N*-METHYL AND *N,N*-DIMETHYL AnTX USING THE COSMIC MOLECULAR MODELLING PACKAGE

Conformation	Molecular state	Energy (kcal/mol)	Torsion angle[1]	Bond length[2] (Å)	Bond order[2]
Chair	a	4.56	2.6	1.45	1.38
	b	5.73	−179.6	1.45	1.38
	c	5.10	1.1	1.45	1.38
	d	6.10	−179.4	1.45	1.38
	e	40.74	−0.4	1.45	1.38
	f	38.55	−174.1	1.45	1.38
	g	41.78	−2.1	1.45	1.38
	h	39.71	−172.0	1.45	1.38
	i	46.99	−2.4	1.45	1.38
	j	44.78	−171.5	1.45	1.38
Boat	a	5.49	0.4	1.45	1.38
	b	6.50	177.9	1.45	1.38
	c	8.33	−0.5	1.45	1.38
	d	9.34	177.6	1.45	1.38
	e	43.94	−0.6	1.45	1.38
	f	42.82	178.1	1.45	1.38
	g	48.84	−2.2	1.45	1.38
	h	47.33	178.3	1.45	1.38
	i	[k]			
	j	[k]			

[1] The torsion angle is defined as τ(C3,C2,C10,Oxygen) (see Fig. 1A).
[2] Bond length and bond order refer to the bond C2–C10 (see Fig. 1A).
[a] Unprotonated, N–CH_3 *anti, s-cis* enone conformer.
[b] Unprotonated, N–CH_3 *anti, s-trans* enone conformer.
[c] Unprotonated, N–CH_3 *syn, s-cis* enone conformer.
[d] Unprotonated, N–CH_3 *syn, s-trans* enone conformer.
[e] Protonated, N–CH_3 *anti, s-cis* enone conformer.
[f] Protonated, N–CH_3 *anti, s-trans* enone conformer.
[g] Protonated, N–CH_3 *syn, s-cis* enone conformer.
[h] Protonated, N–CH_3 *syn, s-trans* enone conformer.
[i] *N,N*-Dimethyl AnTX, *s-cis* enone conformer.
[j] *N,N*-Dimethyl AnTX, *s-trans* enone conformer.
[k] The boat conformation for both *s-cis* and *s-trans* *N,N*-dimethyl-AnTX was not observed. This configuration flipped back into the chair form during geometry optimization.

AnTX analogues (Table 5). For charged *N*-methyl- and *N,N*-dimethyl-AnTX, the *s-trans* conformer was lower in energy by approximately 2 kcal/mol. Once again this was due to intramolecular electrostatic interactions, with the carbonyl oxygen in the *s-trans* conformer closer to the centre of positive charge located close to the nitrogen atom. While the inactivity of the *N*-methylated AnTX analogues may be explained by the Hacksell and Mellin model [10], it is conceivable that the inability of *N,N*-dimethyl AnTX to adopt a boat conformer (Table 5) may imply that the boat form of the azabicycle is itself important for activity.

To aid in determining which enone configuration of AnTX is important for biological activity, the use of rigid and semirigid analogues may play an important role. For example, the enone may be constrained in a ring which preserves either an *s-cis* or *s-trans* conformation. 9-PHT is an example of this approach [40], however, the aromatic nature of the ligands may also distort the electronic environment associated with this area of the ligand. Alternatively, steric obstruction of the enone moiety will alter conformational preferences to either the *s-cis* or *s-trans* configuration, but further progress in this area must await development of the requisite synthetic methodology.

CONCLUSIONS

Our molecular modelling studies for examining the conformation of AnTX have employed both molecular mechanics and MO techniques. Using molecular mechanics, the *s-trans* configuration of protonated AnTX was shown to be the preferred form. As this was found to be due to intramolecular electrostatic forces, these internal interactions may not play a role at the receptor itself. Examination of some AnTX derivatives using molecular mechanics also failed to provide insight into identifying the bioactive conformer of AnTX. The use of semi-empirical MO techniques demonstrated that intramolecular electrostatic interactions influence the conformation considerably. Indeed, the neglect of conjugation in the enone system led us to conclude that MOPAC did not produce realistic molecular geometries within this region. The *s-trans* conformer was also shown by ab initio calculations to be the preferred form, although the difference in the energies of the *s-cis* and *s-trans* forms appeared to be somewhat exaggerated. The situation regarding the bioactive conformation (*s-cis* vs. *s-trans*) of AnTX is, in our opinion, not yet resolved. Further progress in this area must await the emergence of new ligands, specifically designed to probe the configurational demands of the receptor. This computational study has served to focus our attention on a number of potential new ligands and their synthesis, a significant challenge in itself.

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