Cation–π Interaction between the Trimethylammonium Moiety and the Aromatic Ring within Indole-3-acetic Acid Choline Ester, a Model Compound for Molecular Recognition between Acetylcholine and its Esterase: an X-Ray Study

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X-Ray analysis of indole-3-acetic acid choline ester, a model compound for molecular recognition between the neurotransmitter acetylcholine and its esterase, shows that the quaternary trimethylammonium group is folded back to make a close contact with the indole ring through the cation–π interaction; ^{1}H NMR spectroscopy confirms this type of interaction also occurs in solution.

The cation–π interaction has received much attention because of its chemical,[1] biological,[2,3] and theoretical[4] importance. Of special interest is a suggestion[2] that acetylcholine (ACh), a neurotransmitter, could bind to acetylcholinesterase (AChE) through cation–π interaction between the quaternary cationic trimethylammonium group of ACh and the π system of aromatic amino acid residue(s) of AChE. This has been supported by a recent X-ray study[3] of AChE from *Torpedo californica*, which has revealed that there are 14 aromatic rings but no more than one acidic residue at the ACh binding site. However, in spite of increasing interest, X-ray evidence for the existence of such a cation–π interaction between the trimethylammonium group and the π-ring system is still limited: the only two X-ray examples, to our knowledge, are cytidine 5′-diphosphocholine[5,6] and phosphocholine-binding immunoglobulin Fab McPC603.[7] In order to further substantiate such a cation–π interaction in a system relevant to ACh–AChE recognition and to examine more closely the significance of the quaternary trimethylammonium group in the interaction, we have now conducted† a comparative study by using selected model compounds: indole-3-acetic acid choline ester in the form of its chloride **1** and its isosteric uncharged compound, 3,3-dimethylbutyl indole-3-acetate **2**, where the trimethylammonium nitrogen of **1** is replaced by the carbon atom in **2**.

Compound **1** was synthesized by the DCC method[8] in 8% yield from indole-3-acetic acid (2 mmol) and choline chloride (2 mmol) in acetonitrile and recrystallized from acetonitrile–diethyl ether. Compound **2** was synthesized in 90% yield from indole-3-acetic acid (2 mmol) and 3,3-dimethylbutan-1-ol (3 ml; 25 mmol), and was recrystallized from ethanol. 3,3-Dimethylbutyl acetate was synthesized in 83% yield from acetyl chloride (50 mmol) and 3,3-dimethylbutan-1-ol (6 ml; 50 mmol). Crystal structures of **1** and **2** have been determined.‡

A segment of the crystal structure of **1** is shown in Fig. 1. The most interesting structural feature is the folded conformation of the ester molecule, where the indole ring head group and the trimethylammonium tail group are close to each other [the closest contact of 3.699(5) Å is between the C(13) and the C(5) atoms]. The choline moiety adopts the *gauche* conformation about the C(11)–C(12) bond [torsion angle O(1)–C(11)–C(12)–N(2) = 79.7(3)°] due to an $N^+\cdots O$ electrostatic interaction, as usually observed[5,9] for the O–C–C–N^+ system. Thus it appears that the folded shape of the molecule is ultimately determined by the additional *gauche* conformation about the C(9)–C(10) bond [C(1)–C(9)–C(10)–O(1) = 1.1(5)°]. In addition, this trimethylammonium group makes another close contact with the indole ring of the neighbouring molecule in the crystal lattice [the closest contact of 3.429(4) Å is between the C(14) and the N(1) atoms]. This is reminiscent of suggested[3] multiple interactions of the trimethylammonium group of an ACh molecule with more than one aromatic ring at the ACh binding site of AChE. The chloride ion forms a hydrogen bond with the nitrogen atom N(1) of the indole ring [N(1)···Cl = 3.136(3) Å] while its electrostatic interactions with the cationic choline groups may be only of secondary significance [the nearest cationic neighbour of 3.529(3) Å with the C(12) atom (at $x-1, y, z$)]. The ^{1}H NMR spectrum (270 MHz; CD_3OD) of **1** gives a singlet at δ 2.915 for the *N*-methyl protons, while the

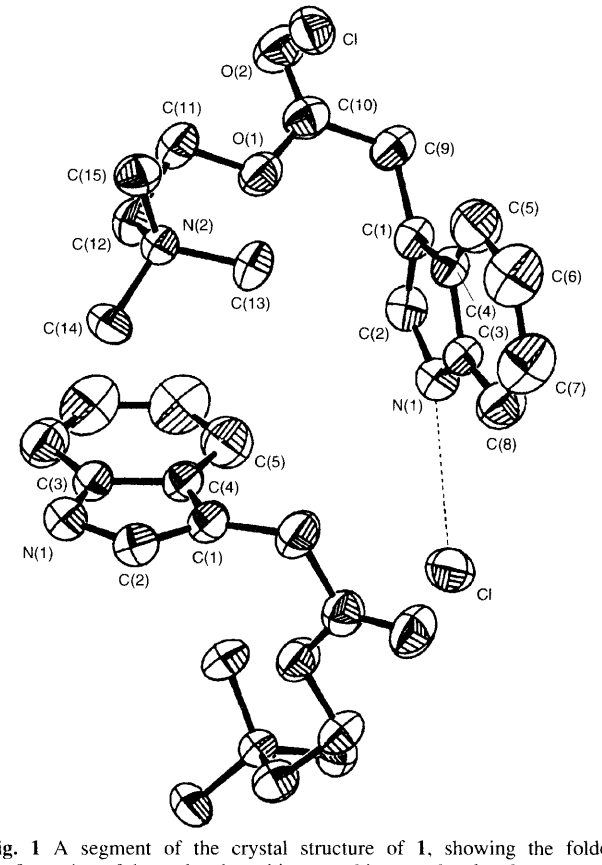
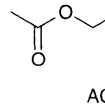
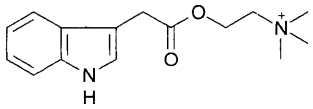


Fig. 1 A segment of the crystal structure of **1**, showing the folded conformation of the molecule and intra- and inter-molecular close contacts between the cationic trimethylammonium group and the indole π rings; close contacts (< 3.8 Å): C(3)···C(4) = 3.764(4), C(13)···C(15) = 3.699(5), C(14)···C(1) = 3.701(4), C(14)···C(2) = 3.570(5), C(14)···C(3) = 3.464(4), C(14)···C(4) = 3.629(4), C(14)···N(1) = 3.429(4) Å. A broken line depicts a hydrogen bond from N(1) to Cl^-.



ACh



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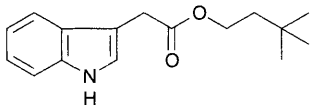

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corresponding signal for acetylcholine itself appears at δ 3.215. This upfield shift of 0.300 ppm for **1** is probably due to the aromatic ring-current effect, indicating that the quaternary trimethylammonium group associates preferentially with the indole ring of the same molecule in a dilute solution (5.5 mmol dm^{-3}).§

On the other hand, the uncharged isosteric compound **2** takes the fully extended conformation in the solid state, keeping the head and the tail groups far apart, as shown in Fig. 2. Thus the *gauche–gauche* conformation about the C(9)–C(10) and the C(11)–C(12) bonds in **1** changes to a *trans–trans* one [C(1)–C(9)–C(10)–O(1) = 169.8(9)° and O(1)–C(11)–C(12)–C(13) = 176.9(9)°] in **2**; the other torsion angles, C(2)–C(1)–C(9)–C(10), C(9)–C(10)–O(1)–C(11) and C(10)–O(1)–C(11)–C(12), are almost the same for **1** and **2**. Furthermore, no intermolecular interaction between the *tert*-butyl group and the indole moiety occurs in the crystal lattice. The ^{1}H NMR spectra (270 MHz; CD_3OD) of **2** and 3,3-dimethylbutyl acetate give only a small upfield shift (0.050 ppm) for the *tert*-butyl protons of **2** (chemical shifts of δ 0.895 and 0.945, respectively), indicating that the contact between the terminal *tert*-butyl group and the indole ring may be minor if it exists at all in solution for **2** (5.9 mmol dm^{-3}).§

The present comparative study demonstrates that, since **1** and **2** are sterically almost equivalent, the structural difference observed for **1** and **2**, the folded and the extended conformations, respectively, might be caused by the electronic difference between the trimethylammonium and the *tert*-butyl groups. This implies that the cationic nature of the quaternary trimethylammonium group could be responsible for this preferable association with the indole ring in **1**, that is, through cation–π interaction. It is of interest to note here that, in the crystal structure[5,6] of cytidine 5′-diphosphocholine, the choline group is folded to make a close contact with the cytosine ring [closest contact of 3.48(3)[6] Å], while in the crystal structure[5] of cytidine 5′-diphosphate, which lacks the choline group, the diphosphate group is extended away from the cytosine base; this implies again the significance of the trimethylammonium group in the cation–π interaction with the π-ring system, although the authors[5,6] do not discuss this point.

X-Ray and solution studies to further examine the electronic effects of the aromatic ring on the cation–π interaction with the choline group, by inducing electron-donating or -withdrawing substituents into the indole ring, are under way.

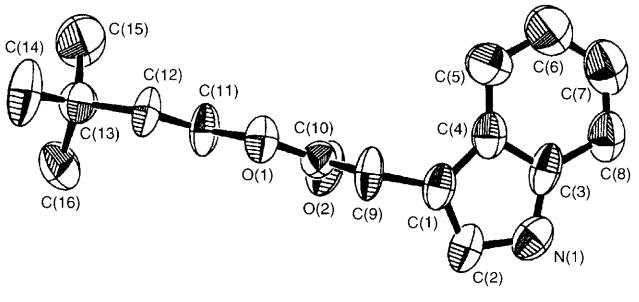

Fig. 2 The molecular structure of **2**, showing the fully extended conformation

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Footnotes

† Our initial efforts to crystallize the molecular complexes expected to be formed between ACh and aromatic compounds involving indole-3-acetate, motivated by an NMR study[10] demonstrating their significant interactions, were unsuccessful.

‡ *Crystal data* for **1**: $C_{15}H_{21}ClN_2O_2$, colourless plates, M = 296.80, monoclinic, space group $P2_1/n$, a = 10.167(2), b = 10.707(4), c = 14.834(2) Å, β = 97.11(2)°, U = 1602.3(6) Å^3, Z = 4, D_c = 1.230 g cm^{-3}, μ(Mo-Kα) = 2.41 cm^{-1}, $F(000)$ = 632. At convergence, R = 0.040, R_w = 0.029 [$w^{-1} = \sigma^2(F)$] for 1855 observed reflections [$I > 3\sigma(I)$]. For **2**: $C_{16}H_{21}NO_2$, colourless plates, M = 259.35, monoclinic, space group $P2_1/a$, a = 11.392(3), b = 10.317(2), c = 13.235(2) Å, β = 102.87(2)°, U = 1516.4(5) Å^3, Z = 4, D_c = 1.136 g cm^{-3}, μ(Mo-Kα) = 0.74 cm^{-1}, $F(000)$ = 560. At convergence, R = 0.084, R_w = 0.074 [$w^{-1} = \sigma^2(F)$] for 934 observed reflections [$I > 3\sigma(I)$]. Intensity data were measured using a Rigaku AFC7R diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71069 Å) by using the ω–2θ scan method. For **2** the diffraction pattern was in general quite weak and did not extend much beyond 2θ = 40°, mostly due to the small crystal size (0.10 × 0.30 × 0.30 mm). The structures were solved by direct methods and refined by using full-matrix least-squares (TEXSAN), with anisotropic thermal parameters for the non-hydrogen atoms; for **1** all the hydrogen atoms were located from the difference Fourier map and refined isotropically, while those for **2** were included but fixed at the calculated positions during the least-squares refinement. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

§ A solution study to quantitatively evaluate exact populations of the folded *vs.* the opened conformers for **1** and **2** remains to be carried out.

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