Synthesis and X-Ray Crystal Structure of 2-Acetyl-9-azabicyclo[4.2.1]nonan-3-one. A Conformationally Locked s-*cis* Analogue of Anatoxin-a

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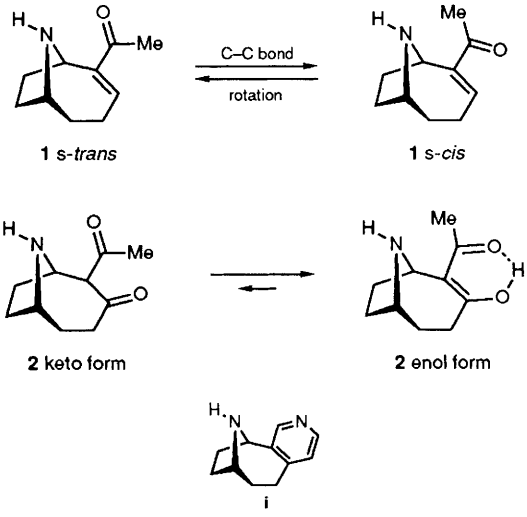
2-Acetyl-9-azabicyclo[4.2.1.]nonan-3-one **2** ('hydroxyanatoxin-a'), which represents a conformationally locked variant of the s-*cis* conformer of the potent cholinergic agonist anatoxin-a, is synthesised and characterised; this molecule lacks both nicotinic and muscarinic potency.

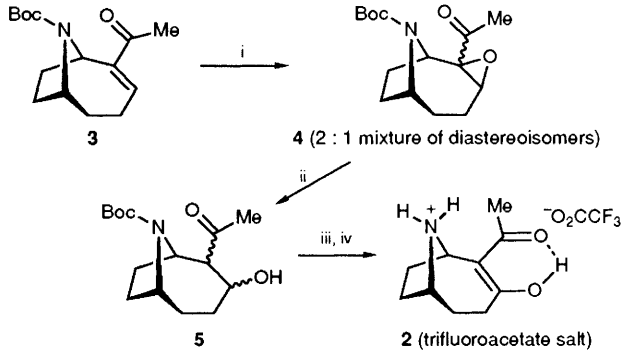
Anatoxin-a **1** is recognised as being one of the most potent nicotinic acetylcholine agonists known,[1] and its neurotoxicity also presents a significant environmental hazard.[2] While structural modifications of this ligand have been generated[3] in order to probe those features crucial for agonist potency, the conformational properties of the enone moiety—the most flexible portion of an otherwise relatively rigid molecule—have also attracted synthetic,[4] as well as computational[5] interest. The enone component of anatoxin-a can adopt two low-energy conformations corresponding to the s-*cis* and s-*trans* forms.

The s-*trans* conformer is observed in the solid state (for both anatoxin-a[5a] and *N*-acetylanatoxin-a[6]) and there is evidence for the presence of both conformers in solution, as judged by ^{1}H NMR spectroscopy. Recent computational studies[5c] suggest that the s-*trans* form of protonated **1** is more stable (by 8 kJ mol^{-1}) than the corresponding s-*cis* conformer. However, the biological relevance of these (or other) conformers is unclear.†

The 1,3-diketone **2** represents an attractive probe for the s-*cis* conformer of anatoxin-a since this ligand would be

† Various computational studies have examined the relationship between s-*cis* and s-*trans* anatoxin-a. Rapoport[5a] has suggested that s-*cis*-**1** is more stable (ΔE = 3.138 kJ mol^{-1}), although our own studies[5c] indicate that the s-*trans* form of protonated **1** is the more stable (ΔE = 8.368 kJ mol^{-1}); anatoxin-a (pK_a 9.36[5a]) is essentially fully protonated at physiological pH. Both studies did conclude that the chair form of 9-azabicyclo[4.2.1]nonane skeleton predominated. Hacksell and Mellin[5b] have also examined the relative importance of these two conformers based on excluded receptor volume, and concluded that the s-*trans*-**1** is biologically viable.





Scheme 1 *Reagents and conditions*: i, H_2O_2, NaOH, methanol, room temp. (64%); ii, Al/Hg, tetrahydrofuran, H_2O, room temp. (77%); iii, pyridinium chlorochromate, 4Å molecular sieves, CH_2Cl_2, room temp. (79%); iv, CF_3CO_2H, CH_2Cl_2, room temp. (quantitative)

expected to exist predominantly as the corresponding enol tautomer, stabilized by an intramolecular hydrogen bond, while retaining a hydrogen bond acceptor function.[7] In this Communication we describe the synthesis (in racemic form) of 'hydroxyanatoxin-a' **2**, together with results of a preliminary biological evaluation of this novel ligand.

The synthesis of hydroxyanatoxin-a **2** is shown in Scheme 1.‡ Epoxidation of *N-tert*-butoxycarbonyl (Boc) anatoxin-a **3** was carried out under basic conditions to give epoxyketone **4** as a 2 : 1 mixture of diastereoisomers. Reductive cleavage of **4** was problematic and a wide range of otherwise commonly used reagents proved to be ineffective in this case. The transformation was, however, successfully accomplished using aluminium amalgam to give a mixture of ketones **5**. Oxidation of **5**, followed by cleavage of the Boc protecting group, gave 2-acetyl-9-azabicyclo[4.2.1]nonan-3-one (hydroxyanatoxin-a) **2** as the trifluoroacetate salt [m.p. 96–98 °C (Et_2O–CH_2Cl_2)] [Found: $M^+ + H$, 182.119. $C_{10}H_{16}NO_2$ requires M, 182.118); δ_H (270 MHz, $CDCl_3$) 17.09 (1 H, s), 10.11 (1 H, br s), 9.13 (1 H, br s), 4.80 (1 H, d, *J* 10 Hz), 4.12 (1 H, m), 2.83–2.58 (2 H, m), 2.42–2.20 (2 H, m), 2.25 (3 H, s), 2.10–1.78 (4 H, m); δ_c (67.8 MHz, $CDCl_3$) 196.5, 190.5, 110.8, 57.6, 54.6, 35.4, 30.6, 27.6, 26.6, 22.8]. The ^1H NMR spectrum of **2** showed only the enol form (δ 17.09) to be present; in [$^2H_6$]DMSO solution approximately 14% of the keto tautomer was observed.§ The structure of **2** has also been established by X-ray crystallographic analysis and contains two crystallographically independent formula units; the two cations are chemically very similar. A diagram of one cation is shown in Fig. 1 with selected bond lengths and angles in the caption.¶

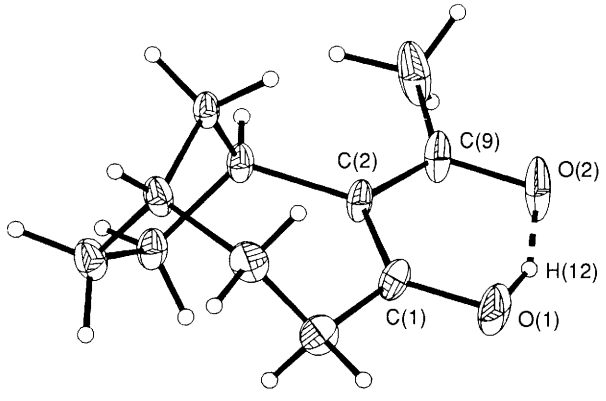


Fig. 1 Diagram of the crystallographically independent cation of **2**. Selected bond lengths (with those corresponding to the second cation in parentheses) are: C(1)–O(1) 1.280(6) [1.281(6)]; C(9)–O(2) 1.299(6) [1.308(6)]; O(1)–H(12) 1.13(2) [1.27(2)]; O(2)–H(12) 1.35(2) [1.26(2)]; C(2)–C(9) 1.389(7) [1.387(6)]; C(2)–C(1) 1.424(7) [1.416(6)].

The experimental determination of hydrogen positions confirms the enol form, with the intramolecular hydrogen bond being almost symmetrical.

With this new ligand available, albeit in racemic form, its ability to bind the nicotinic and muscarinic acetylcholine receptors has been examined. In competition binding assays against [^3H]nicotine binding to rat brain membranes, the IC_{50} of **2** was in excess of 10^{-5} mol dm^{-3}, compared with a value of 7×10^{-9} mol dm^{-3} for anatoxin-a **1**. Clearly the structural modifications associated with **2** drastically diminishes nicotinic potency. This change was not associated with the emergence of any muscarinic potency: both **1** and **2** (examined at concentrations up to 10^{-5} mol dm^{-3}) totally failed to inhibit the binding of 1-quinuclidinyl [4-phenyl-^3H]benzilate and of the M1-selective muscarinic ligand, pirenzepine, to rat brain membranes. These are important findings, given that **2** is structurally more closely related to anatoxin-a than other s-*cis* analogues that have been prepared, although the lack of potency is in conflict with the results of Kanne and Abood.‖

‡ All new compounds are racemic and gave satisfactory spectral data (IR, ^1H and ^{13}C NMR) and were further characterised by high resolution mass measurement; all yields refer to isolated material, homogeneous by TLC.

§ The *endo* enol form of diketone **2** is shown simply to make clear the relationship of this ligand to anatoxin-a. However, the preferred tautomer (*endo vs. exo* enol), which in a variety of other '*endo-exo*-β-diketones' is sensitive to ring size,[8] remains to be defined. In the solid state, no preference is apparent within experimental error (see Fig. 1).

¶ *Crystal data*: $[C_{10}H_{16}NO_2]^+$ $[CF_3CO_2^-]$, M_r = 295.26, triclinic, $P\bar{1}$, a = 9.114(6), b = 12.309(2), c = 14.228(3) Å, α = 113.15(1), β = 101.71(1), γ = 104.49(2)°, U = 1337.1 Å^3, Z = 4, D_c = 1.467 g cm^{-3}, $F(000)$ = 616. Intensity data collected on a FAST area detector diffractometer as previously described.[9] 7999 Data measured, giving 5570 unique of which 2204 were observed [$F_0 > 3\sigma(F_0)$]. The structure was solved by direct methods and refined using full-matrix least-squares analysis. The final R, R_w values for 370 parameters were 0.043, 0.047 respectively. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

‖ Kanne and Abood[4] have reported the synthesis of the racemic nicotine–anatoxin-a hybrid structure **i**. This structure represents a constrained s-*cis* variant of anatoxin-a and **i** is comparable to anatoxin-a in its pharmacologic potency and binding to nicotinic receptors.

Nevertheless, our results do support the biological relevance of the s-*trans* conformer of anatoxin-a, and further progress in defining the biologically active conformations of anatoxin-a will be aided by the continued development of new ligands. The 1,3-diketone function of **2** offers considerable flexibility for the synthesis of a variety of heterocyclic analogues but conformationally-constained s-*trans* analogues are also attractive targets and work towards this goal is underway.

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