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6-CARBOXYMETHYL-2-AZABICYCLO[2.2.1]HEPTANE ENANTIOMERS: MUSCARINIC ACTIVITIES OF RIGID ANALOGUES OF ARECOLINE

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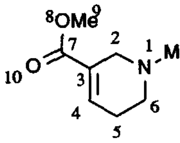
Abstract: (1R,4R,6R)-(-)-6-Carbomethoxy-2-azabicyclo[2.2.1]heptane **8a**, (1R,4R,6R)-(-)-6-carbomethoxy-2-methyl-2-azabicyclo[2.2.1]heptane **2a** and their enantiomers **8b** and **2b** have been prepared as rigid analogues of the enantiomorphic conformations of arecoline, and their activities as muscarinic agonists evaluated.

Betel nuts, the seeds of *Areca catechu*, are extensively used throughout Asia and Oceania as a mild stimulant.[1] Arecoline (**1**), the main alkaloid from the seeds,[2] has long been recognised to be a muscarinic agonist. Pauling and Petcher determined the X-ray crystal structure of arecoline hydrobromide in 1971. In the structure they solved, two independent conformations were present in the asymmetric unit. In both the N-methyl group was occupying a pseudoequatorial position, and the corresponding dihedral angles N1-C2-C3-C7 were 150° and -165°, respectively.[3,4] The arecoline methiodide crystal also contains two enantiomorphic conformers, with the corresponding dihedral angles of 157° and -157°.[5] From molecular mechanics calculations the corresponding torsion angles are between 166° to 170°, depending on the force field used.[6] The possibility that arecoline adopts enantiomorphic conformations when interacting with muscarinic receptors could be a cause for its lack of selectivity.



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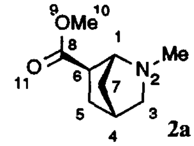

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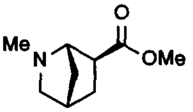

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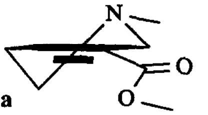

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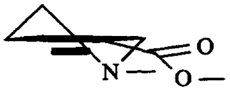
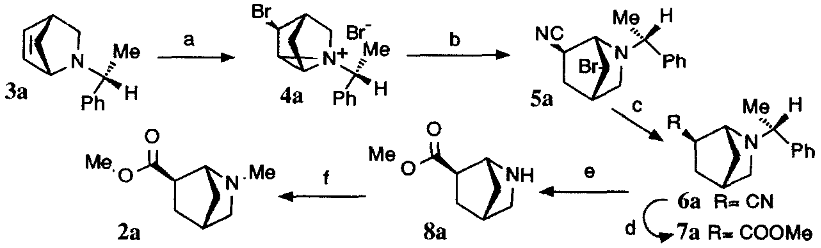


1b

It has been recently shown that muscarinic agonists can accomodate a large degree of rigidity and still be potent and efficacious and several azabicyclic skeletons have been used as templates for muscarinic agonists.[7,8] The 2-azabicyclo[2.2.1]heptane skeleton has received much less attention in this regard, although 6-acetoxy-2-azabicyclo[2.2.1]heptane was proposed by Lattin as a rigid acetylcholine analogue as early as 1970.[9] We recognised that the enantiomers of *exo*-2-methyl-5-carbomethoxy-2-azabicyclo[2.2.1]heptane **2a** and **2b** could mimic the enantiomorphic conformations of arecoline **1a** and **1b**, and provide the basis for selective interactions with the muscarinic receptors. The corresponding torsional angles N2-C1-C6-C8, derived from energy-minimised structures using molecular mechanics,[10] are 160° and -160°, respectively. We therefore decided to synthesise these molecules.

Syntheses: A rapid enantioselective access to the 2-azabicyclo[2.2.1]heptane skeleton is provided by the hetero-Diels Alder reaction described by Grieco and Larsen.[11] In their paper, however, no assignment of the absolute configurations of the products was made. Starting from (*R*)-phenylethylamine hydrochloride, the less polar diastereoisomer 3a ($[\alpha]_D^{20}$=-20° (c= 2.0, CH_2Cl_2)) is obtained in 60% yield. The relative stereochemistry of this compound was demonstrated by the observation of nuclear Overhauser effects between both the aromatic protons and H-3_{endo}, and between the benzylic proton and the vinylic H-6.[12] Independent determination of the absolute configuration was obtained by X-ray crystallographic analysis of the enantiomer of bromide 4a, as reported elsewhere.[13] Grob and Dratva have used the nucleophilic opening of an analogous racemic aziridinium ion to prepare racemic 6-substituted 2-azabicyclo[2.2.1]heptanes.[14] We have here adapted their methods for enantioselective synthesis. Treating the enantiomerically pure 4a (mp 190° dec. $[\alpha]_D^{20}$= 4.7 (c=1.3,CH_2Cl_2)),with tetraethylammonium cyanide, the crystalline 5a (mp 124-126°, $[\alpha]_D^{20}$= 11.5 (c=0.9, CH_2Cl_2)) was obtained in enantiomerically pure form. A clean inversion of the configuration of the ring system occurs, due to the fact that only the less sterically hindered bond of the aziridinium is available for S_N2 nucleophilic attack. Reduction of the bromide was achieved with tributyltin hydride to 6a (mp 59-61°, $[\alpha]_D^{20}$=-30.2 (c=0.65, CH_2Cl_2)). Acidic methanolysis of the nitrile gave the corresponding methyl ester 7a (bp 140°, 0.1 mmHg, $[\alpha]_D^{20}$=-50.1 (c=0.9, CH_2Cl_2)). Hydrogenolysis of the N-ethylphenyl moiety with Pd/C in AcOH at 40°, proceeded smoothly in 18h to give the secondary amine 8a. Reductive methylation with an Eschweiler-Clarke procedure gave 2a.[15] The optical purity was determined by ^{1}HNMR using (-)-(*R*)-(9-anthryl)-2,2,2-trifluoroethanol as the chiral solvating agent. The enantiomeric series of compounds was prepared in analogous fashion from (*S*)-phenylethylamine.



Reagents and conditions: a) Br_2, CH_2Cl_2,73%; b) Et_4NCN, CH_2Cl_2, 65%; c) Bu_3SnH, AIBN, reflux, 3h, 96%; d) HCl,MeOH, reflux 2h, 91%; e) H_2,Pd/C,AcOH 5 atm. 86%; f) H_2CO, HCOOH, 90°, 1h, 90%

Biological activity: The compounds were tested as their hemifumarate salts. The activities in guinea pig ileum contraction (a model for M_3 activity) and depolarisation of rat superior cervical ganglion and of rat hippocampal slice preparations (both models for M_1activity), and binding with [^{3}H]-pirenzepine (PZ, M_1-selective) to homogenised rat cerebral cortex membranes were tested as previously described.[16] Inactive compounds were tested up to concentrations of 10^{-4}M. Efficacies relate to the percentage of the maximal response produced by carbachol (ileum and hippocampus) or muscarine (ganglion). The activities of the compounds prepared and arecoline are given in Table 1. All the bicyclic compounds show lower potencies and efficacies than arecoline. Racemic **8** and arecoline present similar activity in all three preparations. Although in ileum and ganglion **8a** is only about ten-fold more potent than **8b**, all the hippocampal activity in the racemate must be ascribed to **8a**; **8b** is thus at least tenfold less potent in the hippocampus. **8b** also presents lower efficacies in all tissue preparations, posessing highest overall activity in the ileum. The racemate **2** is characterised as a partial agonist, with the highest activity in the ganglial preparation. The enantiomeric **2a** and **2b** show tendencies of opposed selectivities; **2a** is selective for the hippocampus, whereas **2b** is selective for the ganglion. However, the compounds are very weak partial agonists. This may be due to unfavourable steric interactions of the muscarinic receptor with the additional *endo*-hydrogens or with the methylene bridge; to an unfavourable positioning of the ester oxygens, or to unfavourable conformation of the N-methyl group. The last explanation is supported by the fact that the N-nor compounds **8a** and **8b** are more potent than the corresponding N-methyl analogues, but have lost the tendency towards selectivity. In fact, the compound with the greatest similarity to arecoline is **8a**, which lacks this methyl group. Presumably, arecoline interacts with the muscarinic receptors preferentially in a conformation mimicking **8a**, namely **1a**.

TABLE 1: Muscarinic Potency and Efficacy

COMPOUND		ILEUM	GANGLION	HIPPOCAMPUS	[^{3}H]-PZ
		pD_2 (%)	pD_2 (%)	pD_2 (%)	pIC_{50}
1		6.5 (100)	6.6 (80)	6.1 (100)	5.76
8	(+/-)	6.2 (91)	6.3 (57)	5.5 (103)	5.71
8a	(-)	6.0 (120)	6.7 (90)	6.0 (120)	5.85
8b	(+)	5.2 (94)	5.2 (47)	<4	5.59
2	(+/-)	4.2 (58)	5.0 (82)	5.4 (39)	5.55
2a	(-)	<4	4.4 (70)	5.9 (33)	5.41
2b	(+)	4.9 (68)	5.9 (71)	<4	5.72

In this case, increased rigidity of the skeleton has brought about some selectivity, but at the expense of both potency and efficacy. Previous work indicates that muscarinic agonists can have different activities in the ganglion and hippocampus preparations, not readily explained by differences in receptor reserve in both tissues.[17] The stereoselectivity of these compounds does indicate, however, that arecoline itself may be interacting with the muscarinic receptors preferentially with one enantiomorphic conformation. As has been repeatedly pointed out,[18] the use of racemic compounds to explore what is certainly an enantioselective recognition process is bound to obscure the relevant parameters necessary to obtain selectivity in muscarinic agonists.

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15. **(1R,4R,6R)-(-)-6-Carbomethoxy-2-azabicyclo[2.2.1]heptane 8a**. TLC (Silicagel, CH_2Cl_2-MeOH-NH_4OH 9:1:0.1) Rf = 0.1.$[\alpha]_D^{20}$ = -78.7° (c = 0.55 in MeOH) Hydrogenfumarate: mp 141-144°C, $[\alpha]_D$= -35.8 (c = 0.5 in CH_2Cl_2), enantiomeric purity (^{1}HNMR) > 99.5 %. MS (EI, 70EV): 155 (17, M+), 124 (10, [M-OCH_3]+), 98 (13), 79 (16), 68 (40, [C_4H_6N+]), 67 (100), ^{1}HNMR ($CDCl_3$): 1.48, m, 1H (1xH-7), 1.53, m, 1H, (1xH-7), 1.60 m, 1H, (H-5), 1.9, m, 2H, (NH, H-5), 2.49, m, 1H, (H-4), 2.60, m, 2H, (H-6, 1xH-3), 2.90, m, 1H, (1x H-3), 3.64, br s, 1H, (H-1), 3.67, s, 3H, (OCH_3), ^{13}CNMR ($CDCl_3$): 29.69, t, (C-5), 36.24, t, (C-7), 36.32, d, (C-4), 49.59, d, (C-6), 50.55, t, (C-3), 51.72, q, (OCH_3), 59.16, d, (C-1), 174.45, quat, (C=O). **(1S,4S,6S)-(+)-6-Carbomethoxy-2-azabicyclo[2.2.1]heptane 8b**. $[\alpha]_D^{20}$ = 81°(c = 2.4 in CH_2Cl_2). Hydrogenfumarate: mp 142-144°,$[\alpha]_D^{20}$ = + 32.8 (C = 0.5 in CH_2Cl_2);Enantiomeric purity (^{1}HNMR) = 97.8 %. **(1S,4S,6S)-(+)-6-Carbomethoxy-2-methyl-2-azabicyclo[2.2.1]heptane 2b** TLC(Silica gel, CH_2Cl_2-MeOH-NH_4OH 90:10:1) Rf = 0.14 MS (EI, 70 EV): 169 (19, M+), 138 (10), 94 (14) 82 (35), 81 (100), 71 (31), ^{1}HNMR ($CDCl_3$) 0.54, br d, J = 7.2 Hz, 1H, (H-7_{anti}), 0.68, ddd, J = 9.0, 4.4, 1.6Hz, 1H, (H-5_{endo}), 0.76, dm, J = 7.2,0.4 Hz, 1H, (H-7_{syn}), 1.05, dm, J = 9.0 3.6, 1.8Hz, 1H, (H-5_{exo}), 1.25, d, J = 6.0 Hz, 1H, (H-3_{endo}), 1.49, s, 3H, (N-CH_3), 1.54, br s, 1H (H-4), 1.96, ddd, J = 6.0, 1.8, 2.0Hz, 1H, (H-3_{exo}), 2.06, ddd, J = 6.5, 3.6, 0.4 Hz, 1H, (H-6_{endo}), 2.45, br s, 1H, (H-1), 2.80, s, 3H, ($COOCH_3$) ^{13}CNMR ($CDCl_3$): 32.54, t, (C-5), 34.72, t, (C-7), 37.62, (N-CH_3), 40.20, d, (C-4), 42.02, d, (C-6), 51.76, q, (O-CH_3) 59.63, t, (C- 3), 66.13, d, (C-1), 175.04, quat., (C=O). Hydrogenmaleinate : mp 117-119°C, $[\alpha]_D^{20}$ = + 25.0 (c = 0.5 in MeOH). **(1R,4R,6R)-(-)-6-Carbomethoxy-2-methyl-2-azabicyclo [2.2.1]heptane 2a** hydrogenmaleinate mp:115-118°C, dec. $[\alpha]_D^{20}$ = -28.2 (c = 0.55 in MeOH). Enantiomeric purity (^{1}HNMR):99.3 %.

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