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Preliminary Synthetic Studies of Methyllycaconitine, a Potent Nicotinic Acetylcholine Receptor Antagonist: Rapid Syntheses of AE-Bicyclic Analogues

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Abstract

A series of bicyclic analogues incorporating the homocholine motif of methyllycaconitine has been prepared to test the hypothesis that this is the essential pharmacophore of this potent, selective nicotinic receptor antagonist. A double Mannich reaction has been employed to construct the 3-azabicyclo[3.3.1]-nonane ring system, containing an *N*-ethylpiperidine moiety. The neopentyl-like alcohol was then esterified, using isatoic anhydride under basic conditions, to afford the corresponding anthranilate.

There is a long history of the use of *Aconitum* and *Delphinium* by various civilisations as sources of poisons and medicines (Benn & Jacyno 1983). Probably the earliest use of a *Delphinium* preparation, as an insecticide, is the treatment of head and body lice reported by Pliny the Elder in AD 77. A pounded extract of *D. staphysagria* seeds was an efficient treatment for head and body vermin. Possibly significantly, the pounded flowers could be ingested, in wine, to counteract the poison of serpents. Serpent bites, in particular, were treated by applications of *D. staphysagria*.

Methyllycaconitine (MLA) (**1**) is a competitive nicotinic acetylcholine receptor (nAChR) antagonist. As such, it displaces the snake toxin α-bungarotoxin from its binding site on the pentameric ligand-gated ion channel protein receptor. Apparently, a similar *Delphinium* preparation was a standard issue in the British Army at the time of the battle of Waterloo (Benn & Jacyno 1983) and this practice is apparently still recommended for the treatment of lice! *Delphinium* plants are held responsible for more cattle deaths in North America than any other poisonous plant (Nambi Aiyar et al 1979; Keeler 1975). In 1938, Manske examined the aerial portion of *Delphinium brownii* Rydberg and established one of the alkaloids to be MLA (**1**), the 2-[2-(*S*)-methylsuccinimido]-benzoate ester of the norditerpenoid lycoctonine (**2**) (Fig. 1) (Manske 1938). This complex hexacycle has been reported in at least 30 *Delphinium* species. MLA produces mortality in a broad spectrum of insects (Jennings et al 1986), its toxicity resulting from its being a highly potent competitive nAChR antagonist (Nambi Aiyar et al 1979; Jennings et al 1986; Ward et al 1990). nAChR is the principal receptor in the insect central nervous system. Several subtypes of nAChR, in the vertebrate nervous system as well as at neuromuscular junctions, have recently been characterized by molecular biological techniques. MLA (**1**) shows higher affinity for neuronal α-bungarotoxin binding sites both in insects and vertebrates than for any other nAChR subtype (Jennings et al 1986; Ward et al 1990). In contrast, lycoctonine (**2**), the parent neopentyl-like alcohol, derived from MLA by saponification, exhibits markedly less nicotinic activity, indicating that, at least, the anthranilate moiety is significant in the structure-activity profile (Jennings et al 1986; Ward et al 1990).

The portion of the MLA molecule leading from the ester carbonyl function through carbon atoms 18, 4, and 19 to the *N*-ethyl group bears a formal resemblance to an acylated homocholine motif. In our structure-activity relationship (SAR) studies, we aim to explore the roles of this motif and the acylating anthranilic acid moiety in the MLA pharmacophore. We have, therefore, designed and synthesized a series of small molecule bicyclic analogues of MLA, as one part of our preliminary synthetic studies on this norditerpenoid alkaloid. These [3.3.1] bicycles have been designed to incorporate the 3-aminopropan-1-ol motif. In this communication, we present rapid and efficient syntheses of AE-bicyclic analogues which contain the piperidine (E) and cyclohexane (A) rings of MLA (**1**).

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Materials and Methods

General details

Thin layer chromatography was performed on pre-coated plates (Merck TLC aluminium sheets silica 60 F_{254}). The plates were visualized either with ninhydrin in *n*-butanol followed by heating with a hot air gun, or by short wavelength (254 nm) ultraviolet light. Column chromatography was performed according to the method developed by Still and co-workers (1978) using Sorbsil C60-H flash chromatography silica gel (40–60 μm) purchased from Prolabo, Eccles, Manchester. ^{1}H NMR spectra were recorded at 270 MHz using a Jeol GX270 spectrometer. ^{13}C NMR spectra were recorded at 67·8 MHz (GX270) employing 90

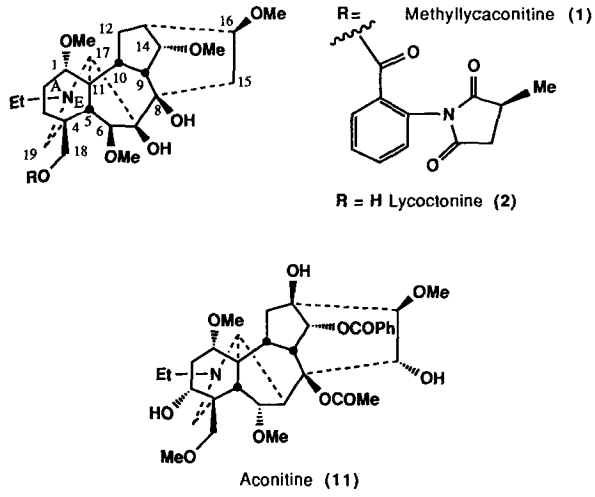

FIG. 1. Comparison of structures.

and 135 DEPT pulse sequences to aid multiplicity determinations. Low resolution mass spectra were recorded on a VG Analytical 7070E with a VG 2000 data system. EI spectra were produced at 70 eV; CI was employed using *iso*-butane as the reagent gas, and +ve and −ve FAB was performed using 3-nitrobenzyl alcohol as the matrix. IR spectra were obtained using thin discs (KBr) on a Perkin-Elmer 782 infrared spectrophotometer and UV spectra were obtained in aqueous solution with a Perkin-Elmer Lambda 3 UV/Vis spectrophotometer. All chemicals and reagents were purchased from Aldrich. Solvents were purchased from Fisons and were either HPLC grade or were purified according to Perrin and Amarego (1988).

Results and Discussion

Synthesis of bicyclic alcohols **7** *and* **10** (Fig. 2)

The 9-substituted bicyclic alcohols (3-alkyl-3-aza-9-(*RS*)-methoxy- and methoxymethylbicyclo[3.3.1]nonane-1-methanols) **7** and **10** were designed to contain the AE ring system found in MLA (**1**). The synthesis of these bicycles, similar to those found in atisine (Ihara et al 1990), cardiopetaline (Shishido et al 1986), and related *Aconitum* and *Delphinium* alkaloids, was achieved in each case in four steps from ethyl 2-cyclohexanone-1-carboxylate (**3**), enabling subsequent conversion into the corresponding anthranilate esters (as seen in Fig. 2).

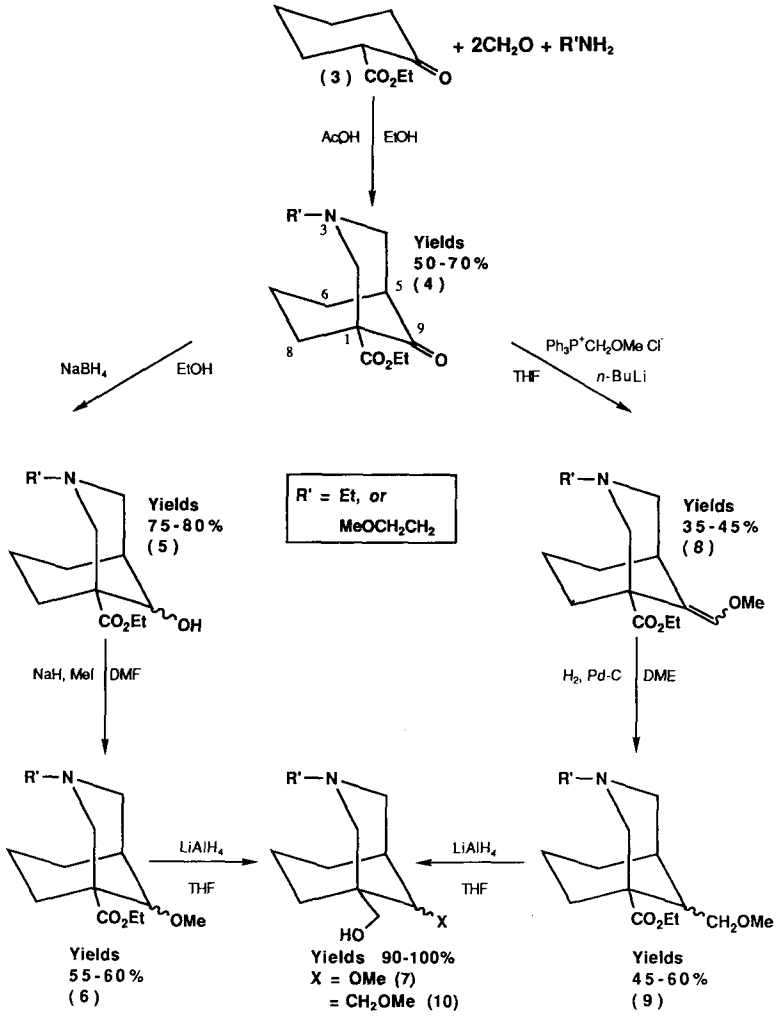

FIG. 2. Synthesis of bicyclic alcohols **7** and **10**.

Thus, a double Mannich condensation between keto-ester (**3**), a primary amine ($R'NH_2$), and aqueous formaldehyde (37%, 2 equivalents, reflux in ethanol in the presence of a catalytic amount of glacial acetic acid) was used to construct the 3-azabicyclo[3.3.1]nonane system (**4**), typical yields 50–70%. Both ethylamine and β-methoxyethylamine were used as the amine to give two parallel sets of analogues (Blicke & M°Carty 1959). The basicity of a tertiary amine can be significantly reduced by the addition of a β-methoxyethyl group and therefore, it was hoped that this series would be more lipophilic at physiological pH (Perrin et al 1981). In one series of AE-bicyclic analogues, we decided to replace carbon C-6 (norditerpenoid numbering) with an *O*-methyl ether at C-5. Thus, reduction of **4** with sodium borohydride (carried out at room temperature in anhydrous ethanol) gave a mixture of epimeric alcohols (**5**), in 75–80% yields, which were then *O*-methylated (using sodium hydride and methyl iodide in anhydrous *N,N*-dimethylformamide) to give **6** in 55–60% yields. In another series of analogues, we extended the [3.3.1]bicycle carbon skeleton from the ketone at C-5 to C-6 (norditerpenoid numbering) by a Wittig reaction. Thus, the *E* and *Z* enol ethers (**8**) were synthesized from **4** by preparing the phosphorus ylid in-situ from *n*-butyllithium and (methoxymethyl)triphenyl phosphonium chloride in anhydrous tetrahydrofuran. Catalytic hydrogenation (5 atm) was then used to reduce the mixture of enol ethers (**8**) to the 9-(*RS*)-methoxymethyl ethers (**9**) using 10% palladium on charcoal in anhydrous 1,2-dimethoxyethane, typical yields 35–45%. Reduction of the ester functionality in **6** and **9** was achieved using lithium aluminium hydride in anhydrous tetrahydrofuran to give, in almost quantitative yields, the corresponding neopentyl-type alcohols **7** and **10**, respectively.

General procedure for synthesis of 2-(RS)-methylsuccinimidobenzoates (Fig. 3)

The efficient preparation of the intermediate 2-aminobenzoates was performed by heating isatoic anhydride, at 60°C, with the appropriate aliphatic alcohol in the presence of the basic catalyst, 4-(*N,N*-dimethylamino)pyridine in *N,N*-dimethylformamide (Perrin et al 1981), in 50–90% yields. Under these basic conditions, the formation of the ester is promoted over the carbamate. The conversion of these esters into their corresponding imides was achieved by fusion with two equivalents of 2-(*RS*)-methylsuccinic anhydride (Morrell 1914), typically for 2–4 h resulting in 40–70% yields of the isolated, desired 2-methylsuccinimidobenzoates.

It was found that at most stages in our synthetic routes only modest separation of the epimers was obtainable by chromatography over silica gel, but *O*-methyl ethers (**6**) were separable which enabled full characterization following from detailed inspection of their ^1H NMR spectra (COSY and nOe experiments). Further synthetic and SAR studies in this research area are ongoing in our laboratories. Other workers in this area recently reporting analogues of these complex, hexacyclic norditerpenoid alkaloids include the research groups of: Wiesner et al (1978), van der Baan et al (1992) Kraus et al (1993), and Whiting et al (1994)

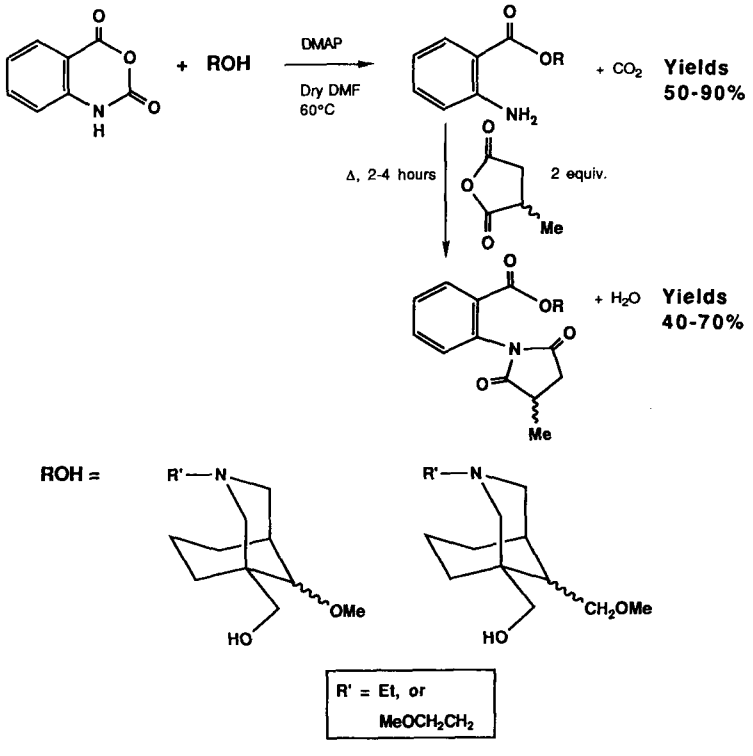

FIG. 3. General procedure for the synthesis of 2-(*RS*)-methylsuccinyimidobenzoates.

MLA (**1**) (free base) isolated in our studies, displayed comparable activity to authentic MLA citrate at both [^{125}I]α-bungarotoxin sites and [^{3}H]nicotine sites ($K_i = 2{\cdot}5 \pm 0{\cdot}9 \times 10^{-9}$ and $1{\cdot}3 \pm 0{\cdot}8 \times 10^{-5}$ M, respectively) (Coates et al 1994). Lycoctonine (**2**), produced by controlled hydrolysis of the ester in MLA, was four orders of magnitude less potent than MLA at the [^{125}I]α-bungarotoxin site ($K_i = 5{\cdot}0 \pm 1{\cdot}0 \times 10^{-5}$ M) (Coates et al 1994). Aconitine (**11**), a closely related neurotoxin from *Aconitum*, which interacts with sodium channels at the site characterized by batrachotoxin (Ward et al 1990), displayed poor activity ($K_i = 1{\cdot}9 \pm 0{\cdot}4 \times 10^{-5}$ and $>10^{-3}$ M, respectively). The striking difference in biological activity between MLA (**1**) and lycoctonine (**2**) however, indicates that the *N*-succinyl anthranilate ester moiety is significant.

MLA (**1**) is, therefore, a potent competitive nAChR antagonist and the first low molecular weight ligand able to discriminate between nAChR subtypes in vertebrates, preferring neuronal over neuromuscular. Our synthetic [3.3.1] bicyclic analogues of MLA, functionalized as their 2-methylsuccinimidobenzoate esters will allow us to test further the significance of the anthranilate moiety and the homocholine motif found around the AE bicycle of MLA (**1**).

Acknowledgements

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