COMMUNICATION

Investigations into nicotinic acetylcholine receptor (nAChR) antagonists: synthesis of a sub-unit of methyllycaconitine

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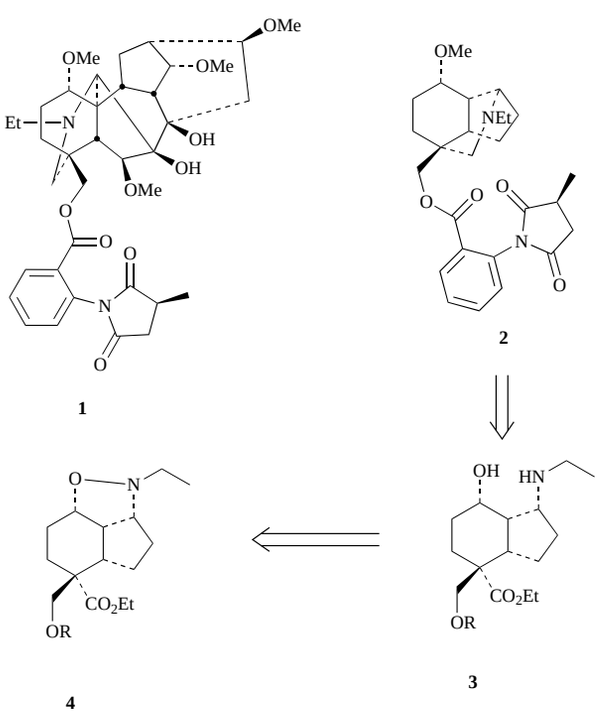
A potentially toxophoric subunit of methyllycaconitine has been synthesised from penta-1,4-dien-3-ol in 14 steps and 5% overall yield.

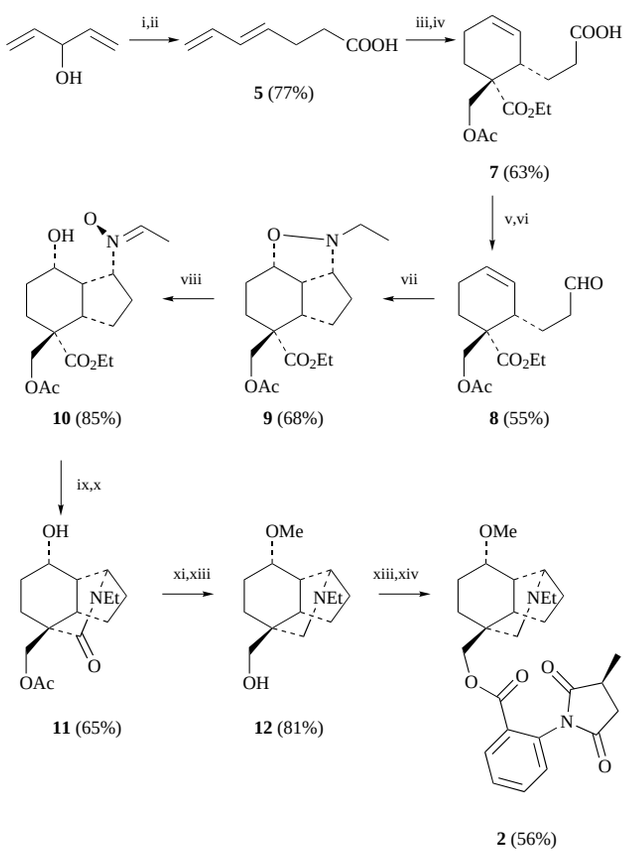
Methyllycaconitine (MLA) **1**, a C_{19} diterpenoid alkaloid, is found in the *Delphinium* and *Aconitum* species,[1,2] and has proved highly toxic to mammals and insects. In both cases, it acts on nicotinic acetylcholine receptors (nAChR) inhibiting neurotransmission and inducing paralysis[3] and it is reported to be the most potent non-protein competitive nAChR antagonist discovered to date.[4] MLA has recently acquired extensive use in distinguishing nAChR subtypes, which may be implicated in Alzheimer's disease,[5] and has been found to have high affinity for the α7 subtype;[6] it is more selective than α-bungarotoxin,[7] a polypeptide neurotoxin isolated from snake venom, but has comparable potency.



Scheme 1 Retrosynthesis

The high toxicity to animals of MLA disbars it as an agrochemical; however, if the inhibitory action is localised in a toxophoric section, a smaller subunit could find practical application if of significantly lower toxicity to mammals. To this end a number of methyllycaconitine analogues have been investigated. Structure–activity relationship investigations have shown that the methyl group on the succinimido ring[8] and the ethyl group of the tertiary amine[9] are important and this led us to investigate the synthesis of the A/E/F tricyclic fragment **2**. Previous work in this laboratory[10] led us to use a strategy based on the disconnection of the C–N bond to the *cis*-fused 6,5-bicarbocycle **3**, which could in turn be derived from the isoxazolidine **4** (Scheme 1).

Scheme 2 shows the forward synthesis. Penta-1,4-dien-3-ol



Scheme 2 *Reagents and conditions*: i, $MeC(OEt)_3$, $EtCO_2H$, 142 °C, 3 h; ii, KOH, MeOH; iii $CH_2C(CH_2OH)CO_2Et$ **6**, LiCl, aq $NaHCO_3$, 60 °C, 120 h; iv Ac_2O, pyr, 12 h; v, (*a*) $(Pr^i)_2NEt$, Bu^iOCOCl, DME; (*b*) $NaBH_4$; vi TPAP, NMO; vii, $EtNHOH{\cdot}CF_3CO_2H$, PhH, reflux, 3 h; viii, MCPBA, CH_2Cl_2, 0 °C; ix, PtO_2, H_2, 1 atm, 48 h; x, xylene, reflux, 24 h; xi, MeI, Ag_2O, reflux, 24 h; xii, $LiAlH_4$, dioxane, reflux, 12 h; xiii, isatoic anhydride, DMAP, DMF; xiv, (*S*)-(−)-methyl succinic anhydride, xylene

was heated with triethyl orthoacetate and a catalytic amount of propionic acid and the resulting ester was subjected to alkaline hydrolysis to afford the (*E*)-hepta-4,6-dienoic acid **5**.[11] Diels–Alder reaction of the sodium salt of the acid with 2 equivalents of the methacrylate **6**[12] in aq. 5 M lithium chloride[13] yielded the

cyclohexene acid as 3.3 : 1 mixture of stereoisomers (*endo* : *exo*).† Much experimentation was required to discover the most suitable substituents, dieneophile, and reaction conditions to minimise polymerisation. Acetylation followed by separation of the diastereomers gave the desired *endo* acid **7**. The acid was converted into the corresponding alcohol in a one-pot procedure whereby the acid was first converted to the mixed anhydride with diisopropylethylamine and isobutyl chloroformate and then reduced with sodium borohydride.[14] Oxidation of the alcohol with TPAP [15] gave the aldehyde **8**, which was heated to reflux with *N*-ethylhydroxylamine in benzene to provide the isoxazolidine **9**. All attempts to cleave the N–O bond reductively were, to our surprise, unsuccessful. Thus we deployed a two-step procedure in which the isoxazolidine was oxidised to the *N*-oxide with MCPBA; a spontaneous elimination/ring-opening resulted in formation of the nitrone **10**. This was reduced to the amine by catalytic hydrogenation over platinum. Subsequent reactions showed that the stereochemical integrity of the C–N bond was retained throughout this sequence. Ring-closure to the amide **11** was effected by heating to reflux in xylene for 24 h. *O*-Methylation of the alcohol functionality was achieved by refluxing with methyl iodide in the presence of silver(I) oxide: performing the *O*-methylation step before the reduction of the amide circumvented problems which arose in the model system in which the quaternary amine salt was formed as a significant side product.[10] Reduction of the amide to the amine **12**, with concomitant removal of the acetyl protecting group was effected with $LiAlH_4$. Reaction of the alcohol functionality with isatoic anhydride gave the anthranilate. The succinimido ring was incorporated by refluxing this intermediate with (*S*)-methyl succinic anhydride in xylene to give the desired A/E/F tricyclic fragment **2** as a mixture of diastereomers.‡

The stereochemistry generated by the Diels–Alder reaction and the 1,3-dipolar addition was confirmed by X-ray crystallographic studies on the nitrone. In addition the heterocyclic ring closure to the amide **11** would have not been possible if the relative stereochemistry of the amine and the ester functionalities had been incorrect.

Thus starting from the commercially available penta-1,4-dien-3-ol, we have synthesised the desired A/E/F tricycle **2** in 14 steps and 5% overall yield. This ester models the A/E/F tricyclic system of methyllycaconitine and contains 6 stereogenic centres. Biological testing of this compound is currently underway.

† To a solution of (*E*)-hepta-4,6-dienoic acid **5** (9.86 g, 78 mmol) in water (30 ml) at 25 °C was added sodium hydrogen carbonate (6.57 g, 78 mmol) portionwise over 30 min. After gas evolution had subsided, ethyl 2-(hydroxymethyl)acrylate **6** (20.34 g, 156 mmol) and lithium chloride (6.14 g, 145 mmol) were added. The mixture was warmed to 60 °C and stirred for 120 h. The mixture was extracted with diethyl ether to remove unreacted methacrylate, then acidified to pH3 with 2 M aqueous hydrochloric acid and extracted into diethyl ether. The extracts were washed with brine, dried and concentrated. The product was subjected to column chromatography (light petrol–ethyl acetate, 7 : 3) to yield the desired compound as a colourless oil (18.16 g, 91%) (*endo* : *exo* 3.3 : 1, as determined by NMR spectroscopy).

‡ All new compounds gave satisfactory spectroscopic and analytical data.

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