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Rapid and Efficient Isolation of the Nicotinic Receptor Antagonist Methyllycaconitine from *Delphinium*: Assignment of the Methylsuccinimide Absolute Stereochemistry as *S*

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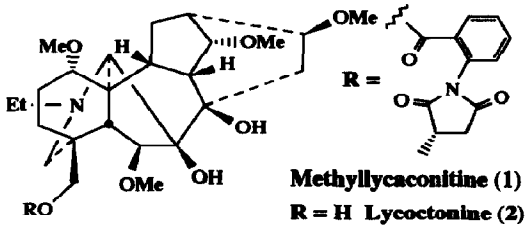
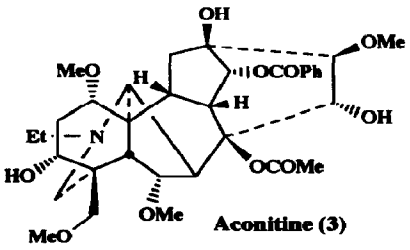
Abstract: Methyllycaconitine (MLA) has been isolated from Garden Hybrid *Delphinium* and purified by vacuum liquid chromatography. ^{13}C NMR and optical rotation have been used to characterize the absolute configuration of the methylsuccinimide moiety as *S*. Ligand binding assays confirmed the potency of MLA and its selectivity for α-bungarotoxin-sensitive neuronal nicotinic acetylcholine receptors.

There is a long history of the use of *Aconitum* and *Delphinium* by various civilizations as sources of poisons and medicines; probably the earliest is the treatment of lice reported by Pliny the Elder.[1] In addition, these plants are held responsible for more cattle deaths in North America than any other poisonous plant.[2] In 1938, Manske examined the aerial portion of *Delphinium brownii* Rydberg and established one of the alkaloids to be methyllycaconitine (MLA) (**1**), the (-)-*N*-(*o*-carboxyphenyl)methylsuccinimide ester of the norditerpenoid alcohol lycoctonine (**2**).[3] MLA (**1**) has been reported in at least 30 *Delphinium* species and also in *Consolida ambigua* and *Inula royaleana*.[4] MLA (**1**) is known to be the principal toxic alkaloid, in these species, and to produce mortality in a broad spectrum of insects.[5] Both its insecticidal action and its toxicity are believed to be a result of nicotinic acetylcholine receptor (nAChR) antagonism and (**1**), at one subset of nAChR, is the most potent, small molecule, competitive antagonist yet reported.[6] Despite one of its trivial names, MLA (**1**) differs from aconitine (**3**) in many respects, and (**1**) has not been found in *Aconitum*.



Methyllycaconitine (1)
R = H Lycoctonine (2)



Aconitine (3)

Due to the high toxicity of norditerpenoid alkaloids[7], the recent use of (**1**) in Russian medicine for its "curariform" activity[8], and the possibilities afforded by (**1**) as a lead compound for the design of pest controlling agents, we require a convenient source of homogeneous (**1**) for structure-activity relationship (SAR) studies. We have therefore undertaken the characterization of (**1**) from a garden hybrid strain of *Delphinium*, closely related to the American cultivar, Pacific Giant, and to the species, *D. elatum*. In this *Letter*, we report a rapid and efficient isolation of the nAChR antagonist (**1**), its saponification to the parent alcohol lycoctonine (**2**), and the unequivocal characterization of the *S*-methylsuccinimide moiety by ^{13}C NMR spectroscopy and by optical rotation.

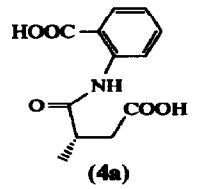
As Benn and his colleagues have consistently drawn[9,10,11], (**1**) apparently contains *S*-(-)-methylsuccinic acid; however, many authors have left the stereochemistry of the substituent on the succinimide as ambiguous. Indeed, as recently as 1989 and 1993, the chirality at this carbon centre has been left undefined and must therefore be supposed to be undefined or insecure.[12,13,14] Early work by Goodson[15] has shown that (-)-methylsuccinic acid is one of the hydrolysis products from (**1**), although the specific optical rotation found was small and no inference was made as to the stereochemistry of the carbon bearing this methyl group. Therefore, we undertook a proof of the configuration of this remaining chiral centre in natural MLA (**1**) to aid in our modelling of the nicotinic pharmacophore for more accurate interpretation of SAR data.

***Extraction of Garden Hybrid* Delphinium *seeds and the isolation of MLA* (1)**: Seeds of Garden Hybrid *Delphinium* (12 g) were ground and defatted with redistilled hexane (210 ml) in a soxhlet extractor (presoak of 21 h). The seeds were then extracted with redistilled chloroform (180 ml) (presoak of 21 h). Reducing the density of the seeds packed into the soxhlet thimble was found to improve the efficiency of the extraction. The extract was concentrated *in vacuo* (to 50 ml) and then extracted with aqueous sulfuric acid solution (0.75 M, 65 ml). The acidic layer was extracted with redistilled chloroform (2 x 50 ml), basified to pH 10 with saturated aqueous sodium carbonate solution and then extracted with diethyl ether (3 x 50 ml). Drying (Na_2SO_4) and evaporation *in vacuo* of the combined organic layers gave crude alkaloidal material as an off-white foam (147 mg, 1.22% weight of seeds taken); tlc on silica gel (5:4:1 cyclohexane-chloroform-diethylamine, detection by Dragendorff Munier spray) showed 3 main bands. The success of this pilot run was followed by a large scale seed extraction (600 g) with essentially equal efficiency.

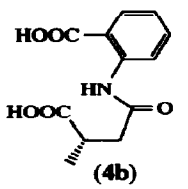
Purification of MLA by vacuum liquid chromatography: Crude alkaloidal material (992 mg), from a large scale seed extraction, was purified by vacuum liquid chromatography[16] over alumina. Elution was performed using a stepped gradient of mixtures of hexane, diethyl ether and methanol, in order of increasing polarity. Fractions were monitored by tlc on silica gel 60. Those fractions containing (**1**) as the sole alkaloid [R_f = 0.30, authentic MLA (**1**) R_f = 0.31, 5:4:1 cyclohexane-chloroform-diethylamine] were combined and evaporated *in vacuo* to yield pure MLA (**1**) (439 mg) homogeneous by tlc and NMR spectroscopy.

Determination of chirality of the methylsuccinimide moiety. Ester (**1**) was saponified with aqueous sodium hydroxide solution to afford lycoctonine (**2**) and the *N*-(methylsuccinyl)anthranilic acids, the half-acid amides (**4a**) and (**4b**), as a mixture of isomers. The diacid (**5**) was then obtained by acid catalysed hydrolysis of (**4a**) and (**4b**) with a little detectable racemization (*vide infra*). The bis-*l*-menthol ester (**6**) of natural (**5**) was prepared[17] and ^{13}C NMR spectroscopic analysis showed that the natural product was the *S*-enantiomer[18] as follows. Racemic-(**5**) was converted into its bis-*l*-menthol ester (**6**) and the methylene carbon of (**5**), i.e. α to the chiral carbon in the succinimide moiety, displayed two signals which could clearly be resolved at 67.8 MHz: *R* = 37.82 and *S* = 37.88 ppm (Δ = 0.06 ppm). The chiral carbon signal itself was not resolved into two signals (δ = 36.07 ppm). Diacid (**5**), obtained from (**1**), was converted into (**6**) and displayed peaks at 37.92 and 37.85 ppm (intensity ratio approx. 14:1 respectively).

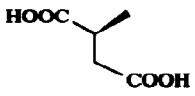
Itaconic acid was hydrogenated in the presence of a $RhCl_3$-BPPM chiral catalyst[19] to afford *S*-(**5**) which was converted into (**6**) whose ^{13}C NMR spectrum displayed 37.94 ppm for the key methylene carbon chemical shift. Inspection of this spectrum, after dilution with one molar equivalent of the bis-*l*-menthol ester (**6**) of racemic (**5**), revealed an additional signal at 37.90 ppm with approximately half the intensity of the higher frequency signal (37.98 ppm; Δ = 0.08 ppm). Additional confirmation of the *S*-configuration at this centre in MLA (**1**) came from the optical rotation of (**5**) obtained from (**1**) *via* (**4a**) and (**4b**). This rotation is small in water ($[\alpha]_D$ = -8.8°, c = 2)[15], but a little larger in ethanol ($[\alpha]_D$ = -15.0°, c = 1.89).[20] Synthetic *S*-(**5**) displayed $[\alpha]_D$ = -14.7° (c = 3.2, EtOH, 25°C). This ^{13}C NMR spectroscopic procedure will be applicable to the analysis of other methylsuccinimides or anhydrides, including half-ester amides and bis-amides.



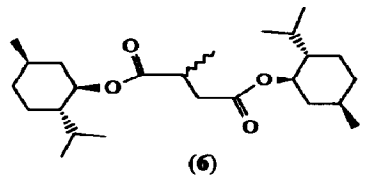
(**4a**)



(**4b**)



(**5**)



(**6**)

Biological activity of purified MLA: The purified MLA (**1**) was assessed for potency at nAChR in ligand binding assays on rat brain membranes.[21] The nAChR subtype identified by [^{125}I]-α-bungarotoxin labelling showed high affinity for (**1**) which inhibited [^{125}I]-α-bungarotoxin binding with a K_i of 3 nM. In contrast, [^{3}H]-nicotine-labelled nAChR bound (**1**) with a K_i of 13 μM. These values agree closely with those previously determined for the citrate salt of (**1**)[22] and confirm the exquisite selectivity of (**1**) for neuronal α-bungarotoxin-sensitive nAChR. The parent alcohol (**2**) exhibited little neuronal blocking action (K_i = 5 μM at [^{125}I]-α-bungarotoxin-labelled nAChR), indicating that MLA's aromatic ester function is a significant haptophore. At [^{3}H]-nicotine labelled nAChR, (**2**) failed to inhibit binding at concentrations up to 1 mM. Therefore, we are investigating the SAR of these alkaloids and, in particular, we are determining the importance of the unusual acyl moiety with respect to potency and selectivity for nAChR subtypes.[23] MLA (**1**) and its many synthetic analogues are useful pharmacological tools as probes for interactions at nAChR.

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