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Acylation of Lycoctonine: Semi-Synthesis of Inuline, Delsemine Analogues and Methyllycaconitine

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Abstract: Lycoctonine has been acylated to afford sequentially inuline, delsemine analogues and methyllycaconitine using isatoic anhydride followed by *S*-(-)-methylsuccinic anhydride. This protocol is a rapid, facile method for the regiospecific introduction of the anthranilate ester moiety found in potent nicotinic acetylcholine receptor antagonists.

Lycoctonine (**1**) is an important hexacyclic norditerpenoid alkaloid which contains a primary alcohol at C-18 and tertiary alcohols at C-7 and C-8.[1] Modified anthranilate esters of (**1**) at C-18 are highly potent pharmacological agents; in particular, MLA (**2**) is the principal toxic alkaloid of several species of *Delphinium* and produces mortality in cattle[2] as well as in a broad spectrum of insects.[3] Both its insecticidal action and its toxicity are believed to be a result of nicotinic acetylcholine receptor (nAChR) antagonism and, at one subset of nAChR, (**2**) is the most potent, small molecule, competitive nAChR antagonist yet reported.[4] Several research groups are actively studying the structure-activity relationships (SAR) of these alkaloids as probes for selective nAChR subtypes. Esters of (**1**) have been prepared[5], and *N*-deacetyllappaconitine has been acylated on the aniline nitrogen atom with a variety of aliphatic and aromatic carboxylic acids[6] in order to increase the lipophilicity of these potential insecticides.[3] There is no literature precedent for the facile introduction of the natural *S*-anthranoylmethylsuccinimide moiety. Acylation of (**1**) will allow access to the esters inuline (**3**), benzoyllycoctonine (**4**), delsemine (**5a** and **5b**)[7], and the half-ester amides (**6a** and **6b**). The latter may be natural products *per se* or might be artefacts of alcoholysis of (**2**) on isolation.[8]

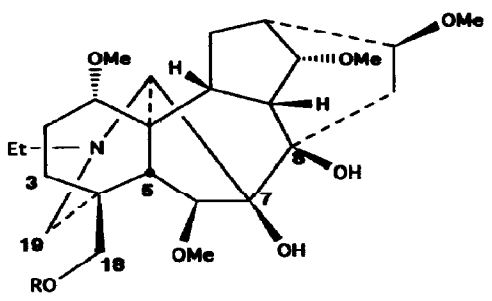
The synthesis of (**3**) can be envisaged by acylation of (**1**)[9], and (**2**) can be derived from (**3**) by formation of the substituted succinimide. In this *Letter*, we report a rapid, facile semi-synthesis of MLA (**2**), via inuline (**3**) and delsemine analogues (**7a** and **7b**) by regiospecific acylation of (**1**). These protocols for the introduction of the anthranilate ester and the subsequent addition of the homochiral succinimide moiety are applicable to the synthesis of related norditerpenoids esterified at C-18 e.g. anhweidelphinine and nudicauline.

The primary alcohol of (**1**) at C-18 should acylate preferentially over the tertiary alcohols at C-7 and C-8. However, it is important to recognize that this nucleophilic alcohol is associated with a neopentyl-like motif and this bulky substituent will affect esterification. We therefore undertook preliminary experiments with neopentyl alcohol (**8**) in order to determine the conditions for the introduction of the anthranilate ester on a milligram scale, suitable for the manipulation of precious natural products. We anticipated that there could be problems attempting to acylate with anthranilic acid, and also with bulky *N*-protecting groups typically used in amino acid chemistry (e.g. tBOC or CBZ) in the *ortho* position of the phenyl ring. These problems can be circumvented by the use of isatoic anhydride which serves to introduce the anthranilate ester with the loss of one equivalent of carbon dioxide. Thus, treatment of (**8**) with (**9**), catalysed by 4-dimethylamino-pyridine (DMAP), in DMF, afforded the anthranilate (**10**) with no trace of the isatoate (**11**) (90°C, 5 h, 64%).[10,11] The C-4-(C-3, C-5, C-19)-C-18 moiety of (**1**) has been mimicked by (**8**) in this successful esterification. Secondary alcohols are sluggish to react with (**9**) and tertiary alcohols are unreactive.[10,11]

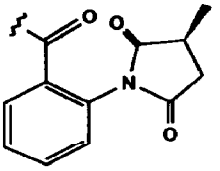
The methylsuccinimide moiety was then introduced by fusion of (**10**) with an excess of neat racemic methylsuccinic anhydride (120°C, 24 h, 79%). Inspection of the ^{1}H NMR spectrum of (**12**) revealed a broad signal for the methyl group (1.46 ppm, half peak height width = 18 Hz), and therefore rotation about the aromatic carbon-nitrogen bond is slow. Further support for this interpretation comes from an inspection of CPK models where the carbonyls on the heterocycle are not free to rotate past the aromatic ester carbonyl functional group. Maleimide (**13**) was prepared (25%) by the fusion of (**10**) with maleic anhydride. This ring closure reaction proceeds via the half-acid amide. Indeed, when succinic anhydride was reacted with (**10**) only (**14**) and not the ring closed product could be isolated. The slow ring-closure step (dehydration) can be accelerated by the addition of carbonyl diimidazole (CDI) (1.2 equiv). The formation of (**12**) and succinimide (**15**) can now be performed from the corresponding anhydride in the presence of CDI, 23 h at 25°C in dichloromethane. In these experiments, racemic methylsuccinic anhydride was used, but, in the synthesis of MLA (**2**), the *S*-enantiomer of methylsuccinic anhydride has been incorporated.[9]

S-(-)-Methylsuccinic acid (**17**) was prepared by hydrogenation of itaconic acid (**16**) in the presence of a $RhCl_3$-BPPM-*S*-methylbenzylamine catalyst[12] (72% yield, >90% ee based on *R*-enantiomer $[\alpha]_D$ = +15.5°).[12] Diacid (**17**) was then converted into anhydride (**18**), $[\alpha]_D$ = -36.5° (c = 3.5, dioxan), with acetyl chloride at 25°C.[13] Lycoctonine (**1**) was esterified with (**9**) catalysed by DMAP, in DMF, 27 h at 70°C, which gave inuline (**3**) (21%). This is the first semi-synthesis of this natural product.[14]

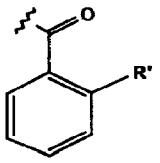
The half-acid amides (**7a**) and (**7b**) were synthesized, but not isolated, by treatment of (**3**) with (**18**) in dichloromethane (28 h at 25°C). The regioisomers (**7a**) and (**7b**) could not be separated by tlc on silica gel 60 using 3:7 methanol-dichloromethane (R_f = 0.8). Closure of the succinimide was achieved by reacting the mixture (**7a**) and (**7b**) with CDI, in dichloromethane, 48 h at 25°C, which gave (**2**), 55% from (**3**), identical in all chromatographic and spectroscopic respects with the natural product.[9,15,16] Thus, the semi-synthesis of MLA (**2**), *via* inuline (**3**), has been achieved by regiospecific acylation of lycoctonine (**1**).



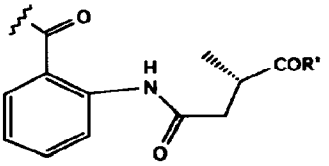
R = H Lycoctonine (1)
(Delsine, Royaline)



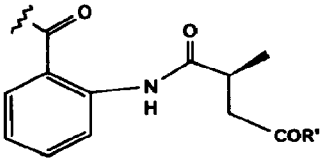
Methyllycaconitine (MLA) (2)
(Delartine, Delsemidine, Mellictine)



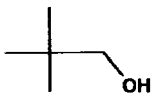
R' = NH_2 Inuline (3)
(Anthranoyllycoctonine)
R' = H Benzoyllycoctonine (4)



R' = NH_2 Delsemine (5a)
R' = OMe/OEt (6a)
R' = OH (7a)



R' = NH_2 Delsemine (5b)
R' = OMe/OEt (6b)
R' = OH (7b)



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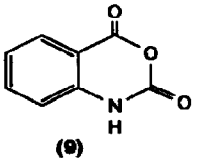
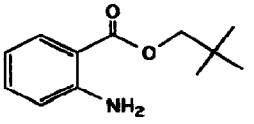
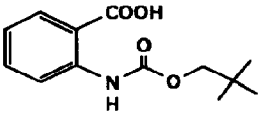


(9)



(10)



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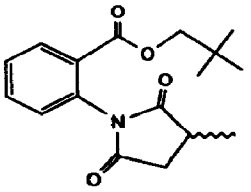

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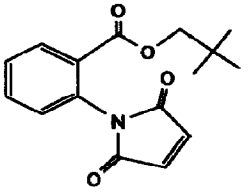

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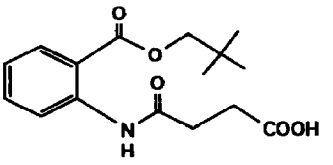
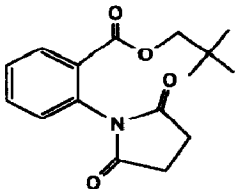


(14)



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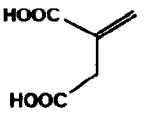

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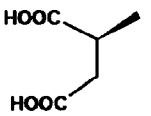
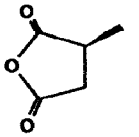


(17)



(18)

In ligand binding assays for rat brain nAChR, (**2**) has markedly higher affinity for neuronal α-bungarotoxin binding sites (K_i = 3 nM), i.e. the α7 subtype, than for any other nAChR subtype.[4] We are therefore investigating the SAR of these alkaloids and we are determining the importance of the unusual acyl moiety with respect to selectivity in binding amongst nAChR subtypes. Lycoctonine (**1**) displays low activity at housefly head nAChR (K_i = 380 nM)[3,17] and synthetic delsemine, as a mixture (**5a**) and (**5b**) from the aminolysis of (**2**), is essentially equipotent with (**2**) at a frog extensor-muscle preparation.[18] MLA (**2**) and its synthetic analogues[19,20] are therefore useful pharmacological tools.

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