Lycaconitine Revisited: Partial Synthesis and Neuronal Nicotinic Acetylcholine Receptor Affinities

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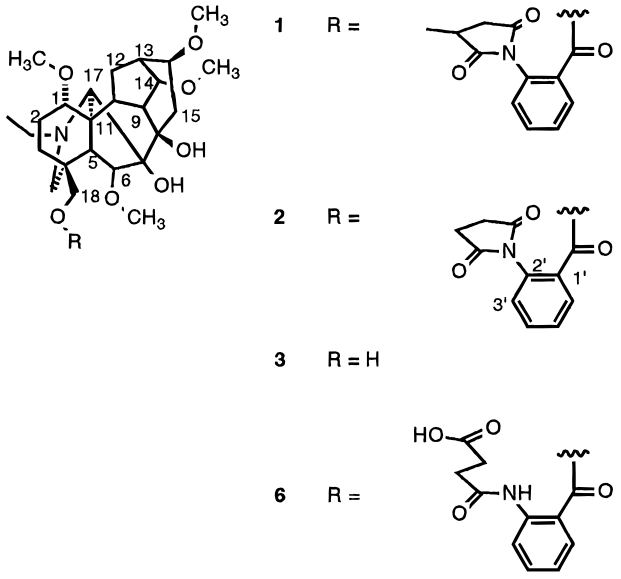
The norditerpenoid alkaloid lycaconitine (**2**) was synthesized from lycoctonine (**3**) and its affinity determined for two neuronal nicotinic acetylcholine receptor subtypes. The structure of **2** was confirmed by a combination of spectroscopic methods.

The norditerpenoid alkaloid, methyllycaconitine (MLA) (**1**), one of the principal toxins of *Delphinium* species ("larkspurs"; Ranunculaceae),[1,2] has recently found extensive use as a ligand for distinguishing neuronal nicotinic acetylcholine receptor (nAChR) subtypes.[3] Such receptors are currently the focus of considerable pharmaceutical interest because of their involvement in Alzheimer's disease and various other aspects of cognitive function.[4] MLA (**1**) displays particularly high affinity for nAChRs of the α7 type and is remarkable in being of comparable potency to, but more selective than, the polypeptide snake neurotoxin α-bungarotoxin.[5]

In continuation of our efforts to elucidate structure−activity relationships in analogues of **1**,[1,2] we turned our attention to lycaconitine (**2**), an alkaloid differing from **1** only in the absence of the methyl substituent from the succinimide ring. Although isolations of **2** from several species of *Aconitum* and *Delphinium* have been reported, there have been virtually no published reports of modern pharmacological investigations on an authentic sample of this compound (*vide infra*), and there are conflicting sets of spectroscopic data in the literature.[6−8]

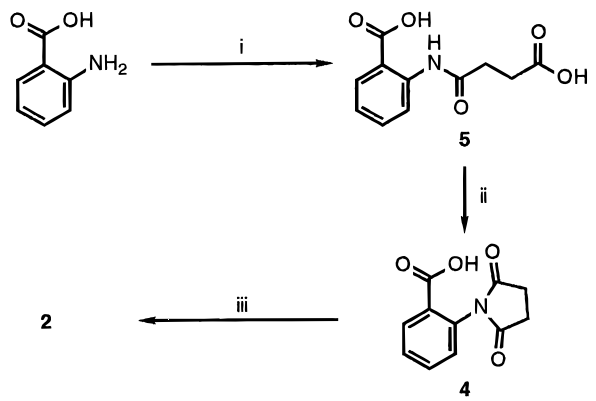
In order to obtain a sample of **2** for biological evaluation and to resolve the spectroscopic inconsistencies, we developed a partial synthesis of **2** from its parent amino alcohol, lycoctonine (**3**). This paper describes the synthesis of **2**, preliminary nAChR-binding data and the complete ^{1}H and ^{13}C NMR assignments for **2**.

Lycoctonine (**3**) was obtained by chromatography of the mixture of diterpenoid amino alcohols produced upon basic hydrolysis of the total alkaloid extract from *D. glaucum* (formerly *D. brownii*).[9] Lycaconitine (**2**) was then prepared by the esterification of **3** with *o*-(*N*-succinimido)benzoic acid (**4**), using *p*-toluenesulfonyl chloride in pyridine (Scheme 1). The imido acid **4**, although mentioned in the literature[7,10,11] and trivially named "lycoctoninsäure",[11] has never been adequately characterized. A commercial sample of **4**[12] proved to be the ring-opened amido diacid **5**. Anthranilic acid and succinic anhydride condense readily to form **5**, which may be isolated or cyclized directly to the imide **4**. It should be noted that **4** hydrolyzes readily in acidic solution and slowly on standing in the solid state.



The IR and ^{1}H 1D NMR spectra of our synthetic sample of **2** were in good agreement with the spectroscopic data for the naturally-occurring alkaloid reported by Sakai et al.[8] and by Yu and Das.[6] However, the IR

Scheme 1[a]



[a] Key: (i) $CHCl_3$, succinic anhydride, Δ; (ii) toluene, NEt_3, Δ; (iii) lycoctonine (**3**), *p*-TsCl, pyridine.

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Table 1. Nicotinic Acetylcholine Receptor Binding Properties of Methyllycaconitine (**1**) and Lycaconitine (**2**) in Rat Brain Tissue

	$\sim K_i$ (M)	
	[^{3}H]cytisine	[^{125}I]-α-bungarotoxin
methyllycaconitine (**1**)	4×10^{-7} ($n = 3$)	4×10^{-9} ($n = 3$)
lycaconitine (**2**)	1×10^{-6} ($n = 3$)	8×10^{-8} ($n = 3$)

and ^{1}H NMR spectra reported by Shamma et al.[7] for "lycaconitine" isolated from *D. cashmirianum* are different and are clearly more consistent with a compound in which the succinimide ring has been opened. In particular, the chemical shift pattern of the four aromatic protons in **2** (d, t, t, d) is highly characteristic of *o*-(*N*-succinimido)benzoates (e.g., the imido acid **4** and MLA (**1**)), in which the Ar-H3 resonance is shifted substantially upfield from its position in the corresponding acyclic compound. This effect is presumably due to a change in shielding as a result of the imide ring adopting a conformation that is orthogonal to the plane of the benzene ring. The aromatic proton chemical shifts (δ 8.47, d; 8.08, d; 7.58, t; 7.25, t) observed by Shamma et al.[7] are more appropriate for the hydrolysis product, puberaconitine (**6**), and are reasonably close to the shifts given for the latter compound by Yu and Das.[6]

Additional evidence for the presence of an imide ring in "authentic" **2** is provided by the IR spectrum: our sample exhibited a strong absorption at 1716 cm^{-1}, together with a weaker one at 1782 cm^{-1}, typical of *N*-aryl imides[13] and in good agreement with the values reported by Sakai et al.[8] On the other hand, the "lycaconitine" of Shamma et al.[7] was described as showing absorptions at 1720 and 1705 cm^{-1}.

The pharmacology or toxicology of **2** are virtually unstudied: an LD_{50} (mouse, iv) of ca. 15 mg/kg has been recorded by Sakai,[14] indicating that this alkaloid is somewhat less acutely toxic than the closely-related **1** (LD_{50}, mouse, iv, ca. 3 mg/kg[1]). A limited investigation[7] of the cardiovascular effects of "lycaconitine" in animals was carried out with a compound whose identity is uncertain (*vide supra*).

Since we were interested in the effects of structural modifications to **1** on its activity in cholinergic systems, we compared the affinity of **2** with that of **1** in nAChR preparations from rat brain.[15−17] Our results are shown in Table 1. Thus, **2** was found to be moderate, with about one half of the potency of **1**, in its inhibition of the binding of [^{3}H]-(−)-cytisine (i.e., in its affinity for the α4β2 nAChR subtype); **2** displayed somewhat higher activity in the inhibition of binding of [^{125}I]-α-bungarotoxin (i.e., to the α7 nAChR subtype), but was still substantially less potent than **1**. The lower affinity of **2** for α4β2 and α7 receptors suggests that there may be a specific interaction between the methyl substituent on the imide of **1** and the receptor proteins. More extensive studies focusing on the relationship between structure and AChR binding properties of lycoctonine derivatives have been carried out and will be reported elsewhere.

Experimental Section

General Experimental Procedures. NMR spectra were obtained using a Bruker AM-400 NMR spectrometer equipped with a 5 mm ^{13}C/^{1}H sample probe on samples dissolved in $CDCl_3$ or CD_3COCD_3. 1D-DEPT, 2D COSY, TOCSY, HETCOR, and COLOC spectra were acquired by means of standard pulse sequences; 1D-selective-INEPT spectra were acquired using the pulse sequence of Bax.[18] IR spectra were recorded on a Nicolet 205 FTIR spectrometer using a diffuse reflectance accessory. Mass spectra were obtained from a Finnigan TSQ7000 instrument in ESI or CI modes. Melting points were determined on a Fisher-Johns apparatus. TLC was carried out on Analtech silica gel G plates using the following solvent systems: toluene−EtOAc−88% HCO_2H, 5:4:1 (S1); cyclohexane−$CHCl_3$−NEt_3, 5:4:1 (S2).

Synthesis. Lycoctonine (3). This alkaloid was isolated from *D. glaucum* (*D. brownii*) as described previously.[9] Physical and spectroscopic properties for **3** were consistent with those in the literature,[19] but since NMR assignments for CD_3COCD_3 solutions of this alkaloid have not been previously published, they are given here: ^{1}H NMR (CD_3COCD_3, 400.1 MHz) δ 3.88 (1H, bs, J < 1 Hz, H-6), 3.58 (1H, m, H-18$_b$), 3.55 (1H, m, H-14), 3.46 (3H, s, OMe-6), 3.31 (1H, bdd, J = 11, 5 Hz, H-18$_a$), 3.28 (3H, s, OMe-14), 3.23 (3H, s, OMe-16), 3.22 (3H, s, OMe-1), 3.12 (1H, bt, J = 8 Hz, H-16), 3.02 (1H, dd, J = 6.7, 5.1 Hz, H-9), 2.96 (1H, dd, J = 9.9, 7.2 Hz, H-1), 2.87 (1H, m, $NCH_{2a}CH_3$), 2.84 (1H, bs, J < 1 Hz, H-17), 2.73 (1H, dd, J = 12.7, 7.0 Hz, $NCH_{2b}CH_3$), 2.55 (1H, d, J = 11.4 Hz, H-19$_b$), 2.51 (1H, m, H-15$_b$), 2.49 (1H, m, H-12$_b$), 2.29 (1H, dd, J = 11.5, 2.0 Hz, H-19$_a$), 2.23 (1H, dd, J = 7.2, 4.5 Hz, H-13), 2.16 (1H, m, H-2$_b$), 2.05 (1H, m, H-2$_a$), 1.94 (1H, m, H-10), 1.82 (1H, dd, J = 14.3, 7.4 Hz, H-12$_a$), 1.68 (1H, bs, J < 1 Hz, H-5), 1.66 (1H, m, H-3$_b$), 1.60 (1H, m, H-15$_a$), 1.52 (1H, m, H-3$_a$), 0.99 (3H, t, J = 7.2 Hz, NCH_2CH_3); ^{13}C NMR (CD_3COCD_3, 100.6 MHz) δ 91.6 (C-6), 89.2 (C-7), 84.9 (C-1), 84.8 (C-14), 83.9 (C-16), 78.0 (C-8), 67.7 (C-18), 65.3 (C-17), 58.1 (6-OCH_3), 57.3 (14-OCH_3), 55.8 (16-OCH_3), 55.6 (1-OCH_3), 53.8 (C-19), 51.4 (NCH_2CH_3), 51.0 (C-5), 49.6 (C-11), 47.1 (C-10), 44.0 (C-9), 39.2 (C-4), 38.9 (C-13), 34.7 (C-15), 32.4 (C-3), 29.5 (C-12), 27.1 (C-2), 14.3 (NCH_2CH_3).

Lycoctoni(ni)c Acid (5). A solution of anthranilic acid (1.37 g, 10 mM) in hot $CHCl_3$ (50 mL) was added to a stirred mixture of succinic anhydride (1.0 g, 10 mM) and hot $CHCl_3$ (60 mL). The reaction mixture was heated for 15 min on a steam bath and allowed to cool. Removal of solvent *in vacuo* yielded an off-white solid (2.4 g) that was recrystallized from aqueous EtOH, giving **5** as colorless needles: mp 181−2 °C (lit.[11] mp 179−180 °C); R_f 0.7 (S1); IR (KBr) ν 3150−2500, 1687, 1598, 1587, 1529, 1452, 1416, 1319, 1255, 1224, 1161 cm^{-1}; CIMS m/z 255 $(M + NH_4)^+$; ^{1}H NMR (CD_3COCD_3, 400.1 MHz) δ 11.28 (1H, br s), 8.72 (1H, dd, J = 8.5, 1.0 Hz, H-3), 8.10 (1H, dd, J = 8.0, 1.7 Hz, H-6), 7.59 (1H, ddd, J = 8.5, 7.3, 1.7 Hz, H-4), 7.14 (1H, bdt, $J \approx$ 7.7, 1.0 Hz, H-5), 2.72 (4H, AA′BB′m, $-CH_2CH_2-$); ^{13}C NMR (CD_3COCD_3, 100.6 MHz) δ 173.8 ($-CH_2CO_2-$)*, 171.1 ($-NHCO-$)*, 170.4 ($ArCO_2-$), 143.0 (C-2), 135.3 (C-4), 132.1 (C-6), 123.0 (C-5), 120.6 (C-3), 115.7 (C-1), 33.2 ($-CH_2CO_2H$), 29.1 ($-NHCOCH_2-$). *Assignments may be reversed.

***o*-(*N*-Succinimido)benzoic Acid (4).** A mixture of lycoctoninic acid (**5**) (0.95 g, 4 mM), toluene (150 mL), and NEt_3 (0.81 g, 8 mM) was refluxed under a Dean−Stark trap for 24 h. TLC of the toluene solution at this point showed complete conversion of starting material

to a single product. The toluene was evaporated *in vacuo* to give a brown oil. This was dissolved in CH_2Cl_2 (100 mL) and washed successively with 1 N HCl (2 × 25 mL), H_2O (2 × 25 mL), and brine (25 mL) and then dried over anhydrous $MgSO_4$. Removal of the solvent *in vacuo* gave an oil that was taken up in a small volume of CH_2Cl_2 and the solution kept at −15 °C for 2 h. The resulting crystals were filtered, washed with a small amount of cold $CHCl_3$, and dried under vacuum to yield **4** as small, colorless rhombs (150 mg): mp 179−180 °C (lit.[11] mp 180−181 °C); R_f 0.6 (S1); IR (KBr) ν 3300−2950, 1781 (w), 1728 (s), 1674, 1602, 1493, 1410, 1387, 1299, 1236, 1208, 1188, 1139, 1083 cm^{-1}; CIMS *m/z* 237 $(M + NH_4)^+$; ^1H NMR (CD_3COCD_3, 400.1 MHz) δ 8.12 (1H, dd, J = 7.8, 1.5 Hz, H-6), 7.71 (1H, dt, J = 7.8, 1.5 Hz, H-4), 7.57 (1H, dt, J = 7.8, 1.2 Hz, H-5), 7.32 (1H, dd, J = 7.8, 1.2 Hz, H-3), 2.83 (4H, AA′BB′ m, $-CH_2CH_2-$); ^{13}C NMR (CD_3COCD_3, 100.6 MHz) δ 177.2 ($NCO-$), 166.2 ($ArCO_2-$), 134.3 (C-2), 133.9 (C-4), 132.2 (C-6), 130.9 (C-3), 129.7 (C-5), 128.8 (C-1), 29.5 ($-CH_2CH_2-$) (atom numbering based on anthranilic acid).

Lycaconitine (2). To a stirred solution of the imido acid (**4**) (22 mg, ∼0.1 mM) in dry pyridine (0.5 mL) was added *p*-toluenesulfonyl chloride (38 mg, ∼0.2 mM). The mixture was cooled on ice, and to it was added lycoctonine (**3**) (47 mg, ∼0.1 mM). The reaction mixture was left to stand at 5 °C for 24 h, whereupon it was partitioned between H_2O (5 mL) and $CHCl_3$ (2.5 mL). The aqueous phase was extracted further with $CHCl_3$ (2 × 2.5 mL), and the combined $CHCl_3$ extracts were washed with H_2O (2 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* yielded a gum that was subsequently chromatographed. VLC on Si gel, eluting with cyclohexane, cyclohexane−$CHCl_3$, and cyclohexane−$CHCl_3$−NEt_3 mixtures, yielded **2** as a chromatographically homogeneous (R_f 0.3; S2) gum (43 mg): IR (KBr) ν 3469, 2940, 2821, 1782 (w), 1722 (s), 1716 (s), 1603, 1494, 1454, 1392, 1266, 1187, 1087, 818, 735, 706 cm^{-1}; ESIMS *m/z* 669 $(M + H)^+$; ^1H NMR (CD_3COCD_3, 400.1 MHz) δ 8.12 (1H, dd, J = 7.8, 1.3 Hz, H-6′), 7.74 (1H, dt, J = 7.8, 1.3 Hz, H-4′), 7.59 (1H, dt, J = 7.9, 1.0 Hz, H-5′), 7.35 (1H, dd, J = 7.8, 1.0 Hz, H-3′), 4.19, 4.11 (2H, ABq, J (obs) = 11.2 Hz, H-18_a, H-18b), 3.91 (1H, bs, J < 1 Hz, H-6), 3.56 (1H, bt, $J \approx$ 4.5 Hz, H-14), 3.40 (3H, s, OCH_3-6), 3.29 (3H, s, OCH_3-14), 3.26 (3H, s, OCH_3-1), 3.24 (3H, s, OCH_3-16), 3.14 (1H, bt, $J \approx$ 8.0 Hz, H-16), 3.04 (1H, m, H-9), 3.02 (1H, m, H-1), 2.94 (1H, m, $NCH_{2a}CH_3$), 2.93 (1H, s, H-17), 2.90 (4H, bm, $NCOCH_2CH_2-$), 2.79 (1H, m, $NCH_{2b}CH_3$), 2.75 (1H, d, J = 12 Hz, H-19_a), 2.56 (1H, d, J = 15 Hz, H-15_a), 2.53 (1H, m, H-12_a), 2.50 (1H, d, J = 12 Hz, H-19_b), 2.26 (1H, dd, J = 4.5, 7.3 Hz, H-13), 2.25 (1H, m, H-2_a), 2.10 (1H, m, H-2_b), 1.97 (1H, m, H-10), 1.85 (1H, dd, J = 7.3, 14.4 Hz, H-12_b), 1.79 (1H, m, H-3_a), 1.76 (1H, bs, J < 1 Hz, H-5), 1.62 (1H, dd, J = 7.5, 15 Hz, H-15_b), 1.62 (1H, m, H-3_b), 1.02 (3H, t, J = 7.2 Hz, (3H, t, J = 7.2 Hz, NCH_2CH_3); ^{13}C NMR (CD_3COCD_3, 100.6 MHz) δ 177.0 ($ArNCO-$), 177.0 ($ArNCO$), 165.3 ($ArCO_2-$), 134.3 (C-2′), 134.0 (C-4′), 131.6 (C-6′), 130.9 (C-3′), 129.9 (C-5′), 128.8 (C-1′), 91.8 (C-6), 89.4 (C-7), 84.9 (C-14), 84.5 (C-1), 83.9 (C-16), 78.0 (C-8), 70.5 (C-18), 65.2 (C-17), 58.7 (6-OCH_3), 57.5 (14-OCH_3), 56.0 (1-OCH_3)*, 55.8 (16-OCH_3)*, 53.3 (C-19), 51.7 (C-5), 51.4 (NCH_2CH_3), 49.8 (C-11), 46.9 (C-10), 44.1 (C-9), 38.9 (C-13), 38.3 (C-4), 35.1 (C-15), 32.6 (C-3), 29.5 ($-COCH_2CH_2CO$), 29.5 (C-12), 26.8 (C-2), 14.3 (NCH_2CH_3).

Since the majority of diterpenoid alkaloid NMR spectra have previously been determined in $CDCl_3$ solution,[19,20] spectroscopic data were also collected for **2** in $CDCl_3$ for comparative purposes and are as follows: ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ 176.5 ($ArNCO-$), 176.5 ($ArNCO$), 164.1 ($ArCO_2-$), 133.7 (C-4′), 132.8 (C-2′), 131.1 (C-6′), 130.0 (C-3′), 129.4 (C-5′), 126.8 (C-1′), 90.7 (C-6), 88.4 (C-7), 83.9 (C-1), 83.8 (C-14), 82.5 (C-16), 77.4 (C-8), 69.5 (C-18), 69.4 (C-17), 58.1 (6-OCH_3), 57.7 (14-OCH_3), 56.2 (1-OCH_3)*, 55.7 (16-OCH_3)*, 52.2 (C-19), 50.9 (NCH_2CH_3), 50.1 (C-5), 48.9 (C-11), 46.0 (C-10), 43.1 (C-9), 38.1 (C-13), 37.4 (C-4), 33.5 (C-15), 32.0 (C-3), 28.8 ($-COCH_2CH_2CO$), 28.6 (C-12), 26.0 (C-2), 14.0 (NCH_2CH_3). *Assignments may be reversed.

Bioassays. [^3H]Cytisine and [^{125}I]-α-bungarotoxin binding to rat brain membranes was determined using modifications of the methods of Pabreza et al.[16] and Marks et al.,[17] respectively, as described by Arneric et al.[15]

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