Syntheses of the Proposed Structures of Poison-Frog Alkaloids 179 and 207E and Their Inhibitory Effects on Neuronal Nicotinic Acetylcholine Receptors

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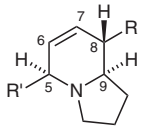
Abstract: Syntheses of the structures postulated for **179** and **207E**, members of a proposed new class of poison-frog alkaloids, 6,7-dehydro-5,8-disubstituted indolizidines, are described. The FT-IR spectrum and GC retention time of the synthetic **207E** were different from those of the natural product; consequently the original structure of **207E** needs to be revised. It is likely that the position of the double bond is instead at the 7,8-position.

Key words: poison-frog alkaloids **179** and **207E**, inhibitory effects on neuronal nicotinic acetylcholine receptors, dehydro-5,8-disubstituted indolizidines

The indolizidine ring is found in a great number of naturally occurring alkaloids, and many of these alkaloids show intriguing biological activities.[1] Many alkaloids containing a 3,5-disubstituted or 5,8-disubstituted indolizidine ring system have been detected in the skin extracts of poison frogs from neotropical regions.[2,3] Very recently, a new class of poison-frog alkaloids possessing a 6,7-dehydro-5,8-disubstituted indolizidine structure has been proposed, based upon mass and FT-IR spectral data[3] (Figure 1). None of these have been isolated for NMR spectral analysis so the position of the double bond was tentative. It now appears that the structures of many of these dehydro-indolizidines must be re-evaluated with respect to the position of the double bond.

As part of our program directed at studying the synthesis of biologically active alkaloids,[4] we report here the first chiral synthesis of the structures proposed for **179** (**1**) and **207E** (**2**), and the evaluations of their inhibitory effects on neuronal nicotinic acetylcholine receptors.



179: R = Me, R' = *n*-Pr (**1**)
207E: R, R' = *n*-Pr (**2**)

R = Me, CH_2OH, Et, *n*-Pr, *n*-Bu
R' = *n*-Pr, *n*-Bu, $CH_2CH{=}CHMe$, $(CH_2)_2CH{=}CH_2$

Figure 1 Proposed structures of 6,7-dehydro-5,8-disubstituted indolizidine poison-frog alkaloids, with representative side chains[2]

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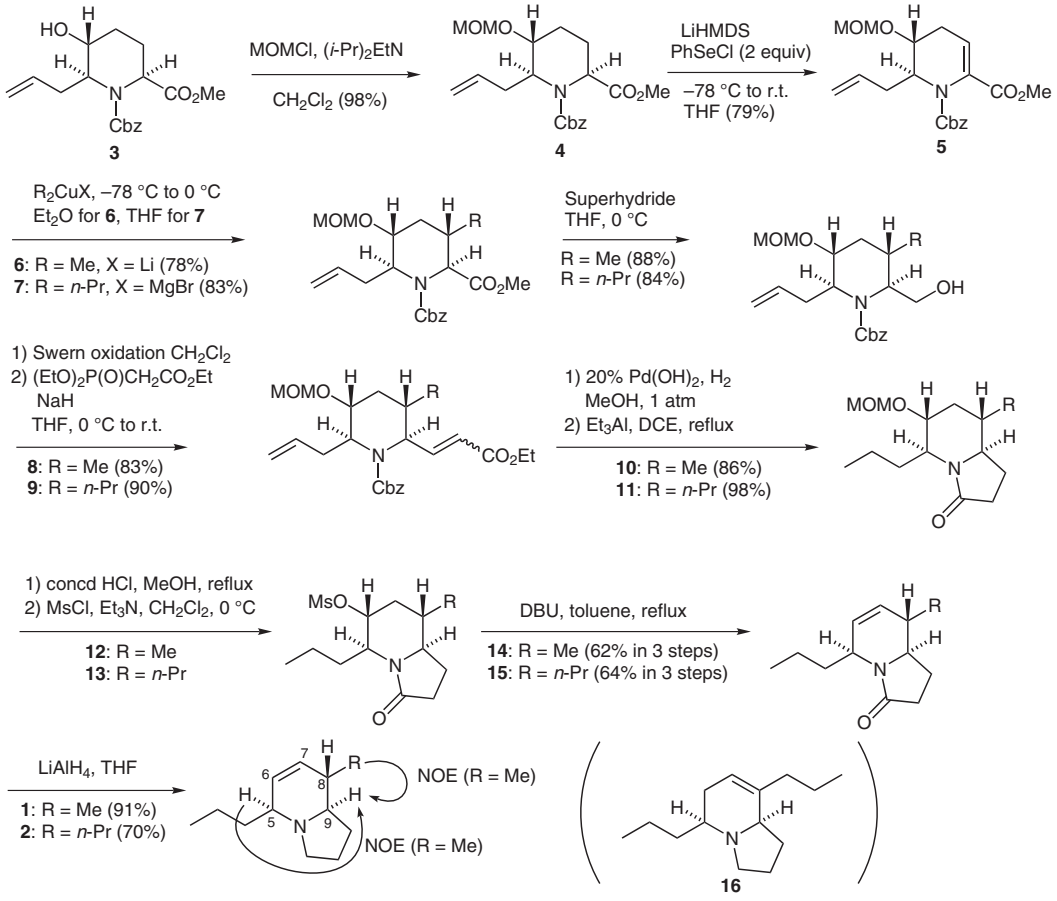
We used the chiral piperidinol **3**[5] as the starting material, and masked the hydroxy group as a MOM ether to afford **4**. Ether **4** was transformed into the enamino ester **5** using Rubio's procedure.[6] The key Michael-type conjugate addition of dimethylcuprate or dipropylcuprate to **5** proceeded smoothly giving rise to the adducts **6** or **7** in about 80% yield as a single isomer.[7] The stereochemistry of **6** was determined based upon NOE experiments on **1**. In difference NOE experiments, NOE enhancement (ca. 2.7%) was observed on the C-9 proton upon irradiation of the methyl protons at C-8, and on irradiation of the C-5 proton a weak but clear NOE was detected on the same C-9 proton, indicating the 5,9-*Z* and 8,9-*E* configurations of **6** as shown in Scheme 1. The stereoselectivity of this key conjugate addition reaction can be rationalized as follows: The conformation of **5** should prefer conformer **A** over that of **B** owing to relief of $A^{(1,3)}$ strain[8] between the urethane moiety and the allyl substituent at the α-position. The stereoelectronically preferred α-axial-attack[9] will occur on **A**, followed by protonation of the resulting enolate to afford adduct **6** or **7**. This selectivity can also be explained by Cieplak's hypothesis.[10] It is noteworthy that the stereochemical course of the attack of the anion is controlled by a stereoelectronic effect despite the severe 1,3-diaxial repulsion between the axial alkoxy substituent at the 3-position and the incoming anion[11] (Scheme 2).

With the key tetrasubstituted piperidines **6** and **7** in hand, we next turned our attention to their conversions into the lactams **10** and **11**. Reduction with Superhydride followed by Swern oxidation and the Horner–Emmons reaction provided the α,β-unsaturated esters **8** and **9**. These esters were hydrogenated over Pearlman's catalyst with concomitant cleavage of the Cbz group. Exposure of the resulting amino esters to Weinreb's reaction[12] formed lactams **10** and **11**. To complete the synthesis of **1** and **2**, the lactams **10**, **11** were converted into dehydropiperidines **14** and **15** using an E2-elimination reaction of the corresponding mesylates **12** and **13** with DBU. Finally, reduction of the lactam moiety in **14** and **15** with $LiAlH_4$ furnished **1**[13] and **2**[14] in 91% and 70% yield, respectively (Scheme 1).

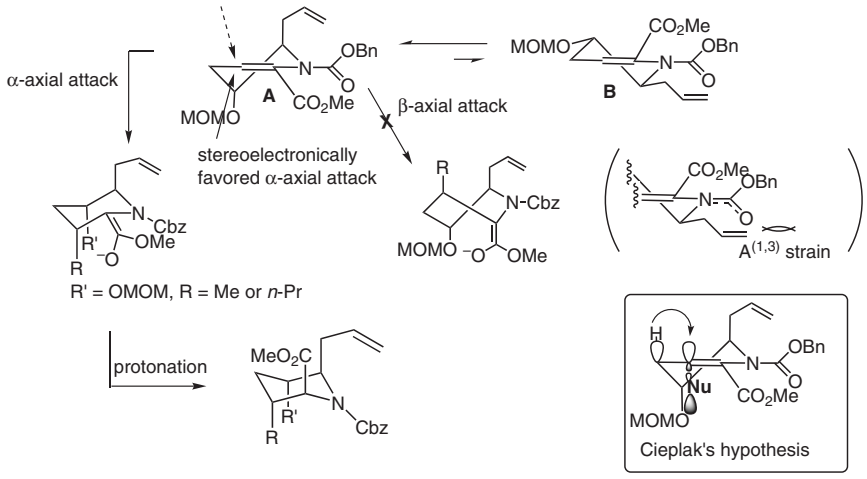
The GC-FTIR spectrum and GC retention time of synthetic **2** proved different from those of natural **207E**, detected in the skin extracts of *Oophaga granulifera*, although the



Scheme 1 Syntheses of structures proposed for the alkaloids **179** (**1**) and **207E** (**2**)

mass spectra were similar. In the GC-FTIR spectra, both synthetic **2** and the natural product showed a characteristic sharp and intense Bohlmann band near 2790 cm^{-1} indicating the 5,9-*Z* configuration in both compounds. Synthetic **2** had an absorption band at 3030 cm^{-1} indicating the vinyl C–H stretching. However, natural alkaloid **207E** showed only weak vinyl C–H stretching and also no enamine absorption. These results strongly indicate that the revised structure of indolizidine **207E** would have a 7,8-double bond (**16**). The synthesis of **16** is in progress.

Electrophysiological experiments were used to examine the effect of the synthetic materials **1** and **2** on nicotinic receptors expressed in *Xenopus laevis* oocytes using the same method as described previously.[15] After pre-incubation with the synthesized indolizidine for 3 minutes, current was elicited by 5 seconds application of acetylcholine (100 μM for α7, 1 μM for α4β2) in combination with the



Scheme 2 Stereochemical course of the Michael-type conjugate addition reaction of enamino ester **5**

indolizidines. Indolizidine **1** (30 μM) reduced the peak amplitude of the α7-nicotinic-receptor-mediated current more effectively than the α4β2-receptor-mediated current (Figure 2, A). When the concentration–inhibition curves were compared between these receptor subtypes, indolizidine **1** blocked the α7-nicotinic-receptor-mediated current [50% inhibitory concentration (IC_{50}) = 6.9 μM, 95% confidence intervals (CI): 5.6–8.5 μM] with 4.0-fold higher sensitivity than the blockade of α4β2-receptor-mediated current (IC_{50} = 26.6 μM, 95% CI: 15.8–44.8 μM; Figure 2, B). The indolizidine (**2**) blocked α7-nicotinic-receptor responses (IC_{50} = 6.5 μM, 95% CI: 4.2–9.9 μM) with >5.0-fold higher sensitivity than the blockade of the α4β2-receptor responses (IC_{50} >30.0 μM; Figure 2, C and D). The blocking effects of **1** and **2** at 30 μM on α7- and α4β2-nicotinic receptors recovered within 10 minutes after removal of the alkaloids (data not shown).

In this study, we investigated for the first time the pharmacological effects of 6,7-dehydro-5,8-disubstituted indolizidines on nicotinic receptors. The compounds **1** and **2** produced a selective inhibition of α7-nicotinic receptors over α4β2 receptors. These results suggest that the structures of 6,7-dehydro-5,8-disubstituted indolizidines have some determinant moiety for selective interaction with α7-nicotinic receptors. Further studies with other analogues are required to identify the moiety.

In conclusion, we achieved the first synthesis of the proposed structure for dehydro-indolizidines **179** and **207E**. Comparison of synthetic **2** and natural **207E** on GC and GC-FTIR revealed that the proposed structure for **207E** should be revised. The dehydro-indolizidine **179** could no longer be found in skin extracts and an IR had not been obtained, so no comparisons with synthetic **1** were possible. Furthermore, we conducted for the first time a study of the pharmacological effects of the 6,7-dehydro-5,8-disubstituted type of indolizidines on nicotinic receptors, and these indolizidines showed selective inhibition of α7-nicotinic receptors over α4β2 receptors. For the confirmation of the structure of natural **207E**, the synthesis of the 7,8-dehydro isomer **16** of **2** is now under investigation, and will be reported.

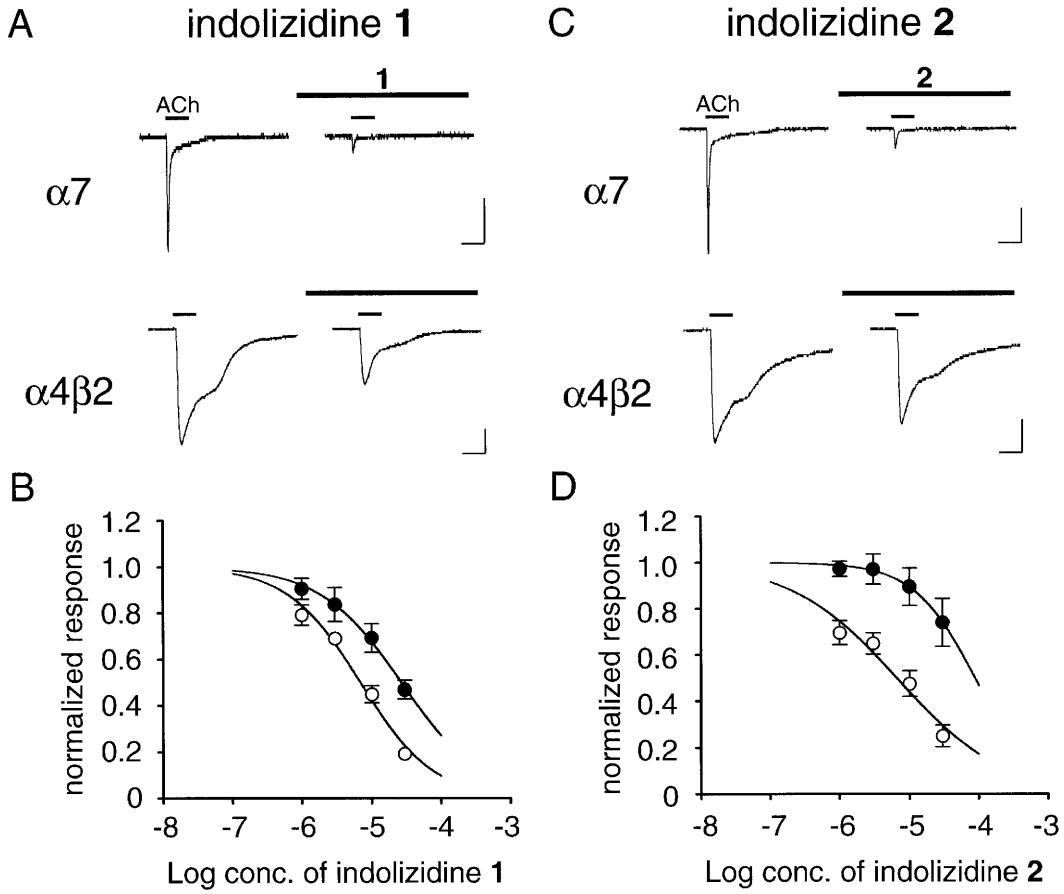


Figure 2 Inhibitory effects of 6,7-dehydro-5,8-disubstituted indolizidines on acetylcholine (ACh)-induced current in *Xenopus laevis* oocytes expressing α7- or α4β2-nicotinic receptors. Current was recorded in voltage-clamp mode at –60 mV. A and C, typical traces showing inhibition by (A) indolizidine **1** or (C) indolizidine **2** at 30 μM of α7 current elicited by 100 μM ACh and α4β2 current elicited by 1 μM ACh. Horizontal bars indicate the period of perfusion with ACh for 5 s. Vertical scale bars represent 200 nA. B and D, concentration–inhibition curves for (B) indolizidine **1** and (D) indolizidine **2** on α7- (○) and α4β2-nicotinic receptors (●). Peak current responses to ACh in the presence of the alkaloid in each oocyte were normalized to the ACh responses (control responses) recorded in the same oocytes. Values represent the mean ± SEM of five separate experiments.

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(13) The spectral and analytical data of synthetic **1** are as follows: IR (neat): 3025, 2957, 2871, 2781, 1456, 1377, 1326, 1287, 1179, 913, 798, 715 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.93 (3 H, t, *J* = 7.5 Hz), 0.94 (3 H, d, *J* = 6.8 Hz), 1.25–1.34 (1 H, m), 1.35–1.41 (1 H, m), 1.42–1.52 (2 H, m), 1.61–1.67 (1 H, m), 1.68–1.75 (1 H, m), 1.76–1.83 (1 H, m), 1.84 (1 H, td, *J* = 9.1, 6.9 Hz), 1.99–2.07 (2 H, m), 2.08–2.14 (1 H, m), 2.64 (1 H, dtd, *J* = 11.0, 3.6, 1.9 Hz), 3.35 (1 H, td, *J* = 8.4, 1.9 Hz), 5.50 (1 H, dt, *J* = 10.2, 1.9 Hz), 5.57 (1 H, dt, *J* = 10.2, 1.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.39 (q), 18.14 (q), 18.64 (t), 20.77 (t), 29.38 (t), 36.04 (t), 37.61 (d), 52.79 (t), 63.00 (d), 67.83 (d), 128.55 (d), 131.53 (d). HRMS: *m/z* calcd for $C_{12}H_{21}N$: 179.1674; found: 179.1686. $[\alpha]_D^{26}$ +129.3 (*c* 2.74, $CHCl_3$).
(14) The spectral and analytical data of synthetic **2** are as follows: IR (neat): 3030, 2957, 2929, 2871, 2781, 1458, 1378, 1329, 1260, 1177, 1105, 929, 803, 713 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.89 (3 H, t, *J* = 6.8 Hz), 0.91 (3 H, t, *J* = 7.3 Hz), 1.12–1.21 (1 H, m), 1.23–1.34 (2 H, m), 1.35–1.40 (2 H, m), 1.43–1.53 (3 H, m), 1.59–1.68 (1 H, m), 1.69–1.74 (1 H, m), 1.76–1.82 (1 H, m), 1.93 (1 H, td, *J* = 9.4, 6.8 Hz), 1.99–2.06 (3 H, m), 2.63 (1 H, dtd, *J* = 11.0, 3.4, 1.7 Hz), 3.34 (1 H, td, *J* = 8.5, 2.1 Hz), 5.60 (1 H, dt, *J* = 9.8, 1.7 Hz), 5.63 (1 H, dt, *J* = 9.8, 1.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.43 (2 × q), 18.64 (t), 19.67 (t), 20.92 (t), 29.69 (t), 34.91 (t), 36.11 (t), 42.54 (d), 52.76 (t), 62.97 (d), 66.13 (d), 129.01 (d), 129.44 (d). HRMS: *m/z* calcd for $C_{14}H_{25}N$ 207.1986; found: 207.1973. $[\alpha]_D^{26}$ +109.1 (*c* 0.32, $CHCl_3$). The GC-MS instrument is a Finnigan-Thermoquest Polaris Q with a Restek-RTX-5MS column (30 m × 0.25 mm i.d.) and the program was 100–280 °C at 10 °C/min with a final hold time of 10 min; t_R = 7.69 min.; natural product from *Oophaga granulifera*: t_R = 8.41 min. MS (EI): *m/z* (%) = 208 (12), 207 (4), 206 (8), 164 (100), 162 (13), 136 (13), 134 (13), 132 (8), 120 (52), 106 (8), 93 (22), 92 (11), 91 (10), 79 (13), 77 (16), 70 (16), 67 (24), 65 (14). MS (EI) of natural product from *Dendrobates granuliferous*: *m/z* (%) = 207 (2), 206 (4), 17 (10), 164 (100), 162 (78), 134 (18), 120 (28), 91 (12), 79 (14), 77 (20), 65 (11).
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