Total Synthesis of (−)-Uniflorine A[⊥]

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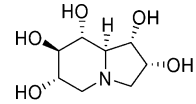
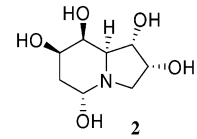
Total synthesis of (−)-uniflorine A (**3**) has been accomplished in nine steps and 11% overall yield from carbohydrate-based nitrone **5**. The key steps of the synthetic strategy were a high regio- and complete stereoselective 1,3-dipolar cycloaddition of alkene **6** with nitrone **5**, a Tamao−Fleming reaction for replacing the silicon substituent with a hydroxy group with retention of configuration, and a Mitsunobu reaction to establish the correct configuration of the target molecule at C-6.

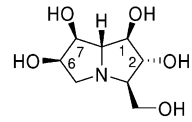
The leaves of *Eugenia uniflora* L. (Myrtaceae), an evergreen tree widely distributed in Paraguay, Uruguay, Argentina, and Brazil, furnish a natural Paraguayan medicine named Nangapiry. Infusions prepared from Nangapiry are used in folk medicines as antidiarrheic, diuretic, antirheumatic, antifebrile, and antidiabetic preparations.[1] Water-soluble extracts obtained from leaves of *E. uniflora* were found to inhibit the α-glucosidases maltase and sucrase, thus accounting for the antidiabetic effect induced by Nangapiry preparations.[1] Two iminosugar alkaloids have been isolated from the water-soluble material, namely, uniflorines A and B, for which the structures of pentahydroxyindolizidines **1** and **2**, respectively, were proposed on the basis of NMR analyses. Compounds **1** and **2** were found to inhibit rat intestinal maltase (IC_{50} values of 12 and 4.0 μM, respectively) and sucrase (IC_{50} values of 3.1 and 1.8 μM, respectively).[1]



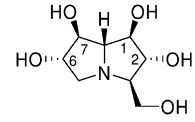
1
originally proposed structure of (−)-uniflorine A



2
originally proposed structure of uniflorine B



3
revised structure of (−)-uniflorine A (6-*epi* casuarine)



4
revised structure of uniflorine B (casuarine)

In 2004, Pyne and co-workers reported the total synthesis of putative uniflorine A (**1**) from L-xylose.[2] However, the NMR spectroscopic data for synthetic **1** did not match those reported for natural uniflorine A, thus proving that the original structural assignment to uniflorine A was incorrect. Other total syntheses of **1** from the group of Dhavale[3] and of diastereoisomers of **1**[4−7] showed that the NMR data of 1,2,6,7,8-pentahydroxyindolizidines differ significantly from those of the natural product named uniflorine A. Pyne and co-workers also recognized that the NMR data reported for uniflorine B and its optical rotation were in good agreement with those of the known alkaloid casuarine **4**,[6] a known inhibitor of α-glucosidases.[8,9] Thus, they suggested that uniflorine B was actually the known alkaloid casuarine and that uniflorine A could possess a similar 1,2,6,7-tetrahydroxy-3-hydroxymethylpyrrolizidine structure, most likely being 6-*epi*-casuarine **3**, on the basis of the greatest difference of the chemical shifts at C-6 in the NMR spectra of uniflorines A and B.[6]

As a part of our program concerning the synthesis of polyhydroxylated indolizidine and pyrrolizidine alkaloids,[10] and in order to shed light on the uniflorine issue, we decided to embark on the total synthesis of the compound proposed to be (−)-uniflorine A (**3**) by Pyne, namely, 6-*epi*-casuarine, by exploiting a strategy that allowed the recent total synthesis of casuarine (**4**).[9] While this work was in progress, Pyne and Ritthiwigrom reported a total synthesis of the non-natural enantiomer of **3**, (+)-uniflorine A, which supported their structural hypothesis.[11] Their synthesis started from D-xylose and involved a boronic acid-Mannich reaction (Petasis reaction) and a ring-closing metathesis (RCM), followed by osmium-catalyzed syn-dihydroxylation and cyclization of the intermediate dihydroxy pyrrolidine derivative as key steps (Scheme 1).[11] The pyrrolizidine obtained *ent*-**3** ($[\alpha]^{22}_D$ +6.6 (*c* 0.35, H_2O)) (lit.[1] for (−)-uniflorine A, $[\alpha]_D$ −4.4 (*c* 1.2, H_2O)) displayed spectroscopic data in good agreement with those of natural uniflorine A, thus confirming the postulated structural assignment as 6-*epi*-casuarine (**3**). Herein we report the first total synthesis of (−)-uniflorine A (**3**) via inversion of configuration of the OH group at C-6 achieved through a Mitsunobu reaction with benzoic acid on a key intermediate previously synthesized in our recent synthesis of casuarine (**4**).[9,12]

The synthesis began with a highly regio- and completely stereoselective cycloaddition of the nitrone **5**[13] with the alkene **6** to yield the isoxazolidine **7** (Scheme 2).[9] Cleavage of the N−O bond and protection of the OH group at C-6 as an acetate afforded the lactam **8**, which was used in the Tamao−Fleming reaction, in order to convert the silicon group into an OH group with complete retention of configuration, to give the lactam **9**, which was orthogonally protected at C-7 as a benzyloxy derivative and then deprotected at C-6 to liberate the OH in **10** (Scheme 2).[9] The intermediate **10** bears a free OH group at C-6, which can be further manipulated independently from the other OH groups. For instance, it could be suitably employed for the first total synthesis of casuarine-6-*O*-α-glucoside.[9]

In order to access (−)-uniflorine A, a Mitsunobu reaction was performed on **10** using THF as solvent, benzoic acid (BzOH) as a nucleophile, triphenylphosphine, and, alternatively, diisopropylazodicarboxylate (DIAD) or diethylazodicarboxylate (DEAD). Reaction of **10** with 1.2 equiv of BzOH, 1.2 equiv of PPh_3, and 1.2 equiv of DIAD at room temperature for 2 h allowed no conversion of starting material. When the mixture was heated at 50 °C for 4 h, a complex mixture was obtained, but isolation of the desired product was unsuccessful. The use of DEAD rather than DIAD

[⊥] Dedicated to Professor Francesco De Sarlo on the occasion of his 70th birthday.

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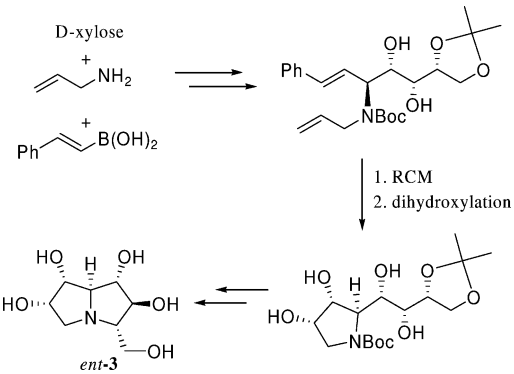
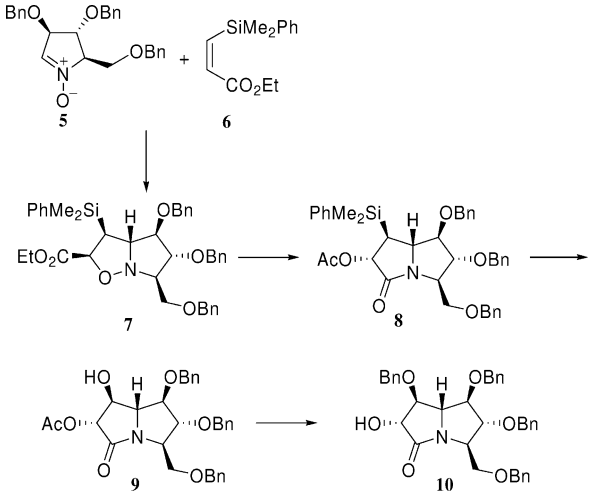
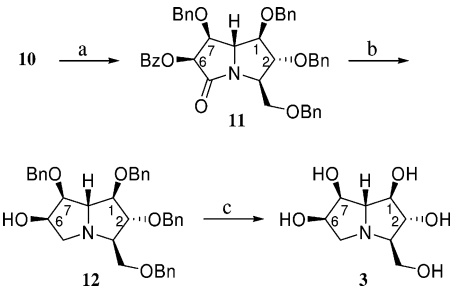
Scheme 1



Scheme 2



Scheme 3[a]



[a] Reagents and conditions: (a) BzOH (1.2 equiv), PPh_3 (1.2 equiv), DIAD (1.2 equiv), THF, rt (75%); (b) $LiAlH_4$ (4 equiv), THF, reflux (45%); (c) H_2, 10% Pd/C, MeOH, HCl, rt then Dowex 50WX8, 6% NH_4OH (71%).

and the same reaction conditions for 18 h gave compound **11** in 63% yield. However, the best yield (75% of isolated product after purification on silica gel) was achieved performing the reaction with DIAD at room temperature for 18 h (Scheme 3). Inversion of configuration at C-6 was confirmed by 1D NOESY experiments performed on compound **11**. Indeed, irradiation of H-6 gave NOE enhancement at H-7, and irradiation of H-7 gave a NOE at H-1 (Figure 1).

Concomitant reduction of the C=O bond and deprotection of the OH group at C-6 with $LiAlH_4$ in refluxing THF gave compound **12** in 45% yield. Finally, catalytic hydrogenation of **12** using 10% Pd on activated carbon in methanol in the presence of 0.1 mL of concentrated HCl (37%), followed by absorption on an acid resin and elution of the free base with 6% aqueous ammonium hydroxide, afforded (−)-uniflorine A (**3**) in 71% yield (Scheme 3). The observed NOEs were consistent with the configuration assigned to

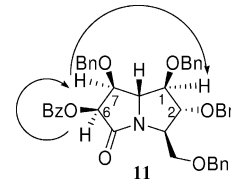


Figure 1. Compound **11**. Irradiation of H-6 gave a NOE at H-7, and irradiation of H-7 gave a NOE at H-1.

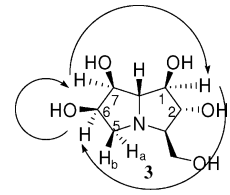


Figure 2. Compound **3**. Irradiation of H-6 gave NOEs at H-7 and H-1, and irradiation of H-7 gave a NOE at H-1.

3, as shown in Figure 2. Indeed, irradiation of H-6 gave NOEs at H-7 and H-1, and irradiation of H-7 gave a NOE at H-1.

The ^1H NMR spectra (D_2O) of **3** and those of natural uniflorine A were essentially identical ($\Delta\delta = 0.01-0.13$ ppm, see Supporting Information, Table 1). All of the ^{13}C NMR signals of **3** (in D_2O with MeCN as an internal reference at δ 1.47) were 1.6−3.0 ppm upfield compared to those reported for the natural product (see Supporting Information); however, a similar difference had been previously noted for synthetic (+)-uniflorine by Pyne and coworkers, who attributed this systematic error to a different reference (not reported) in the spectrum of the isolated natural product. Indeed, the ^{13}C NMR chemical shifts obtained for the compound synthesized by us were in good agreement with those reported for *ent*-**3** synthesized by Pyne ($\Delta\delta = \pm 1$ ppm, see Supporting Information, Table 1).[11] The specific optical rotation of synthetic **3** ($[\alpha]^{21}_D$ −6.9 (H_2O, *c* 0.42)) was essentially identical to that reported for synthetic *ent*-**3** ($[\alpha]^{21}_D$ + 6.6 (H_2O, *c* 0.35)) and matched in sign that of the natural product ($[\alpha]^{21}_D$ −4.4 (H_2O, *c* 1.2)). Further confirmation that natural (−)-uniflorine A is actually 6-*epi*-casuarine (**3**) was furnished by the melting point of synthetic **3** (177−180 °C), which was a near match to that reported for natural (−)-uniflorine A (174−178 °C).[1]

In conclusion, we report the first total synthesis of (−)-uniflorine A in nine steps and 11% overall yield from the carbohydrate-derived nitrone **5** using an efficient and straightforward synthetic strategy that employed a 1,3-dipolar cycloaddition reaction, a Tamao−Fleming reaction, and a Mitsunobu reaction as key steps. These results confirm Pyne's hypothesis on the identity of natural (−)-uniflorine A with 6-*epi*-casuarine (**3**).

Experimental Section

General Experimental Procedures. Commercial reagents were used as received. All reactions were magnetically stirred and monitored by TLC on 0.25 mm silica gel plates (Merck F_{254}). Column chromatography was carried out on silica gel 60 (32−63 μm). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. NMR spectra were recorded on Varian Mercury-400, Varian INOVA-400 (^1H, 400 MHz; ^{13}C, 100 MHz), or Varian Gemini-200 (^{13}C, 50 MHz) spectrometers. Infrared spectra were recorded with a Perkin-Elmer Spectrum BX FT-IR System spectrophotometer. Mass spectra were recorded by direct inlet on a ThermoScientific LCQ Fleet ion trap spectrometer with a Surveyor Plus LC System; relative percentages are shown in brackets. ESI full MS were recorded on a Thermo LTQ instrument by direct inlet; relative percentages are shown in brackets. Elemental analyses were performed with a Perkin-Elmer 2400 analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter.

(1*R*,2*R*,3*R*,6*S*,7*S*,7a*S*)-1,2,7-Tri(benzyloxy)-3-[(benzyloxy)methyl]-5-oxo-hexahydro-5*H*-pyrrolizin-6-yl Benzoate (11). Triphenylphosphine (68 mg, 0.26 mmol) and benzoic acid (31.7 mg, 0.26 mmol)

were added to a solution of **10** (125 mg, 0.216 mmol) in dry THF (4 mL); then DIAD (51 μL, 0.26 mmol) was added at 0 °C. The mixture was stirred at rt for 18 h; then the solvent was removed at reduced pressure and the crude product purified by FCC (eluent petroleum ether/EtOAc, 5:1, then 1:1): 111 mg, 0.162 mmol, 75%; $[\alpha]^{28}_D$ −55.6 (*c* 0.25, CH_2Cl_2); IR ($CDCl_3$) cm^{-1} 1726; ^1H NMR ($CDCl_3$) δ 8.00−7.98 (m, 2H), 7.52−7.48 (m, 1H), 7.37−7.33 (m, 2H), 7.28−7.13 (m, 20H, Ar), 5.63 (d, J = 5.4 Hz, H-6), 4.60 (d, J = 11.2 Hz, 1H, Bn), 4.53−4.29 (m, 7H, Bn), 4.23−4.15 (m, 2H, H-2 + H-3), 4.01 (dd, J = 6.8, 5.4 Hz, 1H, H-7a), 3.90 (dd, J = 6.8, 5.4 Hz, 1H, H-7), 3.70 (dd, J = 5.4, 3.4 Hz, 1H, H-1), 3.63−3.52 (m, 2H, Ha-8 + Hb-8); ^{13}C NMR ($CDCl_3$) δ 168.4 (s), 165.1 (s, C-5), 137.6−136.5 (s, 5C), 133.0 (d, 1C), 139.7−128.9 (d, 2C), 128.1−127.2 (d, 22C), 85.4 (d, 2C, C-1 + C-2), 77.8 (d, C-7), 72.9−71.5 (t, Bn), 72.3 (d, C-6), 68.4 (d, C-7a), 67.9 (t, C-8), 59.2 (d, C-3); MS (ESI) *m*/*z* 706 (100, $[M + Na]^+$); *anal*. C, 75.16%; H, 6.37%; N, 1.95%, calcd for $C_{43}H_{41}NO_7$, C, 75.53%; H, 6.04%; N, 2.05%.

(1*R*,2*R*,3*R*,6*S*,7*S*,7a*S*)-1,2,7-Tri(benzyloxy)-3-[(benzyloxy)methyl]hexahydro-1*H*-pyrrolizin-6-ol (12). $LiAlH_4$ (25 mg, 0.66 mmol) was added to a solution of **11** (111 mg, 0.162 mmol) in dry THF (3 mL). The mixture was stirred at reflux under nitrogen atmosphere for 5 h; then 0.30 mL of a saturated solution of Na_2SO_4 was added dropwise at room temperature. The resulting mixture was filtered through Celite and washed with EtOAc, and then the solvent was evaporated. Purification of the crude product by FCC (eluent petroleum ether/EtOAc, 1:1) gave pure **12** as a pale yellow oil (42 mg, 0.074 mmol, 45%): $[\alpha]^{22}_D$ −6.0 (*c* 0.25, CH_2Cl_2); IR ($CDCl_3$) cm^{-1} 3689, 3605, 2956, 2926, 2854, 1732, 161, 1457, 1378; ^1H NMR ($CDCl_3$) δ 7.35−7.18 (m, 20H, Ar), 4.62−4.44 (m, 8H, Bn), 4.29−4.26 (m, 1H, H-6), 4.03 (pseudo t, J = 4.9 Hz, 1H, H-2), 3.89 (t, J = 4.2 Hz, 1H, H-1), 3.83 (dd, J = 6.9, 4.6 Hz, 1H, H-7), 3.62 (m, 1H, H-7a), 3.57−3.49 (m, 2H, H-8), 3.33−3.30 (m, 1H, Ha-5), 3.03−2.99 (m, 2H, H-3 + Hb-5); ^{13}C NMR ($CDCl_3$) δ 137.6−137.1 (s, 4C, Ar), 128.3−127.4 (d, 20C, Ar), 85.4 (d, 2C, C-1 + C-2), 82.5 (d, C-7), 73.0−71.3 (5C, 4Bn + C-8), 70.5 and 70.3 (d, 2C, C-6 + C-7a), 69.8 (d, C-3), 60.2 (t, C-5); MS (ESI) *m*/*z* 566 (100, $[M + H]^+$); *anal.* C, 76.40%; H, 7.37%; N, 2.75%, calcd for $C_{36}H_{39}NO_5$, C, 76.43%; H, 6.95%; N, 2.48%.

(1*R*,2*R*,3*R*,6*S*,7*S*,7a*S*)-3-(Hydroxymethyl)hexahydro-1*H*-pyrrolizin-1,2,6,7-tetraol ((−)-uniflorine A, 3). HCl 37% (0.1 mL) and 10% Pd on C (33 mg) were added to a solution of **12** (35 mg, 0.062 mmol) in MeOH (4 mL). The suspension was stirred under H_2 atmosphere for 4 days, then filtered through Celite and washed with MeOH. Evaporation of the solvent under reduced pressure afforded crude **3**, which was transferred to a column of Dowex 50WX8 and washed with MeOH (10 mL) and H_2O (10 mL) to remove nonbasic impurities, then with 6% NH_4OH (15 mL) to elute (−)-uniflorine A (**3**) as a yellowish solid (9 mg, 0.044 mmol, 71%): mp 177−180 °C; $[\alpha]^{21}_D$ −6.9 (*c* 0.415, H_2O); ^1H NMR (D_2O) δ 4.23 (q, J = 5.2 Hz, 1H, H-6), 4.08 (pseudo t, J = 4.7 Hz, 1H, H-7), 3.83 (t, J = 7.4 Hz, 1H, H-1), 3.71 (dd, J = 8.8, 7.6 Hz, 1H, H-2), 3.65 (dd, J = 11.9, 3.7 Hz, 1H, Ha-8), 3.50 (dd, J = 11.9, 6.4 Hz, 1H, Hb-8), 3.08 (dd, J = 7.2, 5.0 Hz, 1H, H-7a), 2.97 (dd, J = 12.1, 5.4 Hz, 1H, Ha-5), 2.89 (dd, J = 12.1, 5.0 Hz, 1H, Hb-5), 2.70 (ddd, J = 9.1, 6.4, 3.7 Hz, 1H, H-3); ^{13}C NMR (D_2O) δ 78.7 (d, C-1), 77.5 (d, C-2), 75.7 (d, C-7), 72.0 (d, C-6), 71.8 (d, C-7a), 70.9 (d, C-3), 62.3 (t, C-8), 58.1 (t, C-5); MS (ESI) *m*/*z* 206 (100, $[M + H]^+$), 158 (10), 132 (13), 118 (22), 95 (30), 79 (73), 55 (17); *anal.* C, 46.85%; H, 7.68%; N, 7.11%, calcd for $C_8H_{15}NO_5$, C, 46.82%; H, 7.37%; N, 6.83%.

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Note Added after ASAP Publication: The version of this paper published on October 16, 2009, had refs 12 and 13 mislabeled. The corrected version was published on October 21, 2009.

Supporting Information Available: Comparison of ^1H and ^{13}C NMR data for synthetic and isolated (−)-uniflorine A and synthetic (+)-uniflorine A (Table 1). Synthetic procedures and characterization for compounds **8**, **9**, and **10**. Copies of ^1H, ^{13}C NMR and 1D NOESY spectra for compounds **11** and **3** and ^1H and ^{13}C NMR spectra for compounds **8**, **9**, **10**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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