Isolation and Characterization of Two New Alkaloids, Norpandamarilactonine-A and -B, from *Pandanus amaryllifolius* by Spectroscopic and Synthetic Methods

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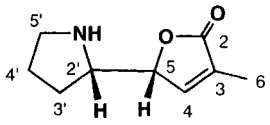
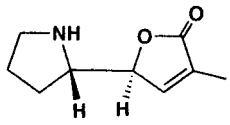
Two new alkaloids, norpandamarilactonine-A (**1**) and -B (**2**), which have a pyrrolidinyl-α,*β*-unsaturated *γ*-lactone moiety as in the known pandamarilactonine alkaloids, were isolated from the leaves of *Pandanus amaryllifolius*. Their structures were determined by spectroscopic analysis and total synthesis.

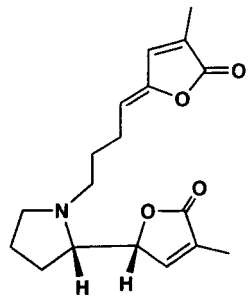
The genus *Pandanus* (Pandanaceae) comprises about 600 species which are widely distributed in tropical and subtropical regions. In a recent pharmacological survey, the hypoglycemic effect of an extract of *P. odorus* was noted.[1] During our chemical studies on the secondary metabolites in *Pandanus* plants,[2] we reported the isolation of pandamarilactonines-A (**3**) and -B (**4**), pyrrolidine alkaloids from *P. amaryllifolius*. Roxb.[3] Further investigation of the minor bases in fresh leaves of this plant resulted in the isolation of two additional alkaloids (**1** and **2**), whose structure elucidation by spectroscopic and synthetic methods are described herein.



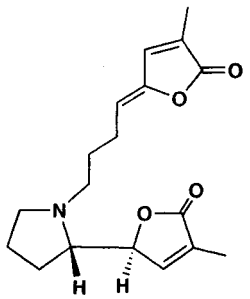
Norpandamarilactonine-A (**1**)



Norpandamarilactonine-B (**2**)



Pandamarilactonine-A (**3**)



Pandamarilactonine-B (**4**)

Compound **1** was obtained as an amorphous powder, $[\alpha]^{19}_D$ 0° (*c* 0.30, $CHCl_3$), and high-resolution FABMS analysis established the molecular formula as $C_9H_{13}NO_2$. The presence of an α-methyl-α,*β*-unsaturated *γ*-lactone residue was shown by characteristic signals in the ^1H and ^{13}C NMR spectra [*δ* 7.13 (1H, ddd, *J* = 0.8, 1.6, 1.6 Hz, H-4), 4.73 (1H, ddd, *J* = 1.6, 1.9, 6.6 Hz, H-5), 1.93 (3H); *δ* 174.3 (C-2), 130.7 (C-3), 147.7 (C-4), 83.8 (C-5), 10.7 (C-6)]. Using the residual four carbons (three methylenes and one methine) and one nitrogen atom, a pyrrolidine ring could be constructed. In the HMBC spectrum, the methine proton (*δ* 3.18, 1H, ddd, *J* = 6.6, 6.6, 7.4 Hz, H-2′) on the pyrrolidine ring correlated with the sp^2 carbon at C-4 (*δ* 147.7) in the α,*β*-unsaturated *γ*-lactone ring. In addition, the methine proton (*δ* 4.73) at C-5 (*δ* 83.8) in the *γ*-lactone ring had connectivity between C-2′ and C-3′ in the pyrrolidine ring. All the above findings enabled us to describe the molecular structure of the new alkaloid as **1**, a pyrrolidinyl-α,*β*-unsaturated *γ*-lactone skeleton, except for the stereochemistry of the vicinal asymmetric centers at C-5 and C-2′. Because of the lack of a *γ-alkylidene*-α,*β*-unsaturated *γ*-lactone moiety as in the known alkaloids, pandamarilactonines (**3** and **4**), we now name the new alkaloid (**1**) norpandamarilactonine-A.

Alkaloid **2** was also obtained as an amorphous powder, exhibiting $[\alpha]_D^{19}$ 0° (*c* 0.70, $CHCl_3$). The UV and mass spectra, as well as the molecular formula obtained by HR-FABMS, were almost identical to those of **1**. The ^1H and ^{13}C NMR spectra were also very similar, indicating that **1** and **2** were diastereomeric at the C-5 and C-2′ positions.

To confirm the structures and relative stereochemistry at C-5 and C-2′ in the new alkaloids, we planned the total synthesis (Scheme 1). According to the procedure of Martin et al.,[4] compound **5** was prepared from 2-pyrrolidone and 3-methylfuran-2(5*H*)-one. The *threo* stereochemistry of the major product (**5**) obtained by vinylogous Mannich coupling reaction has been established by X-ray analysis.[4] The protecting group on the nitrogen in **5** was removed with TMSI in CH_3CN to give the secondary amine in 94% yield, which was identical with the natural product, norpandamarilactonine-B (**2**), by direct comparison of the chromatographic behavior and high-resolution MS and ^1H and ^{13}C NMR spectra. Therefore, the relative stereochemistry of norpandamarilactonine-A (**1**) was determined to be *erythro*.

In summary, two new diastereomeric alkaloids (**1** and **2**) having a pyrrolidinyl-α,*β*-unsaturated *γ*-lactone skeleton were isolated as minor constituents from a tropical medicinal plant, *Pandanus amaryllifolius*. These interesting molecules possessing the substructure of the known alkaloids **3** and **4** were first characterized by spectroscopic analysis and then the structures were confirmed by total synthesis.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-140 polarimeter. UV and IR spectra were recorded on Hitachi U-3400 and JASCO FT/IR-230 spectrophotometers, respectively. EIMS and FABMS were

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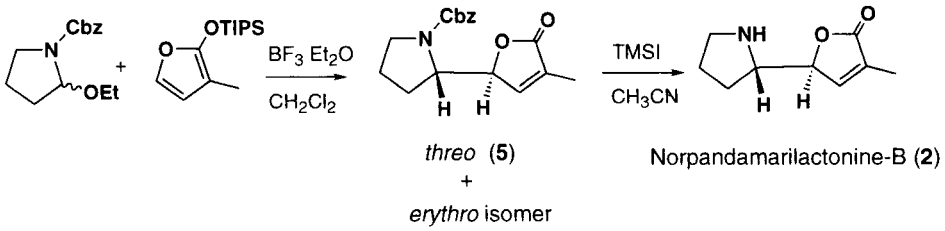
Scheme 1



recorded on JEOL JMS-AM20 and JEOL JMS-HX110 mass spectrophotometers, respectively. The ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOE were recorded on JEOL JNM A-500 and JEOL JNM ECP600 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Kieselgel 60 (Merck, 70−230 and 230−400 meshes) and a silica gel prepacked column (Kusano CPS-HS-221−05) were used for column chromatography.

Plant Material. The fresh leaves of *P. amaryllifolius* were purchased at a flower market in Bangkok (Thailand) and identified by Dr. Kittisak Likhitwitayawuid, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. A voucher specimen was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Chiba University.

Extraction and Isolation of Alkaloids. Fresh young leaves (8.0 kg) of *P. amaryllifolius* were macerated with EtOH (20 L) three times and filtered. The combined filtrates were concentrated under reduced pressure to give a crude extract (201 g), which was then partitioned between Et_2O and 5% aqueous H_2SO_4. The water-soluble fraction was alkalinized with concentrated NH_4OH (pH 10) and exhaustively extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude alkaloidal fraction (10.03 g). A portion of the crude base (1.63 g) was roughly separated by silica gel flash column chromatography using a $CHCl_3$−MeOH/$CHCl_3$ gradient to give seven fractions. The 10% MeOH/$CHCl_3$ eluate was rechromatographed over SiO_2 using the same solvent to give 6 mg of norpandamarilactonine-A (**1**) and 33 mg of norpandamarilactonine-B (**2**).

Norpandamarilactonine-A (1): amorphous powder; $[\alpha]^{19}_D$ 0° (*c* 0.30); UV (MeOH) λ_{max} (log ϵ) 274 (0.44), 252 (0.35), 207 (2.29) nm; IR (neat) ν_{max} 1750 (lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.13 (1H, ddd, J = 0.8, 1.6 and 1.6 Hz, H-4), 4.73 (1H, ddd, J = 1.6, 1.9 and 6.6 Hz, H-5), 3.18 (1H, ddd, J = 6.6, 6.6 and 7.4 Hz, H-2′), 2.96 (1H, ddd, J = 6.3, 6.3 and 10.4 Hz, H-5′), 2.93 (1H, ddd, J = 6.8, 6.8 and 10.4 Hz, H-5′), 1.93 (3H, s, H_3-6), 1.84−1.92 (1H, m, H-3′), 1.72−1.90 (2H, m, H_2-4′), 1.63 (1H, dddd, J = 6.3, 6.3, 6.6 and 12.9 Hz, H-3′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.3 (C-2), 147.7 (C-4), 130.7 (C-3), 83.8 (C-5), 60.4 (C-2′), 47.1 (C-5′), 27.9 (C-3′), 25.6 (C-4′), 10.7 (C-6); FABMS (NBA) *m/z* 168 [M + H]$^+$; HRFABMS (NBA) *m/z* 168.1039 (calcd for $C_9H_{14}NO_2$, 168.1025).

Norpandamarilactonine-B (2): amorphous powder; $[\alpha]^{19}_D$ 0° (*c* 0.70); UV (MeOH) λ_{max} (log ϵ) 274 (0.36), 253 (0.29), 207 (2.58) nm; IR (neat) ν_{max} 1750 (lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.02 (1H, ddd, J = 1.4, 1.7 and 3.0 Hz, H-4), 4.79 (1H, dddd, J = 1.6, 1.9, 3.0, and 6.6 Hz, H-5), 3.20 (1H, ddd, J = 6.6, 7.1, and 7.4 Hz, H-2′), 2.98 (1H, ddd, J = 5.8, 7.1 and 12.9 Hz, H-5′), 2.91 (1H, ddd, J = 6.6, 7.7, and 14.3 Hz, H-5′), 1.93 (3H, s, H_3-6), 1.87 (1H, dddd, J = 3.0, 7.4, 10.7, and 15.4 Hz, H-3′), 1.81 (1H, m, H-4′), 1.74 (1H, m, H-4′), 1.56 (1H, dddd, J = 5.2, 6.9, 7.1 and 15.4 Hz, H-3′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.1 (C-2), 146.6 (C-4), 131.2 (C-3), 84.3 (C-5), 60.2 (C-2′), 46.5 (C-5′), 26.8 (C-3′), 25.1 (C-4′), 10.7 (C-6); FABMS (NBA) *m/z* 168 [M + H]$^+$; HRFABMS (NBA) *m/z* 168.1030 (calcd for $C_9H_{14}NO_2$: 168.1025).

Synthesis of Norpandamarilactonine-B (2). To a solution of diastereomerically pure carbamate (**5**) (30.6 mg, 0.1 mmol), which was prepared according to the procedure by Martin,[4] in CH_3CN (1 mL), was added TMSI (43 μL, 0.3 mmol) at −10 °C under argon. The reaction mixture was stirred at the same temperature for 15 min and then stirred at 0 °C for 15 min. The reaction mixture was poured into a chilled solution of 1 N HCl, and the whole mixture was extracted with Et_2O. The aqueous layer was basified with 1 N NaOH, and the mixture was extracted with $CHCl_3$ three times. The combined organic layers were washed with H_2O, dried over $MgSO_4$, and evaporated to give **2** (16 mg, 94%), which was identical to natural norpandamarilactonine-B by comparison of their chromatographic behaviors, UV, ^{1}H and ^{13}C NMR, and mass spectra.

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References and Notes

(1) (a) Peungvicha P.; Thirawarapan S. S.; Watanabe H. *Biol. Pharm. Bull.* **1996**, *19*, 364−366. (b) Peungvicha P.; Thirawarapan S. S.; Watanabe H. *Jpn. J. Pharmacol.* **1998**, *78*, 395−398. (c) Peungvicha P.; Temsiririrkkul, R.; Prasain, J. K.; Tezuka, Y.; Kadota, S.; Thirawarapan S. S.; Watanabe H. *J. Ethnopharmacol.* **1998**, *62*, 79−84.

(2) (a) Nonato, M. G.; Garson, M. J.; Truscott, R. J. W.; Carver, J. A. *Phytochemistry* **1993**, *34*, 1159−1163. (b) Takayama, H.; Kuwajima, T.; Kitajima, M.; Nonato, M. G.; Aimi, N. *Heterocycles* **1999**, *50*, 75−78. (c) Takayama, H.; Kuwajima, T.; Kitajima, M.; Nonato, M. G.; Aimi, N. *Nat. Med.* **1999**, *53*, 335. (d) Takayama, H.; Ichikawa, T.; Kitajima, M.; Aimi, N.; Lopez, D.; Nonato, M. G. *Tetrahedron Lett.* **2001**, *42*, 2995−2996.

(3) Takayama, H.; Ichikawa, T.; Kuwajima, T.; Kitajima, M.; Seki, H.; Aimi, N.; Nonato, M. G. *J. Am. Chem. Soc.* **2000**, *122*, 8635−8639.

(4) Martin, S. F.; Barr, K. J.; Smith, D. W.; Bur, S. K. *J. Am. Chem. Soc.* **1999**, *121*, 6990−6997.

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