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Alkaloids from *Pandanus amaryllifolius* Collected from Marikina, Philippines

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The dichloromethane extract from a neutral partition of the alcoholic extract of air-dried mature leaves of *Pandanus amaryllifolius* from Marikina, Philippines yielded Pandamarilactone-1 (2), Pandamarilactam-3x, -3y (5-6), Pandamarilactonine-A, -B, -C (7-9), and 6Z-Pandanamine (13). The isolation of these alkaloids by this study corroborated the results from other countries despite the differences in the sites and years of leaves collection and in the extraction and partitioning conditions, which strongly suggest that these alkaloids are natural products of *P. amaryllifolius* and are not experimental artifacts.

Key words: Norpandamarilactonine-A, -B, Pandamarilactam-3x, -3y, Pandamarilactone-1, Pandamarilactonine-A, -B, -C, Pandamarine, Pandanamine

INTRODUCTION

Pandanus amaryllifolius Roxb. (syn. P. odorus Ridl.) is one of the more than 600 *Pandanus* species that are widely distributed in the tropical and subtropical areas (Stone 1978, 1983). Many ethnomedical reports and pharmacological studies of the extracts from the genus *Pandanus* have been documented, and chemical studies have established that the leaves of several species contain alkaloids (Santos et al. 1981; Ysrael et al. 1995; Nonato & Madulid 1997; Jong & Chau 1998; Peungvicha et al. 1998; Azares et al. 1999).

Alkaloid variation was observed in the leaves of *P. amaryllifolius* that were collected from different sites at different times (Figure 1). The following alkaloids have been reported: (+)-Pandamarine (1), a piperidine-type alkaloid with lactam moieties from Isabela, Philippines (Byrne et al. 1992); Pandamarilactone-1 (2) Pandamarilactone-32 (3) and Pandamarilactone-31 (4), also piperidine-type but with lactone instead of lactam moieties from Manila, Philippines (Nonato et al. 1993); Pandamarilactam-3x, and Pandamarilactam -3y (5, 6), pyrrolidinone-type alkaloids with lactone moiety from Jambi, Indonesia (Sjaifullah & Garson 1996); in addition to (2), Pandamarilactonine-A, -B, -C, -D (7-10) and Norpandamarilactonine-A, -B (11-12), all pyrrolidine-type alkaloids with lactone moiety, and 6Z-Pandanamine (13), a symmetrical secondary amine with lactone moieties from Bangkok, Thailand (Takayama et al. 2000, 2001a, 2001b, 2002); and in addition to (7-10) and (13), the 6E-Pandanamine (14) and artifacts (15-16) from West Java, Indonesia (Salim et al. 2004). These reports from 1992 to 2002 used acid-base extraction, while Salim et al. (2004) used neutral partitioning and acid-based methods. The available data show that further studies are necessary to establish whether the structural variation in the reported alkaloids were from biogeographic and/or bioclimatic differences, or mainly from the isolation process.

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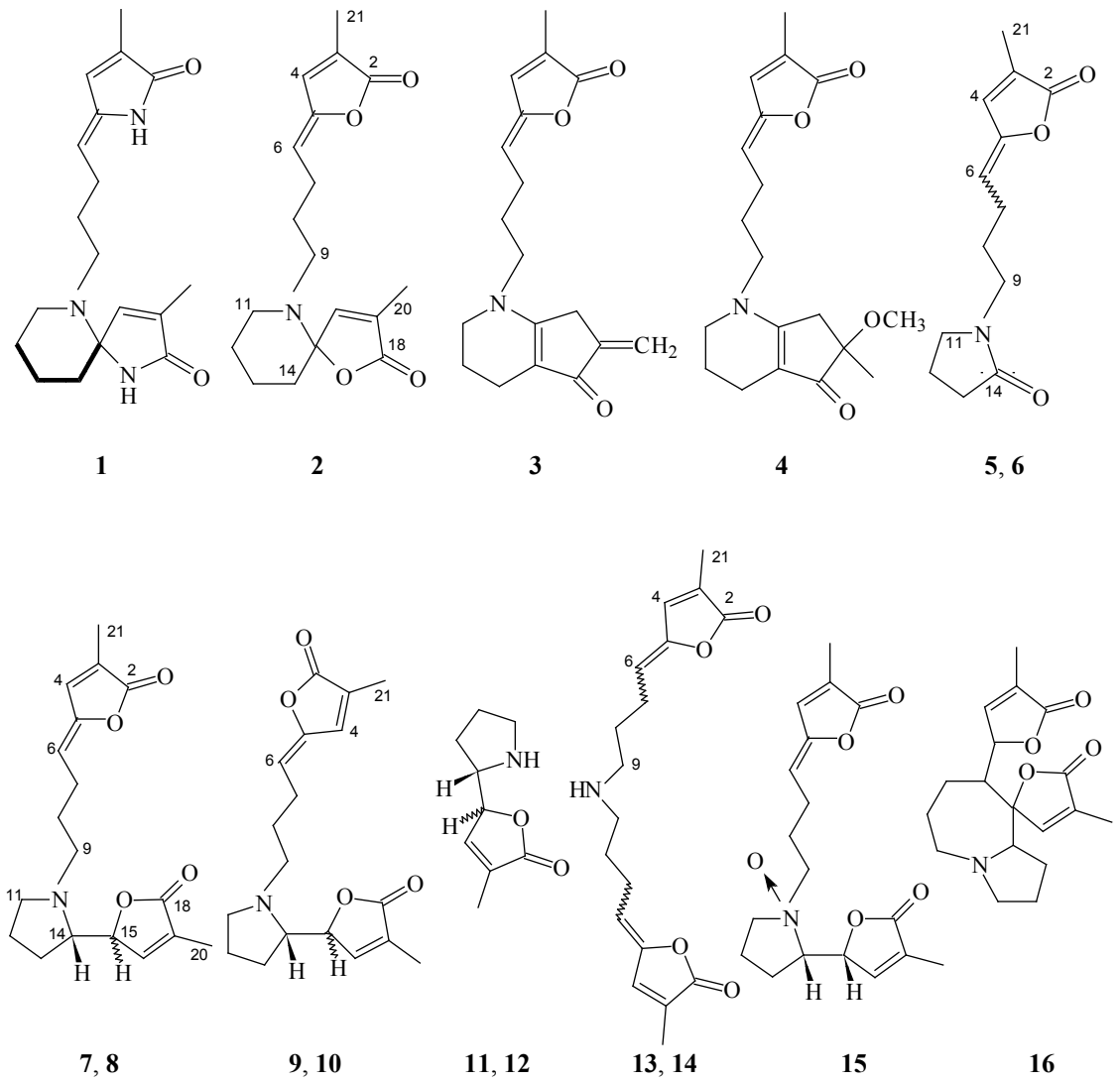

Figure 1. Alkaloids from Pandanus amaryllifolius collected from different sites at different times. (1 Isabela, Philippines 1992; 2-4 Manila, Philippines 1993; 5-6 Jambi, Indonesia 1996; 2, 7-13 Bangkok, Thailand 2000-2002; 7-10, 14-15, 16 West Java, Indonesia 2004)

In this paper, we report the isolation of 2, 5, 6, 7-9, and 13 from the mature leaves of *P. amaryllifolius* that were collected in 1997 from Marikina City, Philippines. As precaution against artifacts formation, neutral condition was used during the solvent partitioning step.

MATERIALS AND METHODS

General Experimental Procedures. EIMS and FABMS were recorded on Jeol JMS-AM20 and Jeol JMS-HX110 mass spectrometers, respectively. The ^{1}H and ^{13}C NMR, HMQC, HMBC, and differential NOE were recorded on Jeol JNM ECP600, Jeol JNM A-500, Jeol JNM GX-400, and Bruker AMX-500 spectrometers. Chromatographic separations used silica gel 60 HF_{254} Merck 7739 (vacuum liquid or VLC); silica gel 60 GF_{254} Merck Art. 7749 (centrifugal thin layer or CTLC); silica gel 60 (70 – 230 mesh) Merck Art. 7734 and (230 – 400 mesh) Merck Art. 9835 (gravity column or GCC); MCI gel CHP 20P (120 - 300 μ, high porous polymer) Mitsubishi Corp. (flash column or FCC); Supelco C18, 4.6 mm x 150 mm, 5μm (RP-HPLC); and silica gel Kusano CPS-HS-221-05 (NP-MPLC). TLC was performed with glass-backed or aluminum-backed plates coated with silica gel F_{254}, and plates were visualized under UV_{254} and by spraying with Dragendorff's reagent.

Plant Material. The mature leaves of *P. amaryllifolius* were collected from the grounds of Marikina Elementary School, Sta. Elena, Marikina City, Philippines on 08

December 1997. The plant was identified and a voucher specimen was deposited at the Herbarium of the Research Center for the Natural Sciences, University of Santo Tomas, Philippines.

Extraction, Isolation, and Identification of Alkaloids. Ground air-dried leaves (690 g) were soaked in single distilled 95% EtOH at room temperature for 24 h, then filtered. The marc was further extracted with fresh portions of EtOH until the extract was negative to Mayer's reagent. The filtrate was concentrated *in vacuo* to give dark green resinous EtOH extract (116.9 g). A 50.5 g portion of the extract was dissolved in EtOH (100 mL), and the ethanolic solution was adjusted to approximately 1:1 EtOH:H_2O, allowed to stand until green resinous materials floated, and was filtered. The EtOH was evaporated *in vacuo*, then the concentrated filtrate was partitioned based on increasing polarity with hexane, CH_2Cl_2, and n-BuOH. The extracts were dried with anhydrous sodium sulfate (Na2SO4), then filtered and concentrated *in vacuo*. The CH_2Cl_2 extract (9.87 g) was fractionated by VLC using hexane, CH_2Cl_2, EtOAc, and MeOH. The CH_2Cl_2:EtOAc (1:1) fraction (216 mg) was chromatographed by CTLC, followed by GCC, using step-gradient with $CHCl_3$:MeOH. The $CHCl_3$ fraction (12.7 mg) was purified by RP-HPLC (from H_2O:CH_3CN [8:2] to [0:100] at 1 mL/min in 10 min) to give alkaloid 2 (5.1 mg). A 1.16 g EtOAc:MeOH (1:1) fraction was chromatographed on MCI gel by FCC using H_2O:MeOH and MeOH. A 55.6 mg MeOH fraction was chromatographed by NP-MPLC with 1% EtOH in $CHCl_3$ to give alkaloid 2 (3.1 mg), alkaloid 7 (8.1 mg), alkaloid 8 (6.5 mg) and a mixture of alkaloids 5, 6, and 7 (1.2 mg). A 45.5 mg MeOH fraction was chromatographed by NP-MPLC with 2% EtOH in $CHCl_3$ to give alkaloid 7 (2.6 mg) and a mixture of alkaloids 7 and 9 (1.2 mg). A 112 mg H_2O:MeOH fraction was chromatographed by NP-MPLC with 5% EtOH in $CHCl_3$ to give alkaloid 13 (14.5 mg). The diagram outlining the extraction, isolation, and purification of alkaloids is shown as Figure 2.

The alkaloid structures were elucidated by comparison of the spectral data of the isolates with the literature.

RESULTS AND DISCUSSION

Seven alkaloids were isolated under neutral condition from the air-dried mature leaves of *P. amaryllifolius*. The aqueous concentrate from the hydroalcoholic solution of the EtOH extract was defatted with hexane, then partitioned with CH_2Cl_2 to give the crude alkaloidal extract. This extract was chromatographed to give alkaloids 2, 7, 8, 13, mixture of 5, 6, and 7, and mixture of 7 and 9.

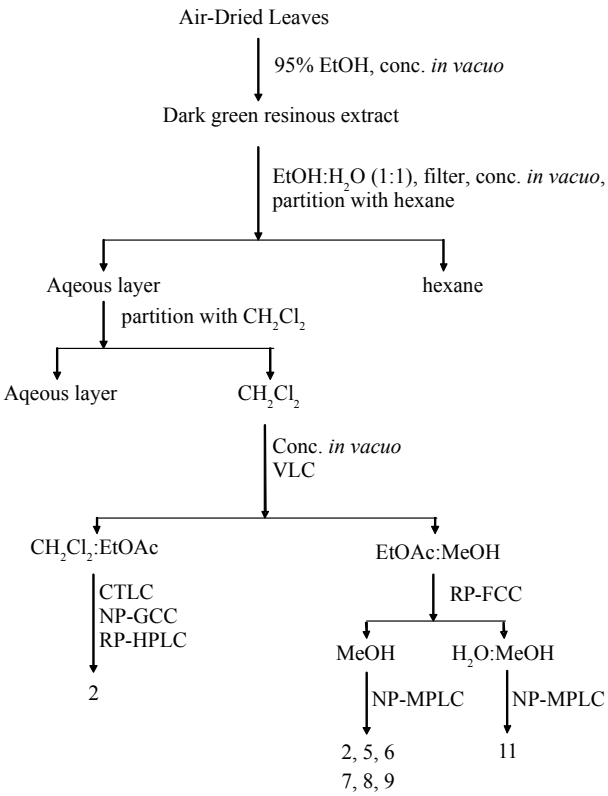

Figure 2. Schematic diagram of the extraction, and isolation of alkaloids from *Pandanus amaryllifollius*

Comparison of the ^{1}H NMR spectral data of the isolates with those of the reported alkaloids from *P. amaryllifolius* (Table 1) showed that the resonances for alkaloids 2, 7, 8, and 13 were in close agreement with those of Pandamarilactone-1 (2), Pandamarilactonine-A (7), Pandamarilactone-B (8) and Pandanamine (13), respectively.

An isolate was found to be a mixture of alkaloids 5, 6, and 7. The ^{1}H NMR resonances at δ 7.29 (1H), δ7.03 (1H), and δ 6.98 (2H) indicated the presence of four λ–substituted α,β-unsaturated γ-lactone moieties; the resonance at δ 5.63 (1H) indicated E-configuration for the H-4 and H-6 of one γ–alkylidine α,β-unsaturated γ-lactone moiety; the resonance at δ 5.19 (2H) indicated Z-configuration for the H-4 and H-6 of two γ–alkylidine α,β-unsaturated γ-lactone moieties; the resonance at δ 4.78 (1H) is characteristic of the H-15 of Pandamarilactonine-A (7); the resonances at δ 3.37 to δ 3.30 (7H) and δ 3.11 to δ2.32 (3H) are characteristics of the H-9 and pyrrolidinone moiety of the Pandamarilactam-3y, -3x (5, 6). The peak heights of 1:2 for H-4 at δ 7.29 (1H) for alkaloid 6 and δ 6.98 (2H) for alkaloids 5 and 7, peak heights of 1:2 for H-6 at δ 5.63 (1H) for alkaloid 6 and δ 5.19 (2H) for alkaloids 5 and 7, and peak heights of 1:1 for H-4 at δ 7.29 (1H)

Table 1. ^{1}H NMRspectral data (δ in ppm, multiplicity, J in Hz, integration) of isolated alkaloids 2, 7, 8, 9, 5, 6, 13 and the identified *P. amaryllifollius* alkaloids

H No	Alkaloid **2**[a]	Pandamari-lactone-**1**[b]	Alkaloid **7**[C]	Pandamari-lactonine-A[d]	Pandamari-lactonine-A[d]	Alkaloid **8**[c]	Pandamari-lactonine-B[d]
4	6.98 (qrt, 1.2, 1H)	6.98 (ddd, 1.2, ~1.0, 1H)	6.98, d, 1.3, 1H	6.99, d-like, 1.5, 1H	6.99, d-like, 1.5, 1H	6.98, d, 1.5, 1H	7.00, d-like, 1.5, 1H
6	5.08 (t, 7.9, 1H)	5.04 (ddd, 8.0, 1.0, 1H)	5.16, t, 7.9, 1H	5.18, dd, 7.9, 7.9, 1H	5.18, dd, 7.9, 7.9, 1H	5.15, t, 7.9, 1H	5.18, dd, 7.8, 8.0, 1H
7	2.30 (qrt, 7.4, 2H)	2.31 (ddd, 8.0, 7.2, 2H)	2.40 ~2.43, m, 2H)	2.43, dd, 7.3, 15.0, 2H)	2.43, dd, 7.3, 15.0, 2H)	2.40~2.43, m, 1H; 2.21-2.28, m, 1H)	2.42~2.48, m, 1H; 2.36, m, 1H)
8	1.50 ~1.56 (m, 2H)	1.54 (dddd, 8.0, 7.2), 2H	1.61~1.66, m, 2H	1.59~1.70, m, 2H	1.59~1.70, m, 2H	1.58~1.68 m, 2H	1.59~1.67, m, 2H
9	2.44 (br s, 2H)	2.45 (dd, 7.0, 2H)	2.80~2.85, m, 1H; 2.40 ~2.43, m, 1H	2.88, ddd, 4.0, 7.9, 12.9, 1H; 2.45, m, 1H)	2.88, ddd, 4.0, 7.9, 12.9, 1H; 2.45, m, 1H)	2.67 ~ 2.74, m, 1H; 2.40~2.43, m, 1H	2.73, m, 1H; 2.43-2.48, m, 1H)
11	2.78 (br t, 5.2, 2H)	2.79 (dd, 7.0, 2H)	3.09, t-like, 7.0, 7.7, 1H; 2.19-2.20, m, 1H	3.12, dd, 6.7, 7.6, 1H; 2.21, m, 1H	3.12, dd, 6.7, 7.6, 1H; 2.21, m, 1H	3.07 ~ 3.10, m, 1H; 2.21 ~2.28, m, 1H	3.12, m, 1H; 2.25, m. 1H)
12	1.69 ~1.75 (br, 2H)	1.72 (m, 2H)	1.70~1.73, m, 1H; 1.39~1.43, m, 1H	1.70~1.80, m, 1H; 1.42, m, 1H	1.70~1.80, m, 1H; 1.42, m, 1H	1.70~1.82, m, 2H	1.73~1.87, m, 2H
13	1.69 ~1.75 (br, 2H)	1.72 (m, 2H)	1.70~1.73, m, 1H; 1.61~1.66, m, 1H	1.70~1.80, m, 1H; 1.59~1.70, m, 1H	1.70~1.80, m, 1H; 1.59~1.70, m, 1H	1.70~1.82, m, 2H	1.73~1.87, m, 2H
14	1.69 ~1.75 (br, 2H)	1.72 (m, 2H)	2.80-2.85, m, 1H	2.83, m, 1H	2.83, m, 1H	2.67-2.74, m, 1H	2.70, m, 1H
15			4.78-4.79, m, 1H)	4.80, ddd, 1.8, 1.8, 5.5, 1H	4.80, ddd, 1.8, 1.8, 5.5, 1H	4.70 ~ 4.71, m, 1H	4.71, ddd, 1.7, 2.0, 5.9, 1H
16	6.72 (qrt, 1.5, 1H)	6.68 (dd, 1.2, 1H)	7.08, s, 1H	7.09, dd, 1.5, 1.8, 1H	7.09, dd, 1.5, 1.8, 1H	7.04, qrt, 1.6, 1H	7.05, dd, 1.5, 1.7, 1H
20	1.90 (d, 1.5, 3H)	1.86 (br s, 3H)	1.92, t-like, 1.7, 1.5, 3H	1.93, dd, 1.5, 1.8, 3H	1.93, dd, 1.5, 1.8, 3H	1.92, d-like, 1.5, 3H	1.93, dd, 1.7, 1.7, 3H
21	2.00 (d, 0.6, 3H)	2.00 (br s, 3H	1.97, s, 3H	1.99, d-like, 0.9, 3H	1.99, d-like, 0.9, 3H	1.98, d-like, 0.8, 3H	1.99, d-like, 0.7, 3H

Table 1 continued . . .

Alkaloid **9**[c]	Pandamari-lactonine-C[d]	Alkaloid **5**[c]	Pandamari-lactam-3y[f]	Alkaloid **6**[c]	Pandamari-lactam-3x[f]	Alkaloid **13**[g]	Pandan-amine[d]
7.30, s, 1H	7.31, dd, 0.9, 1.5, 1H	6.98, d, 1.5, 1H	6.98, dd, 1.0, 0.5, 1H	7.29, s, 1H	7.30, ni, 1.0, 0.5, 1H	7.02 (d, 1.4, 1H)	7.02 (d, 1.5, 2H)
5.64, t-like, 1H	5.64, dd, 8.2, 8.5, 1H	5.19, t, 7.9, 1H	5.18, ni, 7.6, 1.0, 1H	5.63, t, 8.3, 1H	5.58, ni, 8.5, 1.0, 1H	5.13 (t-like, 8.0, 7.7, 1H)	5.14 (dd, 7.8, 8.1, 2H)
2.35~2.47, m, 2H	2.35, ddd, 7.0, 8.2, 14.7, 1H; 2.25, m, 1H)	2.33~2.39, m, 2H	2.30, qrt, 7.6, 7.0, 2H	2.33~2.39, m, 2H	2.25, ni, 8.5, 7.6, 2H	2.41 (qrt-like, 7.7, 7.4, 2H)	2.47 (dd, 15.3, 7.3, 4H)
1.60~1.66, m, 2H	1.62~1.68, m, 2H	1.70~1.80, m, 2H	1.72, qnt, 7.4, 7.0, 2H	1.70~1.80, m, 2H	1.69, ni, 7.6, 7.1, 2H	1.89~1.94 (m, 2H)	2.08 (m, 4H)
2.77~2.92, m, 1H; 2.35~2.47, m, 1H	2.91, ddd, 7.9, 8.2, 11.9, 1H; 2.45, ddd, 5.8, 7.0, 11.9, 1H)	3.30, t, 7.3, 2H	3.30, t, 7.4, 2H	3.30, t, 7.3, 1H; 2.88~2.92, m, 1H	3.31, ni, 7.1, 2H	3.04 (t-like, 7.7, 8.0, 2H)	2.94 (dd, 8.1, 8.1, 2H)
3.12~3.20, m, 1H; 2.20~2.21, m, 1H	3.12, dd-like, 6.7, 7.3, 1H; 2.25, m, 1H)	3.37, t, 7.0, 2H	3.37, t, 7.1, 2H	3.37, t, 7.0, 2H	3.37, ni, 7.0, 2H		
1.74~1.81, m, 1H; 1.40~1.60, m, 1H	1.71~1.82, m, 1H; 1.45, m, 1H	2.01~2.04, m, 2H	2.02, qnt, 7.9, 7.1, 2H	2.01~2.04, m, 2H	2.04, ni, 8.7, 2H		
1.74~1.81, m, 1H; 1.60~1.66, m, 1H	1.71~1.82, m, 1H]; 1.62~1.68, m, 1H	2.33~2.39, m, 2H	2.35, t, 7.8, 2H	2.33~2.39, m, 2H	2.37, ni, 8.0, 2H		
2.77~2.92, m, 1H	2.78, m, 1H						
4.798-4.804, m, 1H	4.80,ddd, 1.8, 1.8, 5.5, 1H						
7.04, t, 1.5, 1H	7.04, d-like, 1.5, 1H						
1.93, s, 3H	1.94, d-like, 1.8, 3H						
2.02, s, 3H	2.02, d, 0.9, 3H	1.99, br, 3H	1.98, d, 0.5, 3H	2.01~2.04, m, 3H	2.03, ni, 0.5, 3H	1.97 (s, 3H)	2.00 (s, 6H)

[a]in $CDCl_3$ at 500 MHz; [b]Nonato et al. (1993), in $CDCl_3$ at 400 MHz; [c]in $CDCl_3$ at 400 MHz; [d]Takayama et al. (2000), in $CDCl_3$ at 500 MHz; [e]Takayama et al. (2002), in $CDCl_3$ at 500 MHz; [f]Sjaifullah and Garson (1996), in $CDCl_3$ at 500 MHz; [g]in $CDCl_3$ at 600 MHz.
d = doublet; m = multiplet; ni = not indicated; qnt = quintuplet; qrt = quartet; s = singlet; t = triplet

for alkaloid 6 and δ 7.03 for H-16 of alkaloid 7 indicated a 1:1:1 mixture of 5, 6, and 7. Table 1 shows that the ^{1}H NMR spectral data for alkaloids 5, 6, and 7 are respectively in agreement with those of Pandamarilactam-3y, -3X (5, 6) and Pandamarilactonine-A (7).

The other isolate was found to be a mixture of alkaloids 7 and 9. The ^{1}H NMR resonances at δ 7.30 (1H), δ 7.09 (1H), δ 7.04 (1H) and δ 6.99 (1H) indicated four γ–substituted α,β-unsaturated γ-lactone moieties; the resonance at δ 5.64 (1H) indicated E-configuration for the H-4 and H-6 of one γ–alkylidine α,β-unsaturated γ-lactone moiety; the resonance at δ 5.17 (1H) indicated Z-configuration for the H-4 and H-6 of another γ–alkylidine α,β-unsaturated γ-lactone moiety, and a multiplet at δ4.80 (2H) is characteristic of the H-15 of the threo isomers Pandamarilactonine-A, -C (7, 9). The peak heights of 1:1 for H-4 at δ 7.30 (1H) for alkaloid 9 and δ 6.99 (1H) for alkaloid 7, peak heights of 1:1 for H-16 at δ 7.04 for alkaloid 9 and δ 7.09 (1H) alkaloid 7, and peak heights of 1:1 for H-6 at δ 5.64 (1H) for alkaloid 9 and δ 5.17 (1H) for alkaloid 7, indicated a 1:1 mixture of 7 and 9. The assignments of the spectral data for alkaloids 7 and 9 were in agreement with those of Pandamarilactonine-A, (7) and, Pandamarilactonine-C (9), respectively, as shown in Table 1.

The ^{13}C NMR peak assignments for alkaloid 2, which consisted of resonances for two C=O of cyclic esters δ 172.9 (C-18) and δ 171.0 (C-2), seven carbons in the olefinic region δ 149.7(C-16), δ 148.5 (C-5), δ 137.6 (C4), δ 131.5 (C-17), δ 129.2 (C-3), δ 113.8 (C-6), and δ 101.7 (C-15), and nine upfield carbons δ 49.5 (C-9), δ 47.1 (C-11), δ 36.1 (C-14), δ 27.1 (C-8), δ 25.0 (C-13), δ 23.9 (C-7), δ 20.7 (C-12), δ 10.7 (C-20), and δ 10.5 (C-21) were in perfect agreement with the data for Pandamarilactone-1 (2) of Nonato et al. (1993). The molecular ion peak $[M]^{+}$. at m/z 317 from LREIMS of alkaloid 2 is also in agreement with the structure of Pandamarilactone-1 (2).

The ^{13}C NMR peak assignments for alkaloid 13, which consisted of resonances at δ 170.91 (C-2), 129.78 (C-3), 137.77 (C-4), 149.30 (C-5), 111.29 (C-6), 23.10 (C-7), 25.36 (C-8), 47.67 (C-9), and 10.53 (C-10) were in agreement with the those for Pandanamine (13) of Takayama et al. (2000). The HMQC spectrum of alkaloid 13 also showed direct correlation of the proton resonances at δ 7.02 (H-4) with the carbon resonances at δ 137.77 (C-4), at δ 5.13 (H-6) with δ 111.29 (C-6), and δ 3.04 (H-9) with δ 47.67 (C-9). The expanded downfield region (δC 170.91 – δC 129.78) of its HMBC spectrum showed correlation of the proton resonance at δ 7.02 (H-4) with the carbon resonances at δ 170.91 (C-2), δ 149.30 (C-5), and δ 129.78 (C-3); it also showed crosspeaks for the correlations of δ 5.13 (H-6) with δ 149.30 (C-5), and δ 137.77 (C-4); correlation of δ 2.41 (H-7) with δ 149.30 (C-5); and the correlations of δ 1.97 (H-10) with δ 170.91 (C-2), δ 137.77, (C-4), and δ 129.78 (C-3). The differential nOe spectrum showed 1.47% enhancement at δ 5.13 (H-6) on irradiation at δ 7.02 (H-4), which confirmed the Z-configuration of the H-4 and H-6 protons. The LRFABMS spectrum gave [M + H]+. ion at m/z 318, which is in agreement with the structure of Pandanamine (13) .

Alkaloid 2, which was at hRf 72 on TLC with SGF_{254} and $CHCl_3$:MeOH (8:2), was the least polar isolate. It was isolated from the CH_2Cl_2:EtOAc fraction as well as from the EtOAc:MeOH fraction, but not from the neat EtOAc fraction, from VLC of the crude alkaloidal extract. The CH_2Cl_2:EtOAc fraction yielded only alkaloid 2, but the EtOAc:MeOH alkaloidal fraction yielded alkaloids 2, 7 (hRf 70), 8 (hRf 70), 13 (hRf 38), mixture of 5, 6, and 7 (hRf 70), and mixture of 7 and 9 (hRf 70). TLC after prolonged storage of alkaloid 2 did not indicate formation of other alkaloids. These results indicated that alkaloid 2 was formed from any of the more polar alkaloids. However, Takayama et al. (2000) has reported that Pandamarilacton[e-1] (2) was not detected from the Pandamarilactonine-A (7) and Pandamarilactonine-B (8) that were acid-treated to test the hypothesis on acid-catalyzed interconversion of 7 and 8 during the isolation process. In addition, the present study observed that 5 and 6 were only minor alkaloids. These results are proofs that alkaloid 2 was formed from alkaloid 13, which is in agreement with the biogenetic route of *Pandanus* alkaloids as proposed by Takayama et al. (2001a).

Pandamarilactone-1 (2) was isolated by Nonato et al. (1993), Azares (1999), and Takayama et al. (2000), which used acid-base extraction, and by this study, which used neutral partitioning. The Pandamarilactonine-A, -B, -C (7, 8, 9) were isolated by Takayama et al. (2000) and this study, which used acid-base extraction and neutral partitioning, respectively. The Pandamarilactam-3x and -3y (5, 6) from acid-base extraction of Sjaifullah & Garson (1996) were isolated by this study as a mixture with alkaloid 7 from neutral partitioning. The 6Z-Pandanamine (13) was isolated from acid-base extraction by Takayama et al. (2001), obtained as a mixture with 6E-Pandanamine (14) from neutral partitioning by Salim et al. (2004), and isolated as pure 13 from neutral partitioning in this study. Salim et al. (2004) did not report isolation nor detection of 2, 5, and 6 from either acid-base extraction or neutral partitioning, but proved that the mixture of 13 and 14 subjected to the acid-base extraction condition yield mixture of the Pandamarilactonines 7-10.

The isolation of 2, 5-6, 7-9, and 13 by this study corroborated the results from other countries despite the differences in the sites and years of leaves collection

and in the extraction and partitioning conditions, which strongly suggest that these alkaloids are natural products of *P. amaryllifolius* and are not experimental artifacts.

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