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New pyrrolidine alkaloids from the roots of *Pandanus amaryllifolius*

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ABSTRACT

Four new alkaloids, pandamarilactonines-E, -F, and -F-*N*-oxide consisting of a pyrrolidine moiety and two α-methyl-γ-lactone residues, and pandamarilactonine-G, possessing a pyrrolidinone function, were isolated from the roots of *Pandanus amaryllifolius*. Their structures were determined based on spectroscopic analyses and chemical syntheses.

Pandanus amaryllifolius Roxb. (Pandanaceae), commonly known as the fragrant screw pine, is distributed in parts of Southeast Asia.[1] Ethnobotanical literature documents this *Pandanus* species to possess several medicinal properties such as antispasmodic, diuretic, and stimulant properties.[2] In the course of our phytochemical efforts to search for novel and biologically active metabolites from the genus *Pandanus*, several novel alkaloids bearing γ-butylidene-α-methyl α,β-unsaturated γ-lactone and pyrrolidinyl α,β-unsaturated γ-lactone motifs were identified from the leaves of *P. amaryllifolius* and *Pandanus dubius*.[3–5] In this Letter, we report the isolation and structure elucidation of four new pyrrolidine-type alkaloids namely, pandamarilactonines-E (**1**), -F (**2**), -F-*N*-oxide (**3**), and -G (**4**) from the roots of *P. amaryllifolius* (Fig. 1).

Chromatographic separation of the crude alkaloid mixture obtained from the MeOH extract[6] of *P. amaryllifolius* roots afforded four new alkaloids, **1–4**. In addition, the known alkaloids, pandamarilactonine-A, -B, -C, and -D, were also identified by comparison of their spectral data with those in the literature.[3a,d]

Pandamarilactonine-E (**1**) was obtained as an amorphous solid and observed to be optically active, $[\alpha]_D^{15}$ +49 (*c* 0.09, $CHCl_3$). Its molecular formula as deduced by HR-ESIMS is $C_{18}H_{29}NO_4$ (*m/z* 346.1977 $[M+Na]^+$, calcd for $C_{18}H_{29}NO_4Na$, 346.1989). Coupled with DEPT and HMQC data, the ^1H and ^{13}C NMR data (Table 1) of **1** showed the presence of two methyls, nine methylenes (two nitrogen-bearing), five methines (one nitrogen-bearing and two oxygenated), and two carbonyls. The spectroscopic data for the upper segment of **1**, which is composed of a 3,5-disubstituted γ-butyrolactone unit, are comparable to that of dubiusamine-B (**5**),[3e] where a *trans* relationship is noted between H-3 and H-5 methine protons. This was confirmed by the ^1H–^1H COSY correlations (Fig. 2) of H_3-21/H-3 to H_2-9, the HMBC correlations of H_3-21 to C-2/C-3, and the NOE correlations between H-5 methine (δ_H 4.50) and H-21 methyl (δ_H 1.27). The lower segment of **1**, which is composed of a γ-pyrrolidinyl-α-methyl-γ-butyrolactone unit, was also established by ^1H–^1H COSY correlations of H_2-11 to H-17/H_3-20 and HMBC correlations of H_2-11/C-14, H-17/C-16/C-18/C-20 and H_3-20/C-16/C-17/C-18. The relative stereochemistry at the lower segment in **1** was elucidated by NOE experiments (Fig. 3). Irradiation of H-15 proton (δ 4.43) resulted in a correlation between H-14 (δ 2.86) and H-17 (δ 2.78) methine protons. NOE analysis of H-17 methine, on the other hand, showed a peak enhancement of H-15 methine and H_3-20 methyl (δ 1.28). These results suggested that the methine protons at H-15 and H-17 were in *syn* orientation. As with known alkaloids pandamarilactonines-A to -D, the stereo centers at C-14 and C-15 in **1** may assume either the *threo* or *erythro* configuration. The observed NOE correlation between H-14 and H-20 proposes a relative *threo* configuration at C-14 and C-15 stereocenters. If an *erythro* configuration, such as that in pandamarilactonine-F (see vide infra), is assumed for **1**, the NOE correlation between H-14 and H-20 should not be observed on the basis of the Dreiding model analysis. The gross structure that was realized by connecting the upper and lower segments of **1** was established through the HMBC correlations observed in H_2-9/C-11 and H_2-11/C-9.

To unambiguously confirm these assignments, including the structure and the absolute configuration of **1**, the stereoselective hydrogenation of dubiusamine-B (**5**),[3e] which was synthesized from D-prolinol in the enantiomerically pure form, was carried out (Scheme 1). Synthetic **1** {$[\alpha]_D^{19}$ +50 (*c* 0.10, $CHCl_3$)} is completely

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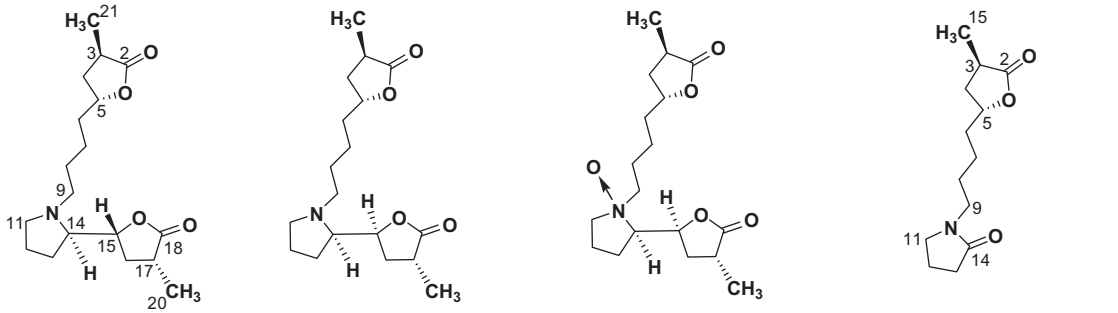


Figure 1. Structures of new alkaloids from the roots of *P. amaryllifolius*.

Table 1
^{1}H (400 MHz) and ^{13}C NMR (125 MHz) data for **1** and **2** in $CDCl_3$

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
2	180.2	—	180.1	—
3	34.0	2.70, overlapped	34.0	2.70, overlapped
4	35.4	2.12, ddd (12.8, 9.2, 5.2) 2.00, m	35.3	2.14, overlapped 2.01, ddd, (12.8, 7.6, 7.6)
5	78.4	4.50, dddd (12.8, 7.6, 7.6, 5.2)	78.4	4.52, dddd (12.8, 7.6, 7.6, 5.2)
6	35.4	1.79–1.64, overlapped 1.61–1.37, overlapped	35.4	1.76, m 1.70–1.37, overlapped
7	23.1	1.61–1.37, 2H, overlapped	23.4	1.70–1.37, 2H, overlapped
8	29.0	1.61–1.37, 2H, overlapped	28.5	1.70–1.37, 2H, overlapped
9	57.3	2.70, overlapped 2.36, overlapped	56.0	2.95, m 2.32, m
11	54.8	3.11, ddd (9.2, 6.4, 2.8) 2.24, ddd (9.2, 9.2, 6.8)	54.6	3.15, m 2.23, m
12	26.9	1.79–1.64, 2H, overlapped	27.1	1.94–1.83, overlapped 1.70–1.37, overlapped
13	24.1	1.88, overlapped 1.61–1.37, overlapped	23.1	1.70–1.37, 2H, overlapped
14	65.8	2.86, ddd (9.6, 4.4, 4.4)	67.0	2.65, m
15	80.9	4.43, ddd (7.6, 4.4, 4.4)	82.3	4.40, br q (6.9)
16	31.3	2.36, overlapped 1.88, overlapped	33.0	2.14, overlapped 1.94–1.80, m
17	34.5	2.78, m	34.3	2.70, overlapped
18	180.6	—	180.2	—
20	16.4	1.28, 3H, d (7.2)	16.4	1.28, 3H, d (7.6)
21	16.0	1.27, 3H, d (7.2)	15.9	1.28, 3H, d (7.6)

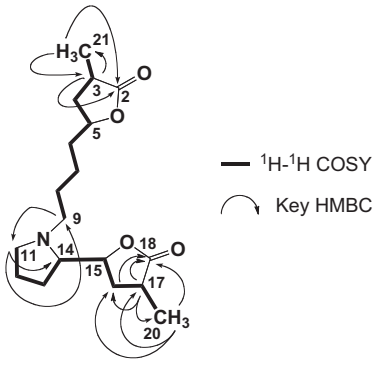


Figure 2. COSY and selected HMBC correlations of **1**.

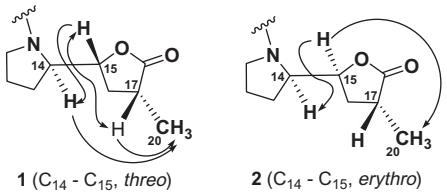
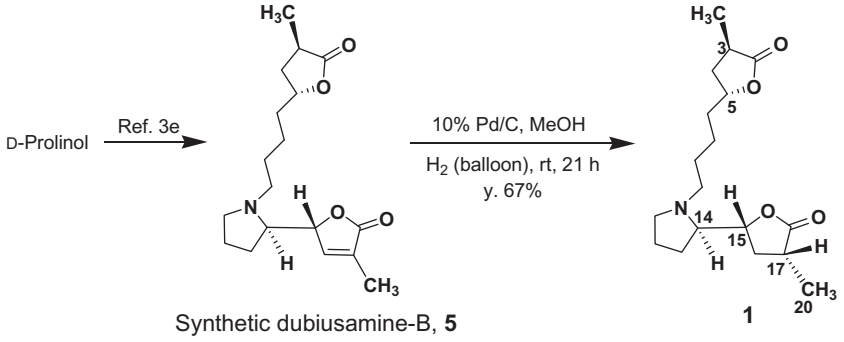


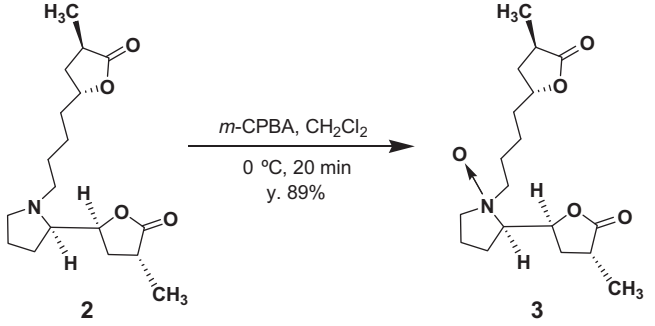
Figure 3. Important NOE correlations of **1** and **2**.

identical with the natural product (^{1}H NMR, ^{13}C NMR, MS). Thus, the structure and the absolute configuration (3*R*, 5*R*, 14*R*, 15*R*, 17*R*) of **1** were established.

Pandamarilactonine-F (**2**), an amorphous solid, $[\alpha]_D^{15}$ +6.7 (*c* 0.12, $CHCl_3$), was determined to have the molecular formula $C_{18}H_{29}NO_4$ (*m/z* 346.1981 $[M+Na]^+$, calcd for $C_{18}H_{29}NO_4Na$, 346.1989) by HR-ESIMS. Analysis of ^{1}H and ^{13}C NMR data (Table 1), as well as its molecular formula, suggested that **2** is a stereoisomer of **1**. NOE experiments on **2** (Fig. 3) revealed a *syn* relationship for H-15 and H_3-20 protons. A relative *erythro* configuration at positions C-14 and C-15 was inferred based on the observed pattern of the chemical shifts and the coupling constants in known *Stemona*[7,8]



Scheme 1. Hydrogenation of **5** into **1**.



Scheme 2. Chemical transformation of **2** into **3**.

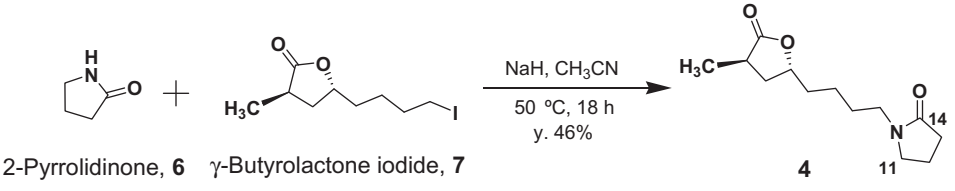
alkaloids and pandamarilactonines-A to -D[3d] that also contained the pyrrolidinyl-γ-butyrolactone unit. Thus, the H-14 methine peak is shifted upfield for the *erythro* isomer (**1**, δ_H 2.86; **2**, δ_H 2.65), while the C-14 peak is shifted upfield for the *threo* isomer (**1**, δ_C 65.8; **2**, δ_C 67.0). The coupling constant ($J_{14,15}$) between the two stereogenic centers at C-14 and C-15 also differentiated the *threo* and *erythro* isomers, as in the case of the diastereomeric pairs of alkaloids saxorumamide ($J_{11,12}$ = 2.0 Hz, *threo*) and isosaxorumamide ($J_{11,12}$ = 6.9 Hz, *erythro*),[8a] or 11(*S*),12(*R*)-dihydrostemofoline ($J_{11,12}$ = 3.0 Hz, *threo*) and 11(*S*),12(*S*)-dihydrostemofoline ($J_{11,12}$ = 7.0 Hz, *erythro*).[8b] For pandamarilactonines-E and -F, $J_{14,15}$ = 4.4 Hz (**1**, *threo*) and $J_{14,15}$ = 6.9 Hz (**2**, *erythro*) were observed, respectively. The above-described arguments hence suggested a relative *erythro* configuration at C-14 and C-15 in **2**. Based on these findings and in correlation to those of **1**, the configuration for pandamarilactonine-F is assumed to be 3*R**, 5*R**, 14*R**, 15*S**, 17*R**.

New alkaloid **3**,[9] $[\alpha]_D^{15}$ +53 (*c* 0.04, $CHCl_3$), had the molecular formula $C_{18}H_{29}NO_5$ (*m/z* 340.2114 $[M+H]^+$, calcd for $C_{18}H_{30}NO_5$, 340.2118) as determined by HR-ESIMS. The presence of an additional oxygen atom and the downfield-shifted signals of the protons at positions H-9 (δ 3.57, δ 3.33), H-11 (δ 3.65, δ 3.16), H-14 (δ 3.42), and H-15 (δ 5.47) in comparison with those of alkaloids **1** and **2**, including its polar behavior in chromatography, suggest that **3** is an *N*-oxide of either pandamarilactonine-E or -F. To verify this hypothesis, alkaloids **1** and **2** were oxidized to their corresponding *N*-oxide derivative using *m*-CPBA (Scheme 2). The spectral data, including the optical rotation {$[\alpha]_D^{18}$ +55 (*c* 0.1, $CHCl_3$)} of the semi-synthetic product obtained from **2**, were completely identical with those of the natural product. NOE correlations observed for H-14, H-15, and H_3-20 in **3** were also in agreement with those of **2**. Thus, new alkaloid **3** is pandamarilactonine-F-*N*-oxide and has the same stereochemistry as that of **2**.

Pandamarilactonine-G (**4**)[10] was isolated as a colorless oil {$[\alpha]_D^{14}$ +61 (*c* 0.08, $CHCl_3$)} and its molecular formula was determined to be $C_{13}H_{21}NO_3$ by HR-ESIMS (*m/z* 262.1406 $[M+Na]^+$, calcd for $C_{13}H_{21}NO_3Na$, 262.1414). ^{13}C NMR and DEPT spectra showed 13 carbon signals that were assignable to one methyl group and eight methylene, two methine, and two carbonyl groups. Analysis of the NMR data revealed that **4** had the same 3,5-disubstituted γ-butyrolactone moiety as alkaloids **1** and **2**, including the *trans* stereochemical relationship of the H-3 and H-5 protons, as evidenced by NOE analysis. The pyrrolidinone unit of **4** was deduced on the basis of the ^1H–^1H COSY correlations of H-11/H-12/H-13 and HMBC correlations of C-14/H-11/H-12/H-13. Finally, the HMBC correlations of C-14/H-9, C-11/H-9, and C-9/H-11 permitted the elucidation of the gross structure of pandamarilactonine-G as that of **4**.

For a conclusive assignment of the structure and the absolute stereochemistry of pandamarilactonine-G, its synthesis utilizing the condensation of 2-pyrrolidinone (**6**) and γ-butyrolactone iodide[3e] (**7**) was attempted (Scheme 3).[11] The spectral data including the optical rotation {$[\alpha]_D^{15}$ +70 (*c* 0.05, $CHCl_3$)} of the synthetic **4** were completely identical with those of the natural product. Thus, the structure including the absolute configuration (3*R*, 5*R*) of pandamarilactonine-G was unambiguously determined.

In conclusion, four new pyrrolidine alkaloids, pandamarilactonine-E (**1**), -F (**2**), -F-*N*-oxide (**3**), and -G (**4**), together with pandamarilactonines-A to -D, were isolated from the roots of *P. amaryllifolius*. The structure, including the absolute configuration of **1** and **4** were established by chemical syntheses.



Scheme 3. Synthesis of **4**.

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6. The whole plant of *P. amaryllifolius* was collected in Nueva Vizcaya, Philippines, and identified by Assistant Professor Rosie Madulid, Department of Biological Sciences, College of Science, University of Santo Tomas. A voucher specimen (USTH-3728) was deposited at the Plant Sciences Herbarium, Research Center for the Natural Sciences, University of Santo Tomas. The air-dried, ground roots (523 g) were exhaustively extracted with MeOH (8.6 L) five times and filtered. The combined filtrates were concentrated under reduced pressure to obtain the MeOH extract (116 g). This was dissolved in 1 N HCl and extracted thrice with EtOAc. The aqueous layer was basified with Na_2CO_3 (pH 7–8) and exhaustively extracted with 5% MeOH/$CHCl_3$. The combined organic layers were dried with anhydrous $MgSO_4$ and evaporated to obtain the crude base (2.1 g). A portion of the crude base (2 g) was initially separated by silica gel flash column chromatography using $CHCl_3$/MeOH gradient. The 2–5% MeOH/$CHCl_3$ eluate was separated by silica gel flash column chromatography using $CHCl_3$/EtOH gradient to give four fractions, F1–F4. Fractions F1, F2 and F3 were separated by MPLC (60% EtOAc/hexane or 80% EtOAc/hexane) to afford pandamarilactonines-A (9.9 mg), -B (9.6 mg), -C (1.1 mg), and -D (3.0 mg). F4 was separated by silica gel flash column chromatography (EtOAc–2% EtOH/$CHCl_3$) and amino-silica gel chromatography (40–50%–90% EtOAc/hexane) to afford pandamarilactonine-E (**1**, 4.4 mg), -F (**2**, 3.6 mg) and -G (**4**, 1.7 mg). The 50% MeOH/$CHCl_3$–MeOH eluate was separated by amino-silica gel (10% EtOH/$CHCl_3$) and silica gel (20% MeOH/$CHCl_3$) open column chromatography to afford pandamarilactonine-F-*N*-oxide (**3**, 1.2 mg).
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9. Pandamarilactonine-F-*N*-oxide (**3**). Amorphous solid; $[\alpha]_D^{15}$ +53 (*c* 0.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.47 (1H, m, H-15), 4.55 (1H, dddd, *J* = 10.4, 8.0, 8.0, 5.2, H-5), 3.65 (1H, ddd, *J* = 12.4, 12.4, 4.4, H-11), 3.57 (1H, m, H-9), 3.42 (1H, ddd, *J* = 11.2, 8.0, 8.0, H-14), 3.33 (1H, m, H-9), 3.16 (1H, m, H-11), 2.69 (2H, overlapped, H-3, H-17), 2.38 (1H, m, H-12), 2.24 (1H, m, H-8), 2.15 (3H, overlapped, H-4, H-12, H-16), 2.03 (3H, overlapped, H-4, H-8, H-16), 1.79–1.48 (6H, overlapped, H_2-6, H_2-7, H_2-13), 1.32 (3H, d, *J* = 7.6, H_3-20), 1.29 (3H, d, *J* = 7.2, H_3-21); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 180.1 (C=O, C-2), 179.1 (C=O, C-18), 79.1 (CH, C-14), 77.8 (CH, C-5), 74.7 (CH, C-15), 67.3 (CH_2, C-9), 66.9 (CH_2, C-11), 35.4 (CH_2, C-4), 34.8 (CH_2, C-6), 34.3 (CH, C-17), 34.0 (CH_2, C-3), 33.4 (CH_2, C-16), 24.0 (CH_2, C-13), 23.7 (CH_2, C-8), 22.7 (CH_2, C-12), 19.6 (CH_2, C-7), 15.9 (CH_3, C-21), 15.8 (CH_3, C-20); HR-ESIMS: calcd for $C_{18}H_{30}NO_5$ $[M+H]^+$: 340.2118, found: 340.2114.
10. Pandamarilactonine-G (**4**). Colorless oil; $[\alpha]_D^{14}$ +61 (*c* 0.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 4.49 (1H, dddd, *J* = 13.2, 8.0, 8.0, 5.6, H-5), 3.37 (2H, dd, *J* = 7.0, 7.0, H_2-11), 3.28 (2H, m, H_2-9), 2.69 (1H, m, H-3), 2.39 (2H, dd, *J* = 8.0, 8.0, H_2-13), 2.10 (1H, ddd, *J* = 13.2, 9.2, 5.2, H-4a), 2.01 (3H, overlapped, H-4b, H_2-12), 1.69 (2H, m, H_2-6), 1.57 (2H, m, H_2-8), 1.42 (2H, m, H_2-7), 1.28 (3H, d, *J* = 7.6, H_3-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 180.0 (C=O, C-2), 175.0 (C=O, C-14), 78.2 (CH, C-5), 47.1 (CH_2, C-11), 42.1 (CH_2, C-9), 35.5 (CH_2, C-4), 35.0 (CH_2, C-6), 34.0 (CH, C-3), 31.0 (CH_2, C-13), 26.9 (CH_2, C-8), 22.7 (CH_2, C-7), 17.9 (CH_2, C-12), 15.9 (CH_3, C-15); HR-ESIMS: calcd for $C_{13}H_{21}NO_3Na$ $[M+Na]^+$: 262.1414, found: 262.1406.
11. To a solution of **6** (5 mg, 0.059 mmol) in CH_3CN (0.5 mL) at 0 °C was added NaH (3 mg, 0.071 mmol, 1.2 equiv) and the mixture was stirred at room temperature for 30 min. A solution of **7** (22 mg, 0.077 mmol, 1.3 equiv) in CH_3CN (0.3 mL) was added to the mixture and the whole was heated for 18 h at 50 °C. The mixture was filtered using Celite (EtOAc) and the filtrate was evaporated under reduced pressure. Purification by silica gel column chromatography (50% EtOAc/hexane to 5% MeOH/$CHCl_3$) afforded synthetic **4** as colorless oil in 46% yield (6.5 mg). All the spectroscopic data including the optical rotation obtained for synthetic **4** were in excellent agreement with those of natural **4**.