CHAPTER 4

Pandanus Alkaloids: Chemistry and Biology

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Contents

I. INTRODUCTION

The plant family Pandanaceae, otherwise known as the screw pine family, is comprised of four genera, *Freycinetia* (*ca*. 200 species), *Sararanga* (two species),

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Pandanus (1,2), and the latest addition to the family, the genus *Martellindendron* (seven species) (3–5). The king of the family, the genus *Pandanus*, is made up of about 700 known species distributed mainly in tropical and subtropical regions; 52 species of the genus are found in the Philippines. The word ''pandans'' comes from Malay, and is applied to any member of the family Pandanaceae, a family of arborescent or lianoid dioecious monocotyledons.

A certain degree of confusion is found in the nomenclature of *Pandanus* species, since several names are known for the same species (2). At the commencement of our research program on the genus *Pandanus*, little was known regarding the chemistry and biological activities of this genus, with only four species reported in the literature. From the constituents of *Pandanus* plants, essential oils from *Pandanus latifolius* (6), lignans and ionones (7) and essential oils (8) from *Pandanus tectorius*, lignans and benzofurans from *Pandanus odoratissimus* (9), as well as 4-hydroxybenzoic acid from *Pandanus odorus* (10) were previously characterized.

P. tectorius (11) and *P. latifolius* (6) were found to contain sterols and the terpene, linalool, respectively. Alkaloids were detected in *P. veitchii* (12) and *P. amaryllifolius* (13). *P. boninesis* Warb. is indigenous to Bonin island in Japan, and has been used as a roadside tree with the fruits used as food (14). Two novel triterpenoids were isolated from the leaves of *P. boninesis* and their structures were elucidated as (24*S*)-24-methyl-25,32-cyclo-5α-lanost-9(11)-en-3*β*-ol and (24*S*)-24-methyl-25,32-cyclo-cycloartan-3*β*-ol (15).

The genus *Pandanus* is present throughout Southeast Asia and Northern Australia. Fiber from the leaves is used to make mats and baskets throughout the region, while local tribes and native animals eat the nut kernel in the fruits (16). The most studied plant in the genus is *P. amaryllifolius* Roxb.

P. amaryllifolius (Pandanaceae) is the only reported *Pandanus* species with scented leaves (Figure 1) (2). This plant is also known as fragrant screw pine, toei hom (Thailand), pandan mabango (Philippines), pandan wangi (Malay), and daun pandan (Indonesia). The leaves are used as a food flavoring, and in traditional medicine in the Philippines, Thailand, and Indonesia. *P. amaryllifolius* is used popularly as a flavoring for rice because it emits a peculiar odor similar to ''ambermohor'' rice (17). The alkaloid 2-acetyl-1-pyrroline was identified as the flavoring agent (13,18). The leaves are used medicinally in Southeast Asia to refresh the body, reduce fever, and relieve indigestion and flatulence (19). The oil of the leaf is described as a purgative, as a treatment for leprosy, and as a stimulant and antispasmodic. It is also reported to be effective against headaches, rheumatism, and epilepsy, and as a cure for sore throat (20). The seeds are reported to strengthen the heart and liver, while the roots are used as a diuretic and an aphrodisiac (20,21). In Indonesia, the volatile oil of *P. amaryllifolius*, known as ''pandan wangi,'' is used as a remedy for toothache, rheumatism, and as a tranquilizer (22). Hot water extracts of the root of this plant (reported as *P. odorus* Ridl.) show hypoglycemic activity, and 4-hydroxybenzoic acid has been isolated as the active principle (23,24).

The leaves contain essential oils, carotenoids, tocopherols and tocotrienols (25), quercetin (26), and non-specific lipid transfer proteins (27). A lectin,

Figure 1 *Pandanus amaryllifolius* Roxb. plant.

pandanin, was recently isolated from the saline extract of the leaves of *P. amaryllifolius* using ammonium sulfate precipitation affinity chromatography on mannose–agarose and molecular size exclusion by gel filtration. The unglycosylated protein pandanin exhibits hemagglutinating activity toward rabbit erythrocytes, and its activity could be reversed exclusively by mannose and mannan. Pandanin also possesses antiviral activities against the human viruses herpes simplex virus type-1 (HSV-1) and influenza virus (H1N1) with 3-day EC_{50} values of 2.94 and 15.63 μM, respectively (28).

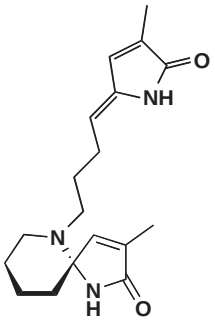
Two other non-specific lipid transfer proteins were also isolated from the saline extract of the mature leaves of *P. amaryllifolius* using affinity chromatography on fetuin-agarose and Affi-gel Blue gel anion exchange chromatography as well as gel filtration. The proteins were demonstrated as non-glycoproteins, with a molecular mass of 18 and 13 kDa, respectively, comprising peptide subunits from 6.5 to 9 kDa in the forms of a heterodimer and a homodimer. The proteins exhibit weak to moderate hemagglutinating activity toward rabbit erythrocytes, however, this activity could not be reversed by mannose. They thus could be easily differentiated from the mannose-binding lectin even though all three proteins have subunits with similar molecular weight (27).

II. ISOLATION AND STRUCTURE ELUCIDATION

A. (±)-Pandamarine from *Pandanus amaryllifolius* (29)

Phytochemical screening of Philippine medicinal plants revealed the presence of alkaloids in the leaves of *P. amaryllifolius* as detected by the Culvenor–Fitzgerald

test. A specimen collected from Isabela, Philippines was extracted with ethanol, and the 5% aq. H_2SO_4 fraction basified with aq. NH_3 and extracted into chloroform, yielding a gum, which crystallized on standing. The crystals were identified as (±)-pandamarine (**1**); this component was the major alkaloid isolated from the leaves of the plant sample. In the ^{1}H NMR spectrum, signals at δ 6.60 (bs), 6.72 (bs), and 5.19 (t, J=8 Hz) identified three trisubstituted alkenes, as did ^{13}C signals at 117.1 (d), 133.6 (s), 134.4 (d), 135.4 (s), 138.9 (s), and 148.0 (d) ppm. The compound also possessed two methyl substituents (^{1}H: δ 1.89 (bs) and 1.78 (d, J=1.5 Hz)) and two amide groups (^{13}C: δ 175.2 (s) and 175.0 (s)). A signal at δ 80.0 was assigned to the spiro carbon center. After recrystallization from a mixture of MeOH, EtOAc, and ether, the complete structure of pandamarine (m.p. 210–211°C) was determined by an X-ray diffraction study. Diffractometer data at 295 K were refined by least squares techniques to a residual of 0.049 2244 "observed" reflections. Crystals of (±)-pandamarine were triclinic, and of space group P 1, a=13.077(2), b=9.857(5), c=7.214(2) Å, α=106.91(3), β=96.22(2), γ=100.01(2)°, Z=2 (29). From the X-ray structure, it was shown that pandamarine contains two γ-alkylidene-α,β-unsaturated lactam moieties, with a piperidine ring in a chair conformation and perpendicular to one of the lactam rings. The C-5/C-6 alkene bond has a *Z*-configuration. Full ^{1}H and ^{13}C assignments were not provided for this compound since HMBC data were not available (29).



(±) Pandamarine (**1**)

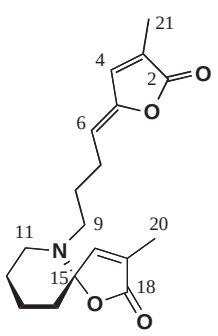
B. Pandamarilactones (30)

Attempts to identify other alkaloids in the leaves of *P. amaryllifolius* afforded three novel alkaloids known as pandamarilactones. The alcohol extract of *P. amaryllifolius* leaves collected from Manila, Philippines, on concentration under vacuum, yielded a dark green resinous material. Partitioning between Et_2O and 5% H_2SO_4 gave a green resinous organic extract. Alkalinization of the green resinous extract, and subsequent extraction with $CHCl_3$, gave a brownish-green

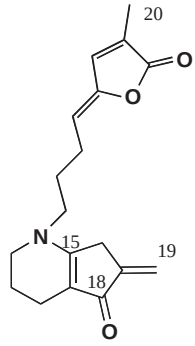
resinous material. A series of chromatographic purifications using $CHCl_3$ with increasing concentrations of MeOH, and reverse-phase HPLC eluting isocratically in 80% MeCN in H_2O, gave a yellow amorphous solid, which was identified as pandamarilactone-1 (**2**). Two other alkaloids pandamarilactone-32 (**3**) and pandamarilactone-31 (**4**) were purified by reverse-phase HPLC using a linear gradient from H_2O to MeCN with UV detection at 254 nm (30).

Pandamarilactone-1 (**2**) was optically active with $[\alpha]_D^{23}=-35$ (*c*. 1.00, MeOH). High-resolution mass spectrometry gave an *m/z* of 317.1635 and established the molecular formula as $C_{18}H_{23}NO_4$, which corresponds to pandamarine ($C_{18}H_{25}N_3O_2$), but with two nitrogen atoms instead of two oxygen atoms. The IR spectrum of **2** showed absorption bands indicative of the α,β-unsaturated five-membered ring lactone at 1764 cm^{-1}, and the enol ester structure at 1695 cm^{-1}. These IR data indicated that pandamarilactone-1 has the structure **2**, in which the two lactam rings of **1** were instead replaced by lactone rings. The ^1H NMR spectrum was in agreement with the proposed structure for **2** and was similar to that of pandamarine (**1**). All other 2D NMR data supported the structure of **2**. ROESY data revealed a correlation between H-4 at δ 6.98 and H-6 at δ 5.04, confirming the *Z* geometry of the C-5/C-6 double bond.

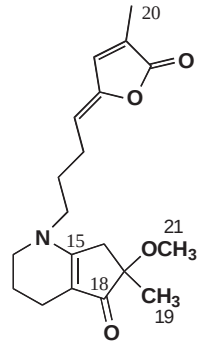
Pandamarilactone-32 (**3**), for which an $[\alpha]_D$ was not recorded, was obtained as a white amorphous solid and was the major alkaloid based on its yield. The alkaloid purified by reverse-phase HPLC gave an *m/z* of 299.1521 by high-resolution EI mass spectrometry, consistent with the molecular formula $C_{18}H_{21}NO_3$ for nine double bond equivalents. The ^1H and ^{13}C NMR spectra revealed identical signals to those of **2** for the upper part of the structure, but that there were different signals for the lower portion. The UV absorption at λ_{max} 325 nm was in agreement with an α,β-unsaturated five-membered ring carbonyl with a nitrogen auxochrome at the β-position (31), while the absorption at 279 nm was in accordance with a five-membered lactone, as in **2**. The IR spectrum revealed the presence of carbonyl signals at 1764 cm^{-1} and 1710 cm^{-1} for a vinyl ester and an enol ester, respectively. A signal at 1667 cm^{-1} was suggestive of a vinylogous amide. The lower ring system had four quaternary carbons (three olefinic carbons and one carbonyl carbon), and five methylenes, of which the one at δ 110.8 (C-19) was an alkene based on the APT ^{13}C spectrum. Important ^{13}C signals at δ 168.0 and 188.0, were assigned to the β-carbon and the carbonyl carbon of a β-substituted vinylogous amide, respectively (32). HMBC data provided the structural information that established the lower part of Pandamarilactone-32. The exo-methylene signals for H-19 at δ 5.89 and 5.14 and the two-proton singlet at δ 3.16 (H-16) all correlated with the signals at δ 188.0 (C-18) and δ 141.0 (C-17). The H-16 signal also correlated to carbons at δ 113.0 (s) and 168.0 (s) assigned to C-14 and C-15, respectively, while the H-19 protons correlated to C-16 at δ 30.9. Pandamarilactone-32 thus contained a *N*-alkyl-6-methylene-hexahydro-5*H*-pyrindin-5-one unit. A *Z* geometry of the C-5/C-6 alkene was revealed by the ROESY correlation between H-4 at δ 6.98 and H-6 at δ 5.09.



Pandamarilactone-1 (**2**)



Pandamarilactone-32 (**3**)



Pandamarilactone-31 (**4**)

Pandamarilactone-31 (**4**), $[\alpha]_D^{23}=-2.0$ (*c*. 1.0, $CHCl_3$) showed the presence of a vinyl lactone from its UV absorption at 295 nm. From its HREI mass spectrum of *m/z* 331.1940 for $C_{19}H_{25}NO_4$, the addition of methanol to the molecular formula of **3** was apparent, resulting in eight double bond equivalents. The ^1H NMR spectrum showed similar resonances with **3**, but lacked the exo-methylene proton signals corresponding to H-19. Instead, four new proton resonances were observed. These were the two signals at δ 2.69 (d, *J*=17.0 Hz) and 2.45 (d, *J*=17.0 Hz) for the non-equivalent geminal protons, the methoxy signal at δ 3.20 (s, 3H) and a methyl singlet at δ 1.37. Comparison of the NMR data of **4** with **3** together with 2D NMR experiments confirmed the structure of pandamarilactone-31. In particular there were correlations between the H-16 protons and carbons at δ 188.0 (C-18) and at δ 23.0 (C-19), and between the methoxy or methyl proton signals and the quaternary carbon at δ 80.0 (C-17). Based on ROESY data, the stereochemical assignment at C-5/C-6 was again *Z* as in alkaloids **1**–**3**. The absolute configuration at C-17 was not defined. Tables I and II present the ^1H and ^{13}C NMR assignments for **2**–**4**.

Pandamarilactonine-31 (**4**) was unlikely to be an isolation artifact since an alternative orientation for the addition of methanol to the reactive enone moiety of pandamarilactonine-32 (**3**) would have been expected on electronic grounds.

All three pandamarilactones, together with pandamarine (**1**), derive from a C9–N–C9 precursor with modifications around the lower portion of the molecules. The skeletal structure of alkaloids **3** and **4** had not previously been reported in the literature. The five-membered lactone ring in the alkaloids **2**–**4**, or the lactam ring of alkaloid **1**, are most likely to be derived from 4-hydroxy-4-methyl glutamic acid. This amino acid has been identified in *P. veitchii* (33) and its biosynthesis has been studied by Peterson and Fowden (34). Further discussion of the biosynthetic pathway that may lead to alkaloids **3** and **4** is provided in Section IV.

C. Pyrrolidinones (35)

The isolation of novel piperidine alkaloids from the leaves of *P. amaryllifolius* collected in various parts of the Philippines prompted Garson *et al*. (35) to look at

Table I ^{1}H NMR spectral data of alkaloids **2–4**[a]

#	**2**[b]	**3**	**4**
4	6.98, ddd, 1.2, 1.0	6.98, ddd, 2.0, 1.0	6.99, ddd, 1.2, 1.0
6	5.04, ddd, 8.0, 1.0	5.09, ddd, 8.0, 1.0	5.10, dd, 8.0, 1.0
7	2.31, ddd, 8.0, 7.2	2.39, ddd, 8.0, 8.0	2.39, ddd, 8.0, 7.6
8	1.54, dddd, 8.0, 7.2	1.77, dddd, 8.0, 8.0	1.76, dddd, 7.6, 7.6
9	2.45, dd, 1.0	3.30, m	3.26 dd, 7.6
11	2.79, dd, 7.0	3.27, m	3.25, m
12	1.72, m	1.85, m	1.80–1.90, m
13	1.72, m	2.33, dd, 6.0	2.25, br dd, 4.8, 1.2
14	1.72, m		
16	6.68, dd, 1.2	3.16, br s	2.69, d, 17.0 2.45, d, 17.0
19		5.89, ddd, 1.0, l.0 5.14, ddd, 1.0, l.0	1.37, s
20	1.86, br s	2.00, d, 2.0	2.00, d, 1.2
21	2.00, br s		3.20, s

[a]400 MHz; solution in $CDCl_3$ referenced at δ 7.25.
[b]J values in Hz.

Table II ^{13}C NMR spectral data of alkaloids **2–4**[a]

#	**2**	3	HMBC correlations	**4**
2	171.0, s	171.0, s	H20, H4	171.0, s
3	129.2, s	129.0, s	H20, H4	130.0, s
4	137.6, d	137.0, d	H20, H6	137.0, d
5	148.6, s	149.0, s	H4, H6	149.0, s
6	113.7, d	111.6, d	H7, H4	112.0, d
7	24.0, t	23.6, t	H6, H8	23.0, t
8	27.2, t	27.8, t	H7, H9	28.0, t
9	50.7, t	50.9, t	H11	51.0, t
11	47.2, t	48.0, t	H9	48.0, t
12	20.8, t	21.1, t	H11, H13	21.0, t
13	25.1, t	18.0, t	H12	18.0, t
14	36.2, s	113.0, s	H16, H13, H12	108.0, s
15	101.7, s	168.0, s	H16, H13, H11, H9	168.0, s
16	149.7, d	30.9, t	H19	37.0, t
17	131.5, s	141.0, s	H16, H19	80.0, s
18	173.0, s	188.0, s	H16, H19	188.0, s
19		110.8, t	H16	23.0, q
20	10.6, q	10.6, q	H4	10.4, q
21	10.4, q			52.0, q

[a]100 MHz; solution in $CDCl_3$ referenced at δ 77.0.

Pandanus species from other regions of Southeast Asia. Plant samples from Jember, East Java and from Jambi, Sumatra, Indonesia were both found to contain alkaloids.

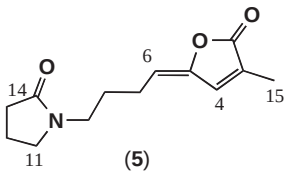
Acid–base extraction and chromatographic separations yielded two new alkaloids named as pandamarilactam-3x (**5**) and -3y (**6**). These alkaloids were related structurally to the pandamarilactones in that they also contained the γ-alkylidene-α,β-unsaturated lactone moiety, but they differed in the lower part where there was a pyrrolidinone ring in each alkaloid instead of the lactone ring that had previously been found. Both pyrrolidinones were isolated from the chloroform fraction of an aqueous ethanolic extract from *P. amaryllifolius* leaves collected near Jambi, and purified by reverse-phase flash chromatography then by reverse-phase HPLC using 80% CH_3CN in H_2O (35).

The molecular ions of pandamarilactam-3x (**5**) and pandamarilactam-3y (**6**) both corresponded to the molecular formula $C_{13}H_{17}NO_3$ with six double bond equivalents. DQFCOSY and HMQC data afforded assignments for the γ-alkylidene-α,β-unsaturated lactone moiety of each compound, while HMBC cross-peaks together with the DQFCOSY data enabled the formation of the fragment containing C-6 to C-9. The pyrrolidinone ring containing the carbonyl at δ 175.0 and three methylenes at δ 47.2, 18.0, and 31.0 was also evident from the COSY and HMBC data. The connectivity of C-9 to the amide carbonyl of the pyrrolidinone ring and other long-range correlations shown in Table III were established through the HMBC data.

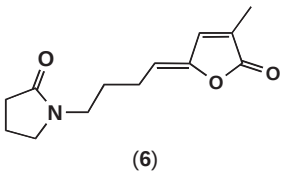
Table III ^1H and ^{13}C NMR assignments for pandamarilactam-3x (**5**) and -3y (**6**)

#	**5**		**6**		HMBC
	$^1H^{a,b}$	$^{13}C^c$	$^1H^{a,b}$	$^{13}C^c$	
2	–	171.0	–	171.0	H4, H15
3	–	131.0	–	129.4	H15
4	7.30 (1.0, 0.5)	135.6	6.98 (1.0, 0.5)	137.7	H6, H15
5	–	148.5	–	148.8	H4, H6, H7
6	5.58 (8.5, 1.0)	112.3	5.18 (7.6, 1.0)	112.8	H7, H8
7	2.25 (8.5, 7.6)	24.0	2.30 (7.6, 7.0)	23.4	H8, H9
8	1.69 (7.6, 7.1)	27.4	1.72 (7.4, 7.0)	26.7	H6, H7, H9
9	3.31 (7.1)	42.0	3.30 (7.4)	41.8	H7, H8
11	3.37 (7.0)	47.1	3.37 (7.1)	47.2	H9, H12, H13
12	2.04 (8.0, 7.0)	17.4	2.02 (7.9, 7.1)	18.0	H11, H13
13	2.37 (8.0)	31.0	2.35 (7.8)	31.0	H11, H12
14	–	175.0	–	175.0	H9, H11, H12, H13
15	2.03 (0.5)	10.8	1.98 (0.5)	10.5	H4

[a]500 MHz; solution in $CDCl_3$ referenced at δ 7.25.
[b]J values (Hz) in brackets.
[c]Inverse detection at 500 MHz; solution in $CDCl_3$ referenced at δ 77.0.



(**5**)



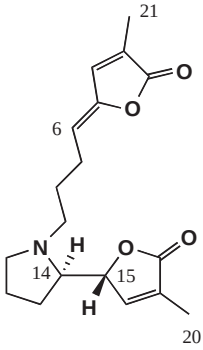
(**6**)

Pandamarilactam-3x (*E*-isomer) (**5**)
Pandamarilactam-3y (*Z*-isomer) (**6**)

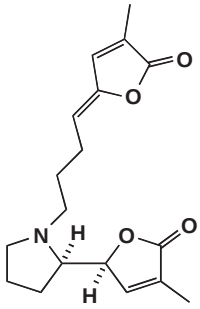
The minor alkaloid pandamarilactam-3x (**5**) showed ^{1}H and ^{13}C spectra, which were closely similar to those of pandamarilactam-3y (**6**), except for the alkene signals at H-4 and H-6 which resonated at δ 7.30 and 5.58, respectively, instead of at δ 6.98 and 5.18 in **6**. The H-6 signal of pandamarilactone-1 (**2**), for which *Z* stereochemistry has been determined by a ROESY experiment, resonated at δ 5.04, which was only 0.14 ppm upfield of the chemical shift value for H-6 in (**6**), but was 0.54 ppm upfield of the H-6 signal in (**5**). In nuclear Overhauser effect (NOE) experiment on a mixture of pandamarilactams-3x and -3y, irradiation of the δ 5·18 signal of alkaloid **6** resulted in enhancement of H-4 at δ 6.98, but there was no NOE enhancement of H-4 at δ 7.30 when the δ 5.58 signal of alkaloid **5** was irradiated. Thus, alkaloids **5** and **6** were the *E*- and *Z*-isomers, respectively. The carbon signal for C-4 of **5** was at δ 135.6 compared to δ 137.7 in **6**.

D. Pandamarilactonines (36–43)

Fresh young leaves of *P. amaryllifolius*, purchased at the flower market in Bangkok, Thailand, afforded two new alkaloids, pandamarilactonine-A (**7**) and pandamarilactonine-B (**8**), both possessing a pyrrolidinyl α,β-unsaturated-γ-lactone residue and a γ-alkylidene-α,β-unsaturated-γ-lactone residue. The alkaloids were isolated, together with the known alkaloid pandamarilactone-1 (**2**), by a series of SiO_2 column chromatography procedures on the alkaloidal fraction obtained by acid–base extraction of an EtOH extract of the sample (36).



Pandamarilactonine-A (**7**)



Pandamarilactonine-B (**8**)

Pandamarilactonine-A (**7**) was obtained as an amorphous powder and was optically active with $[\alpha]_D^{23}$=+35 (*c*. 4.37, $CHCl_3$). The high-resolution FAB-MS gave an *m/z* 318.1721 $[M+H]^+$ for $C_{18}H_{23}NO_4$ suggesting that **7** was an isomer of the co-occurring alkaloid pandamarilactone-1 (**2**). The γ-alkylidene-α-methyl-α,β-unsaturated-γ-lactone moiety of **7** was suggested by UV absorption at 275 nm and was constructed from the diagnostic ^1H and ^{13}C NMR signals that were closely similar to those of **1**–**4**. The three-carbon methylene chain C-7 through C-9 was connected to C-6 of the γ-alkylidene-α-methyl-α,β-unsaturated-γ-lactone moiety through the analyses of the COSY, HMQC, and HMBC data. Likewise, the presence of the α-methyl-α,β-unsaturated-γ-lactone residue was recognized from the ^1H and ^{13}C NMR signals that were similar to those found in pandamarilactone-1 (**2**). The residual four carbons comprising three methylenes (C-11 to C-13), one methine (C-14), and the sole nitrogen atom were suggested to comprise a pyrrolidine ring. The downfield proton signals at δ 2.83 (m) and at δ 4.80 (ddd, *J*=1.8, 1.8, 5.5 Hz) were assigned to H-14 and H-15, respectively. The HMBC spectrum showed correlations from H-14 of the pyrrolidine ring to the methylene carbon at δ 55.0 assigned to C-9 and to the signal at δ 147.0 assigned to C-16 of the α,β-unsaturated-γ-lactone ring. In addition, H-15 of the γ-lactone ring showed connectivity with C-14 and C-13 (δ 23.8) in the pyrrolidine ring. The *Z*-configuration of the γ-alkylidene-α,β-unsaturated-γ-lactone moiety was apparent from the correlation between H-4 and H-6 in the NOESY spectrum. All the observed spectral data pointed to the structure **7** for the new alkaloid, having a novel pyrrolidinyl α,β-unsaturated-γ-lactone skeleton, except that the relative configuration of the vicinal asymmetric centers (C-14/C-15) remained to be determined.

The isomer pandamarilactonine-B (**8**) was also obtained as an amorphous powder but was found to be optically inactive with $[\alpha]_D$ ^{23}=0 (*c*. 0.20, $CHCl_3$). Analyses of the UV, mass, and ^1H and ^{13}C NMR spectra suggested that **8** had an identical carbon skeleton to **7**. When an NOE difference experiment revealed a *Z*-configuration for the γ-alkylidene-α,β-unsaturated-γ-lactone moiety of **8**, it became apparent that **7** and **8** were diastereomers that differed in configuration at either C-14 or at C-15. Thus, **7**/**8** represented a *threo*/*erythro* pair of stereoisomers. Complete ^1H and ^{13}C assignments for the diastereomers **7** and **8** are presented in Table IV. In particular the downfield proton signals at δ 2.70 (m) and at δ 4.71 (ddd, *J*=1.7, 2.0, 5.9 Hz) were assigned to H-14 and H-15, respectively, of **8**.

In synthetic work on alkaloids of the Stemonaceae family, Martin *et al*. (37) assigned the relative configuration of *threo*/*erythro* diastereomers with a pyrrolidinyl α,β-unsaturated-γ-lactone skeleton by comparison of their ^1H and ^{13}C and ^1H chemical shifts at positions equivalent to C-14 and C-15 of the pandamarilactonines. However, the chemical shifts values for H-14/H-15 and for C-14/C-15 of **7** and **8** were closely similar, and so could not be used to define the stereochemistry of **7** and **8**. Instead, the use of *J*-resolved configurational analysis was applied. Three possible staggered conformations for each diastereomer (*erythro*: E1, E2, and E3; *threo* T1, T2, and T3) are shown in Figure 2. The NMR evidence required to distinguish these stereochemical possibilities relies on measurement of $^3J_{H-H}$ values, together with $^3J_{C-H}$ values from PFG J-HMBC 2D

Table IV ^{1}H and ^{13}C assignments for pandamarilactonine-A (**7**) and -B (**8**)

#	**7**		**8**	
	^{1}H $\delta^{a,b}$	$^{13}C^{c}$	^{1}H $\delta^{a,b}$	$^{13}C^{c}$
2		171.1		171.1
3		129.1		129.1
4	6.99 (1H, d, 1.5)	137.7	7.00 (1H, d, 1.5)	137.7
5		148.6		148.5
6	5.18 (1H, dd, 7.9, 7.9)	114.1	5.18 (1H, dd, 7.8, 8.0)	114.1
7	2.43 (2H, dd, 7.3, 15.0)	24.0	2.42–2.48 (2H, m) 2.36 (1H, m)	24.0
8	1.59–1.70 (2H, m)	28.3	1.59–1.67 (2H, m).	28.4
9	2.88 (1H, ddd, 4.0, 7.9, 12.9) 2.45 (1H, m)	55.0	2.73 (1H, m) 2.42–2.48 (1H, m)	55.8
11	3.12 (1H, dd, 6.7, 7.6) 2.21 (1H, m)	54.2	3.12 (1H, m) 2.25 (1H, m)	54.2
12	1.70–1.80 (1H, m) 1.42 (1H, m)	25.7	1.73–1.87 (2H, m)	27.1
13	1.70–1.80 (1H, m) 1.59–1.70 (1H, m)	23.8	1.73–1.87 (2H, m)	24.0
14	2.83 (1H, m)	65.3	2.70 (1H, m)	66.3
15	4.80 (1H, ddd, 1.8, 1.8, 5.5)	83.4	4.71 (1H, ddd, 1.7, 2.0, 5.9)	83.4
16	7.09 (1H, dd, 1.5, 1.8)	147.0	7.05 (1H, dd, 1.5, 1.7)	147.5
17		131.2		130.8
18		174.3		174.3
20	1.93 (3H, dd, 1.5, 1.8)	10.7	1.93 (3H, dd, 1.7, 1.7)	10.8
21	1.99 (3H, d, 0.9)	10.5	1.99 (3H, d, 0.7)	10.5

[a]500 MHz, $CDCl_3$.
[b]*J* values (Hz) in brackets.
[c]125 MHz, $CDCl_3$.

spectroscopy (38), which enables measurement of the torsion angle between two heteroatoms, such as *H*–C–C–*C* (39), in combination with NOE difference measurements that confirm spatial relationships in individual rotamers. In acetone, medium-sized coupling constants were observed between H-14 and H-15 in each diastereomer (**7**, *J*=4.9 Hz; **8**, *J*=3.9 Hz). A large coupling constant (*J*=5.6 Hz) between H-14 and C-16 and a small coupling constant (*J*=3.7 Hz) between H-15 and C-13 were measured for pandamarilactonine-A (**7**) by PFG J-HMBC 2D spectroscopy, indicating their *anti* and *gauche* orientations, respectively (40–42). For pandamarilactonine-B (**8**) small coupling constants of 2.7 Hz ($^{3}J_{CH}$ H-14/C-16) and 3.3 Hz ($^{3}J_{CH}$ H-15/C-13), supported the *gauche* relationships of both H-14/C-16 and H-15/C-13. In alkaloid **7**, NOEs were observed between H-13 and H-16, while in alkaloid **8** there were NOEs between H-14 and H-16. Initially, these data were interpreted in favour of **7** and **8** as the *erythro* and *threo* isomers, respectively, with rotamers E1 and T1 as the

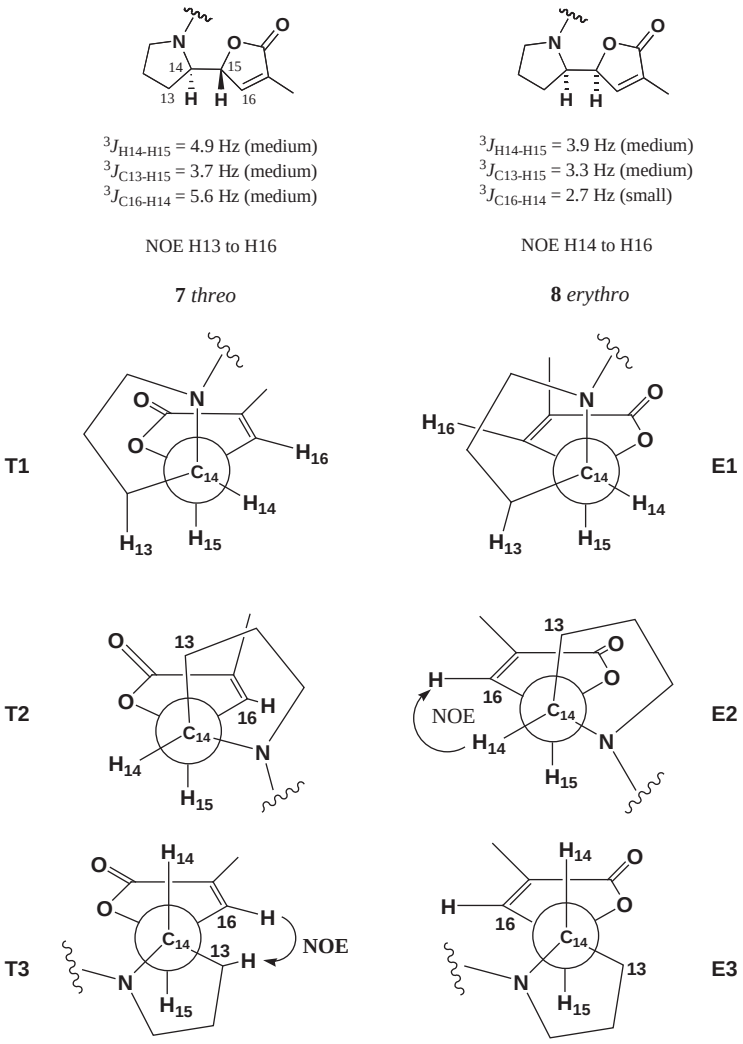


Figure 2 Rotamers of pandamarilactonine-A **7** and -B **8**.

predominant conformers, respectively (36). However, the NMR data measured in $CDCl_3$ hinted that the conformational situation was more complex since the $^3J_{H-H}$ values (**7**, J=5.5 Hz; **8**, J=5.9 Hz) were larger than those measured in acetone. This suggested that the rotamers E3 and T3, in both of which H-14 and H-15 are diaxial, contributed to the overall conformational equilibrium. A synthetic study of racemic pandamarilactonines-A and -B was undertaken as described in detail later (in Section III, A) and showed that pandamarilactonine-A was in fact the *threo* isomer, as shown above for **7**, while pandamarilactonine-B was the *erythro* isomer **8** (43). With this knowledge in hand, the conformational picture for pandamarilactonine-A could be reinterpreted as a mixture of rotamers T2 and T3, and for pandamarilactonine-B as a mixture of rotamers E2 and E3.

By use of these conformational preferences, the NOEs between H-13 and H-16 in **7** (rotamer T3) and between H-14 and H-16 in **8** (rotamer E2) were explained as shown in Figure 2.

Pandamarilactonine-A (**7**) exhibited $[\alpha]_D^{23}$ +35.0, in contrast, the specific rotation of pandamarilactonine-B (**8**) was almost zero. The optical purity of **7** and **8** was later studied using racemic samples prepared in a synthetic study (Section III, A). Chiral HPLC analysis of synthetic pandamarilactonine-A (**7**) yielded two peaks at retention times of 43.2 and 51.7 min with Chiralcel OB while synthetic pandamarilactonine-B (**2**) showed two peaks at 18.7 and 20.4 min using Chiralcel OD. The natural pandamarilactonine-A sample contained predominantly the (+) enantiomer in a ratio of 63:37 while the natural **8** was a racemate (36). Finally, the absolute configuration of (+)-pandamarilactonine-A has been established as (14*R*, 15*R*) by chiral synthesis as described in Section III A (44). The absolute configuration of (+)-pandamarilactonine-B is as yet unknown; consequently the structure **8** shown implies relative stereochemistry alone.

The synthetic work of Takayama *et al.* (Section III, A) (36) implied that there might be an acid-catalyzed interconversion of **7** and **8** during the isolation process so that **8** might be an isolation artifact of **7**. The C-15 position of the α-methyl-α,β-unsaturated-γ-lactone fragment would be sensitive to epimerization under either acidic or basic conditions (45), which suggested that the two alkaloids differed in configuration at C-15 rather than at C-14. However, when the natural alkaloids **7** and **8** were treated with 5% H_2SO_4 under the same conditions as used for the acid–base partitioning of the alkaloids, NMR of the recovered products did not reveal any interconversion of the two alkaloids. It was therefore concluded that **8** was not an isolation artifact since the optical purity of the recovered **7** was unchanged (36).

Subsequent work by Takayama *et al.* (43) provided two additional pandamarilactonine metabolites pandamarilactonine-C (**9**) and pandamarilactonine-D (**10**) from the sample of *P. amaryllifolius* that had earlier yielded **7** and **8**. As before (36), the crude alkaloid fraction was separated by SiO_2 column chromatography using a $CHCl_3$/MeOH gradient, with the 2–5% MeOH/$CHCl_3$ eluate then subjected to SiO_2 medium pressure liquid chromatography using 2% EtOH/$CHCl_3$. Pandamarilactonine-C (**9**) was obtained as an amorphous powder with $[\alpha]_D^{23}$ +26.2 (*c*. 0.99, $CHCl_3$) while Pandamarilactonine-D (**10**) was an amorphous powder, $[\alpha]_D^{25}$ 0 (*c*. 0.21, $CHCl_3$).

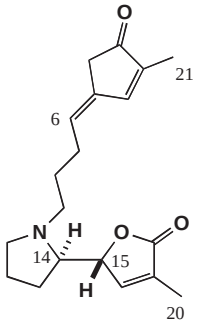
These two new alkaloids gave respectively *m*/*z* 318.1691 $[M+H]^+$ (calcd 318.1704) and *m*/*z* 318.1704 $[M+H]^+$ (calcd 318.1704) by HRFAB-MS (NBA). This established the molecular formulae of the two alkaloids as $C_{18}H_{23}NO_4$, and indicated that they were isomers of the co-occurring alkaloids, pandamarilactonine-A (**7**) and -B (**8**) (36). The spectroscopic data of **9** and **10** were similar to **7** and **8**, revealing an identical carbon skeleton, except that there were upfield shifts in the signals for C-4 in **9** (δ 130.3) and **10** (δ 133.9) compared to those of **7** (δ 137.7) and **8** (δ 137·7). These data were rationalized in terms of the γ-gauche effect of C-7 on C-4 arising from the *E*-configuration of the γ-alkylidene-γ-lactone moiety, an interpretation that was supported by the NOE observed between H-4 and H-7 in both **9** and **10**. Using COSY, HMQC, and HMBC

Table V ^{1}H and ^{13}C assignments for pandamarilactonine-C (**9**) and -D (**10**)

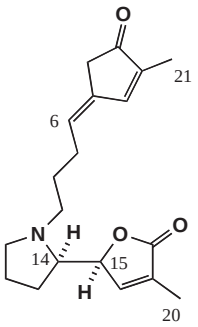
#	**9**		**10**	
	$^{1}H^{a,b}$	$^{13}C^{c}$	$^{1}H^{a,b}$	$^{13}C^{c}$
2		171.0		171.0
3		129.1		130.3
4	7.31 (1H, dd, 0.9, 1.5)	130.3	7.35 (1H, m)	133.9
5		148.7		149.0
6	5.64 (1H, dd, 8.2, 8.5)	113.4	5.60 (1H, dd, 8.3, 8.8)	113.3
7	2.35 (1H, ddd, 7.0, 8.2, 14.7) 2.25 (1H, m)	24.2	2.29 (2H,m)	24.0
8	1.62–1.68 (2H, m)	29.1	1.63 (2H, m).	28.7
9	2.91 (1H, ddd, 7.9, 8.2, 11.9) 2.45 (1H, ddd, 5.8, 7.0, 11.9)	55.2	2.77 (1H, m) 2.42 (1H, m)	55.1
11	3.12 (1H, d-like, 6.7, 7.3) 2.25 (1H, m)	54.2	3.08 (1H, m) 2.25 (1H, m)	54.0
12	1.71–1.82 (1H, m) 1.45 (1H, m)	26.1	1.78–1.92 (2H, m)	26.3
13	1.71–1.82 (1H, m) 1.62–1.68 (1H, m)	23.8	1.78–1.92 (2H, m)	23.9
14	2.78 (1H, m)	65.6	2.72 (1H, m)	65.9
15	4.80 (1H, ddd, 1.8, 1.8, 5.5)	83.8	4.74 (1H, m)	83.0
16	7.04 (1H, d, 1.5)	146.7	6.99 (1H, dd, 1.5, 1.7)	147.1
17		131.3		131.0
18		174.2		174.3
20	1.94 (3H, d, 1.8)	10.8	1.94 (3H, dd, 1.5, 2.0)	10.8
21	2.02 (3H, d, 0.9)	10.8	2.01 (3H, m)	10.7

[a]500 MHz, $CDCl_3$.
[b]*J* values (brackets) in Hz.
[c]125 MHz, $CDCl_3$.

experiments, the proton and carbon signals of **9** and **10** were unambiguously assigned, as shown in Table V.



Pandamarilactonine-C (**9**)

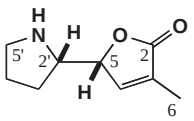


Pandamarilactonine-D (**10**)

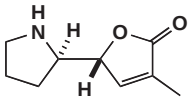
In $CDCl_3$, alkaloid **9** showed a coupling of 5.5 Hz between H-14 and H-15, suggesting a multi-conformer equilibrium, while the multiplet appearance of the H-15 signal in alkaloid **10** prevented measurement of the equivalent $^3J_{H-H}$ value. Confirmation of the relative configuration of the vicinal asymmetric centers at the C-14 and C-15 positions of **9** and **10** therefore involved a total synthesis that is detailed in the next section. Working in the *threo* series, the final product of the synthesis was found to be a mixture of *E*- and *Z*-pandamarilactonines those were identical to pandamarilactonine-C (**9**) and pandamarilactonine-A (**7**), respectively. Thus, pandamarilactonine-C and pandamarilactonine-D were *threo* and *erythro* isomers, respectively. The optical activities of the *E*-isomers showed a similar trend to the *Z* isomers; the *threo* compound was optically active and enriched in the (+)-isomer, while the *erythro* compound was optically inactive. The structures shown for **9** and **10** imply relative configuration only.

E. Norpandamarilactonines (46)

In addition to the four pandamarilactonine metabolites, acid–base extraction of the EtOH extract of fresh young leaves of *P. amaryllifolius* obtained from a market in Bangkok, Thailand followed by chromatography gave two minor alkaloids named norpandamarilactonine-A (**11**) and norpandamarilactonine-B (**12**). The alkaloids were characterized by PFG J-HMBC 2D spectroscopy, and the structures then confirmed by total synthesis. Both norpandamarilactonines lacked optical activity from which it could be concluded that they were racemates. Thus, the structures shown for natural **11** and **12** imply relative configuration alone.



Norpandamarilactonine-A (**11**)



Norpandamarilactonine-B (**12**)

Norpandamarilactonine-A (**11**), obtained as an amorphous powder, gave *m/z* 168.1039 by HRFAB-MS (NBA) analysis to fit the molecular formula $C_9H_{13}NO_2$. The UV ((MeOH) λ_{max} (log ε) 274 (0.44), 252 (0.35), 207 (2.29) nm) and IR signal at 1750 cm^{-1} both suggested an α,β-unsaturated-γ-lactone. Characteristic signals in the ^1H and ^{13}C NMR spectra for the presence of an α-methyl-α,β-unsaturated-γ-lactone residue were observed while the pyrrolidine ring was constructed from the residual four carbons (three methylenes and one methine) and the sole nitrogen atom. The HMBC spectrum provided the connectivity of the methine proton at δ 3.18 for H-2′ with C-4 (δ 147.7) of the α,β-unsaturated-γ-lactone ring, while the methine proton (δ 4.73) for H-5 of the γ-lactone ring showed correlations to both C-2′ (δ 60·4) and C-3′ (δ 27·9) of the pyrrolidine ring. These data provided the complete structure except for the relative configuration of the

Table VI ^{1}H and ^{13}C assignments for norpandamarilactonine-A (**11**) and -B (**12**)

#	**11**		**12**	
	$^{1}H^{a,b}$	$^{13}C^{c}$	$^{1}H^{a,b}$	$^{13}C^{c}$
2		174.3		174.1
3		130.7		131.2
4	7.13 (1H, ddd, 0.8, 1.6, 1.6)	147.7	7.02 (1H, ddd, 1.4, 1.7, 3.0)	146.6
5	4.73 (1H, ddd, 1.6, 1.9, 6.6)	83.8	4.79 (1H, dddd, 1.6, 1.9, 3.0, 6.6)	84.3
2′	3.18 (1H, ddd, 6.6, 6.6, 7.4)	60.4	3.20 (1H, ddd, 6.6, 7.1, 7.4)	60.2
5′	2.96 (1H, ddd, 6.3, 6.3, 10.4) 2.93 (1H, ddd, 6.8, 6.8, 10.4)	47.1	2.98 (1H, ddd, 5.8, 7.1, 12.9) 2.91 (1H, ddd, 6.6, 7.7, 14.3)	46.5
6	1.93 (3H, s)	10.7	1.93 (3H, s).	10.7
3′	1.84–1.92 (1H, m)	27.9	1.87 (1H, dddd, 3.0, 7.4, 10.7, 15.4)	26.8
	1.63 (1H, dddd, 6.3, 6.3, 6.6, 12.9)		1.56 (1H, dddd, 5.2, 6.9, 7.1, 15.4)	
4′	1.72–1.90 (2H, m)	25.6	1.81 (1H, m) 1.74 (1H, m)	25.1

[a]500 MHz, $CDCl_3$.
[b]J values (brackets) in Hz.
[c]125 MHz, $CDCl_3$.

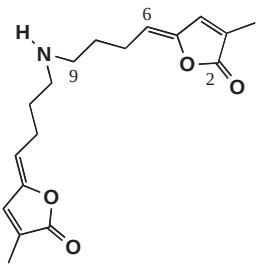
vicinal asymmetric centers at C-2′ and C-5. Since the γ-alkylidene-α,β-unsaturated-γ-lactone unit that was present in the pandamarilactonines (**7**–**10**) was missing, the new alkaloid was named norpandamarilactonine-A (**11**) (46).

Norpandamarilactonine-B (**12**) was also obtained as an amorphous powder with UV and MS data almost identical to those of **11**. Analysis of the NMR data indicated that **11** and **12** were diastereomeric at the C-5 and C-2′ positions. In $CDCl_3$, both **11** and **12** showed a coupling of 6.6 Hz between H-2′ and H-5, and the chemical shift values of C-2′ and C-5′ were closely similar; the *erythro* and *threo* compounds could not be safely distinguished from these values. Thus, confirmation of the structures and relative configuration at C-5 and C-2′ of **11** and **12** was carried out by a total synthesis that is described in detail in Section III. In this way, norpandamarilactonine-A and -B were assigned *erythro* and *threo* stereochemistry, respectively. Table VI presents the ^{1}H and ^{13}C NMR assignments for **11** and **12**.

F. Pandanamine (47)

An alkaloidal extract prepared in the same way from fresh leaves of *P. amaryllifolius* was subjected to silica gel column chromatography. The polar fraction, obtained after elution of the fractions containing pandamarilactonines, was further purified using reverse-phase column chromatography to give the

secondary amine pandanamine **13** as an amorphous powder in 0.21% yield from the crude alkaloid fraction.



Pandanamine (**13**)

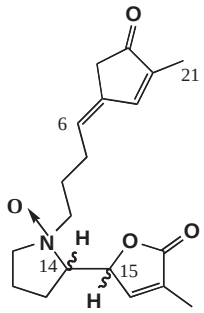
The new alkaloid **13** gave an m/z 318.1721 $[M+H]^+$ by HRFAB-MS for $C_{18}H_{23}NO_4$, and showed a UV maxima at 273 nm. The ^{13}C spectrum showed only nine signals indicative of a symmetrical structure. The characteristic ^1H and ^{13}C NMR signals for the γ-alkylidene-α-methyl-α,β-unsaturated-γ-lactone moiety [δ 7.03 (2H, d, J=1.5 Hz, H-4), 5.14 (2H, dd, J=7.9, 7.9 Hz, H-6), 1.99 (6H, s); δ 170.9 (C-2), 129.8 (C-3), 137.8 (C-4), 149.3 (C-5), 111.3 (C-6), 10.5 (C-21)] were observed and accounted for six of the nine carbons; the remaining carbons (δ 47.7, 254, and 23.1) were all methylenes with the signal at δ 47.7 assigned to C-9 on chemical shift grounds. Analysis of 2D spectra established that the signal at δ 23.1 (C-7) was connected to C-6 of the γ-alkylidene-γ-lactone moiety. The observation of an NOE between H-4 and H-6 demonstrated the *Z*-configuration in the γ-alkylidene-α,β-unsaturated-γ-lactone moiety. All of the above findings allowed the construction of the symmetrical molecular structure pandanamine **13** (47). This lactone compound was identical with a synthetic compound previously prepared as an intermediate for the synthesis of pandamarilactonines (36).

The isolation of pandanamine was of significant interest since its lactam equivalent had earlier been proposed as a biogenetic intermediate in the biogenesis of pandamarine **1** by Byrne *et al*. (29). Pandanamine (13) itself was also postulated as the common biogenetic precursor of pandamarilactonines-A and -B (**7** and **8**) and of pandamarilactone-1 (**2**). Thus, the isolation of this compound from Nature strongly supported the proposed biogenetic pathways to the alkaloids (**2**, **7**, and **8**). This is discussed further in Section IV.

G. Additional Pyrrolidine Alkaloids (48)

Salim *et al*. (48) isolated three new alkaloids, the two pyrrolidine-type alkaloids (**14** and **15**) together with the 6*E*-isomer of pandanamine (**16**), and five known alkaloids (**7**–**10** and **13**) from *P. amaryllifolius* leaves collected from West Java, Indonesia. Evidence that the three new alkaloids all possessed two α-methyl-α,β-unsaturated-γ-lactone functionality was apparent from the spectroscopic studies.

Alkaloid **15** also contained a seven-membered ring, a structural feature which had not been encountered previously in *Pandanus* alkaloids, but which is reminiscent of alkaloids of the Stemonaceae (37).



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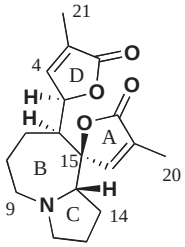

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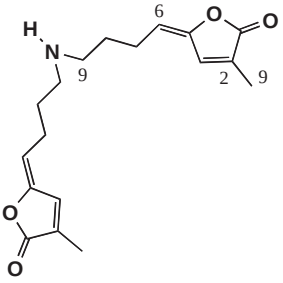


16

Alkaloids **14** and **15** were isolated from dried *P. amaryllifolius* leaves using conventional acid–base extraction methods and purification by reverse-phase HPLC using a gradient of H_2O/CH_3CN that contained 0.1% TFA to improve peak resolution.

Alkaloid **14** was isolated as a colorless amorphous solid; no optical rotation was obtained. The compound had a molecular formula of $C_{18}H_{23}NO_5$ (HRESIMS), which corresponded to that of the pandamarilactonines except for an additional oxygen atom. Characteristic ^1H and ^{13}C NMR signals revealed the presence of both γ-alkylidene-α-methyl-α,β-unsaturated-γ-lactone moiety and α-methyl-α,β-unsaturated-γ-lactone ring, while the pyrrolidine ring was constructed from the remainder of the NMR signals. Overall, the NMR data of **14** in $CDCl_3$ were closely similar to those of pandamarilactonines-A and -B (**7** and **8**), except that there were significant differences at δ 3.78 (H-9a), 2.93 (H-9b), 4.01 (H-11a), 3.03 (H-11b), 3.08 (H-14), and 5.86 (H-15), where the chemical shifts were approximately 1 ppm lower than the published values for **7** and **8**. These NMR data indicated that the nitrogen in **1** was positively charged, and thus **14** was determined to be an N_b-oxide. In their paper, the authors proposed *erythro* stereochemistry at C-14/C-15 of **14**, but they did not provide any evidence that directly supported this stereochemical conclusion. Complete NMR data are given in Table VII.

Alkaloid **15** was isolated as a colorless amorphous solid of low optical activity, $[\alpha]_D^{23}=-4.35$ (*c*. 0.16, $CHCl_3$), and with the molecular formula $C_{18}H_{23}NO_4$ by HRESIMS. Characteristic ^1H and ^{13}C NMR signals again indicated the presence of an α-methyl-α,β-unsaturated lactone moiety but the C-5/C-6 alkene functionality was absent; in particular, the HMBC data showed a correlation between a proton at δ 4.67 assigned to H-5 and C-4 at δ 147.0. The signal at δ 79.2 for C-5 suggested a methine carbon adjacent to an oxygen atom, confirming the presence of a γ-lactone residue. The DQFCOSY data linked H-5 to another methine proton

Table VII NMR assignments of alkaloid **14**

#	$\delta^{13}C$[a]	$\delta^{1}H$ (J)[b,c]	HMBC[d,e]	COSY
2	170.9	–	4, 21	
3	130.0	–	4, 21	
4	137.7	7.04, d (1.3)	6, 21	21
5	149.5	–	4, 6, 7	–
6	110.5	5.20, dd (7.8, 7.8)	7, 21	7
7	23.2	2.51–2.45, m	6, 8, 9	6, 8a/b
8a	24.6	2.21, m	6, 7, 9	7, 8b, 9a/b
8b		1.96, m	–	7, 8b, 9a/b
9a	54.7	3.78, ddd (12.1, 12.1, 4.6)	7, 8, 11, 14	8a/b, 9b
9b		2.93, m	–	8a/b, 9a
11a	54.1	4.01 ddd (7.3, 4.6)	9	11b, 12a/b
11b		3.03, m	–	11a, 12a/b
12a	21.7	2.33, m	13	11a/b, 12b, 13a/b
12b		2.10, m		11a/b, 12a, 13a/b
13a	26.5	2.23, m	11, 14	12a/b, 13b, 14
13b		2.21, m		12a/b, 13a, 14
14	70.5	3.08, ddd (7.8)	15, 20	13a/b
15	78.5	5.86, dd (7.8)	14, 20	20
16	144.4	7.07, br s	15, 20	20
17	132.9	–	15, 16, 20	–
18	172.1	–	16, 20	–
20	10.8	1.95, br s	16	16
21	10.5	2.00, br s	4	4

[a]125 MHz, $CDCl_3$, referenced to ^{13}C at δ 77.0 ppm.
[b]500 MHz, referenced to ^{1}H at 7.26 ppm.
[c]Coupling constants in Hz.
[d]HMBC connectivity from C to H.
[e]Correlations observed for one bond J_{C-H} of 135 Hz and long range J_{C-H} of 7 Hz.

at δ 3.27 (H-6), which in turn, was coupled to signals at δ 1.64 (H-7a) and 1.59 (H-7b). The C-7 to C-9 and C-11 to C-14 portions were then established by DQFCOSY and HSQC data. The ^{13}C shifts of C-11 (δ 59.0) and C-14 (δ 69.7) suggested that these carbons were each adjacent to a nitrogen atom. In this way the pyrrolidine ring was inferred. The remainder of the signals provided a second α-methyl-α,β-unsaturated-γ-lactone moiety; there were HMBC correlations between a methine proton at δ 8.06 (H-16) and both C-15 (δ 87.0) and C-17 (δ 134.3). The signal at δ 87.0 for C-15 was consistent with a quaternary carbon next to an oxygen atom. In the HMBC spectrum, the signal at δ 58.2 (C-9) showed HMBC correlations to both H-11 at δ 4.06 and H-14 at δ 3.92, while C-15 was correlated to H-5, H-6, H-7, H-13, H-14, H-16, and H-20. These data generated the carbon skeleton of **15** as shown.

The relative configuration of **15**, as shown in Figure 3, was assigned from coupling constant and NOESY data together with modeling studies.

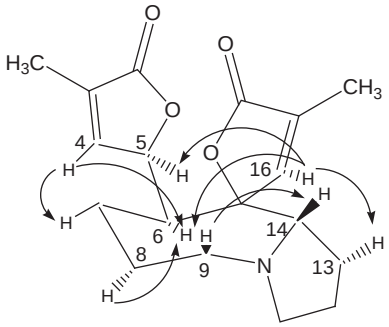


Figure 3 Selected NOESY correlations for alkaloid **15**.

Since alkaloid **15** had four chiral centers, the 16 possible stereoisomers were modeled and individual stereostructures excluded if their NOESY-correlated protons were separated by >4 Å. The model with 5*R*,6*S*,14*S*,15*R* stereochemistry and a chair conformation in the seven-membered ring, was found to fit the experimental NOESY data, with all the inter-proton distances that corresponded to observed NOESY correlations in the range 2.6–3.2 Å. The lowest energy conformation indicated a dihedral angle of 70° between H-5 and H-6, which was consistent with the 1.7 Hz coupling constant measured from the ^{1}H spectrum. Complete HMBC, DQFCOSY, and NOESY data are given in Table VIII.

Diagnostic signals at δ 8.06, 4.67, 4.06, 3.92, and 3.75 that were markers for alkaloid **15** were not observed in the spectrum of the extract before HPLC. Similarly for alkaloid **14**, there was also no evidence for this alkaloid prior to HPLC. Therefore, the isolated alkaloids **14** and **15** were not the ''natural products'' of *P. amaryllifolius*, and were assumed to be artifacts isolated as a result of the acidic conditions used during HPLC. This evidence suggested that the ''natural'' *Pandanus* alkaloids were sensitive to the acidic conditions used in the HPLC purification.

A comparative study of the alkaloids isolated from two different extraction methods was then carried out, involving a solvent partitioned method (method A; sequential partitioning between 50% aq. EtOH with hexane followed by chloroform, then normal phase flash chromatography) and an acid–base extraction method (method B; HCl/ether and $NH_4OH/CHCl_3$ followed by normal phase flash chromatography). An ethanolic extract prepared from a second batch of dried *Pandanus* leaves was divided in half; one portion was further worked up with method A, and the other portion was purified using method B. In method A, acid and base were excluded in order to minimize the formation of artifacts. The two extraction methods gave different alkaloid products.

The solvent partitioned method (method A) produced a mixture of two alkaloids. The major component was pandanamine (**13**) (47) while the second alkaloid was identified as the 6*E*-isomer **16**. In **16**, the alkene signals at δ 7.43 (H-4) and 5.51 (H-6) appeared at lower field [δ 7.00 (H-4) and 5.14 (H-6)] than for pandanamine **13**. Earlier studies on *P. amaryllifolius* alkaloids had showed that an *E*-configuration of the γ-alkylidenebutenolide moiety results in lower field proton

Table VIII NMR assignments of alkaloid **15**

#	$\delta^{13}C$[a,b]	$\delta^{1}H$ (*J*)[c,d]	HMBC[e,f]	COSY	NOESY
2	173.2 (s)	–	4, 21	–	–
3	132.6 (s)	–	4, 5, 21	–	–
4	147.0 (d)	7.03, dd (3.3,1.6)	5, 6, 21	5, 21	5, 7b, 21
5	79.2 (d)	4.67, dd (3.3, 1.7)	6, 7, 21	4, 6, 21	4, 6, 7b, 16
6	44.6 (d)	3.27, ddd (10.2, 3.0, 1.7)	5, 7, 20, 21	5, 7a/b	5, 7a/b, 8a, 16
7a	21.6 (t)	1.64 m	5, 6, 9	6, 7b, 8a/b	–
7b		1.59, m		6, 7a, 8a/b	4, 5, 6, 8b
8a	26.5 (t)	2.24, m	6, 7, 9	7a/b, 8b, 9a/b	6, 7a, 8a
8b		2.08, m		7a/b, 8a, 9a/b	8b
9a	58.2 (t)	3.75, m	7, 8, 11, 14	8a/b, 9b	9a
9b		2.95, m		8a/b, 9a	9b, 14
11a	59.0 (t)	4.06 ddd (10.7, 6.3, 4.4)	12, 13	11a, 12a/b	11a, 12a
11b		2.92, m		11a, 12a/b	11b
12a	23.3 (t)	2.03, m	11, 13, 14	11a/b, 12b	
12b		1.93, m		11a/b, 12a	11b
13a	26.5 (t)	2.14, m	11, 12, 14	12a/b, 13b, 14	14
13b		1.64, m		12a/b, 13a, 14	16
14	69.7 (d)	3.92, dd (9.5, 5.1)	9, 11, 12	13a/b	9b, 13a
15	87.0 (s)	–	5, 6, 7, 13, 14, 16, 20	–	–
16	148.2 (d)	8.06, d (1.3)	14, 20	20	5, 6, 13b, 20
17	134.3 (s)	–	16, 20	–	–
18	171.3 (s)	–	16, 20	–	–
20	10.6 (q)	2.04, d (1.3)	16	16	16
21	10.7 (q)	1.91 d (1.6)	4	4, 5	4

[a]125 MHz, $CDCl_3$, referenced to ^{13}C at δ 77.0 ppm.
[b]Multiplicity from DEPT.
[c]500 MHz, referenced to ^{1}H at 7.26 ppm.
[d]Coupling constants in Hz.
[e]HMBC connectivity from C to H.
[f]Correlations observed for one bond J_{C-H} of 135 Hz and long range J_{C-H} of 7 Hz.

chemical shifts for H-4 and H-6 (35,43). Additionally, the C-4 signal (δ 134.0) for **16** appeared at higher field than in pandanamine. In contrast, four alkaloids were isolated from the acid–base extraction (method B) including pandamarilactonines-A and -B (**7** and **8**) (major components) and

pandamarilactonines-C and -D (**9** and **10**) (minor components) (36,43). Changing the acid used in the extraction (HCl vs. H_2SO_4) did not affect the type of alkaloids obtained from method B.

The absence of pandamarilactonine products from the solvent partitioned method suggested that they were artifacts formed during the acid–base treatment. The most likely precursors of these alkaloids were the pandanamines **13** and **16**, which were isolated from the solvent partitioned method (47,48). In a biomimetic synthesis, Takayama *et al*. had previously shown that **13** cyclized to **7** and **8** on treatment with a catalytic amount of TFA in CH_3CN (36). When the pandanamine products **13** and **16** isolated using the method A procedure were subjected to acid–base treatment similar to that in the acid–base extraction procedure (method B), the products were found to be the pandamarilactonines **7**–**10**. Conversion of the pandanamines to the pandamarilactonines was proposed to involve Michael addition of the nitrogen onto the double bond at C-6 followed by protonation at C-15.

The pandamarilactonines-A and -B (**7** and **8)** isolated from the crude alkaloidal fraction under acid–base conditions could themselves be the precursors to alkaloid **15**. Under acidic conditions (such as in the presence of TFA), the lactone ring of **7** or **8** may enolize, followed by attack of C-15 on C-6, ultimately leading to the formation of **15** after reprotonation at C-5 (48).

The authors commented that the use of conventional acid–base extraction to isolate *Pandanus* alkaloids should be avoided since it can lead to the formation of artifacts, and speculated whether the alkaloids isolated in earlier studies (29,30,35,36,43,46) were ''natural product'' or artifacts formed during the isolation process.

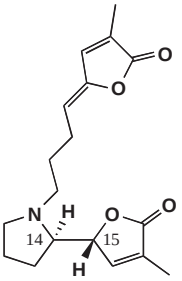
The mixed pandamarilactonine sample isolated from the method B procedure using HCl was anticipated to be a racemate, but the sample in fact had low optical activity, $[\alpha]_D$ +4.0 (*c*. 2.56, $CHCl_3$) that at the time was attributed to the difficulty in removing trace impurities during the chromatography (48). The configurational instability of pandamarilactonines is further explored below (Section III).

In summary, it appears that *P. amaryllifolius* has several subspecies or chemotypes, which may produce different types of alkaloids. Since the very first isolation of pandamarine **1** from *P. amaryllifolius* (29), a number of structural variations in the alkaloids associated with *P. amaryllifolius* has been observed from plants collected in the Philippines, Thailand, and Indonesia. Piperidinyl alkaloids with lactam (29) or lactone (30) moieties have been isolated from Philippine specimens of *P. amaryllifolius*. Pyrrolidinone (35) and pyrrolidine (36,43,46,48) rings feature in alkaloids from *P. amaryllifolius* collected in Indonesia and Thailand, respectively. The majority of *P. amaryllifolius* alkaloids have at least one α,β-unsaturated-γ-lactone ring, and are suggested to be derived from the same common precursor (36), a symmetrical secondary amine called pandanamine (**13**), which has also been isolated from Nature (47). Some of the interesting ''natural'' product structures may in fact be artifacts of isolation or epimerization products since some compounds show sensitivity towards acidic and/or basic conditions.

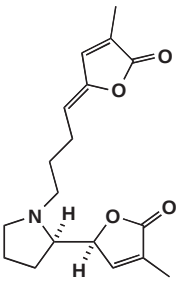
III. SYNTHESIS

A. Pandamarilactonines

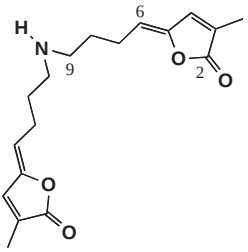
The pandamarilactonine metabolites (**7**–**10**) (36,43) consist of two moieties, a γ-butylidene-α-methyl-α,β-unsaturated-γ-lactone and a structurally unique pyrrolidinyl-α-methyl-α,β-unsaturated-γ-lactone, the latter of which corresponds to norpandamarilactonines (**11** and **12**) (46). Five independent methods for the construction of the norpandamarilactonine unit have been developed, and these can be classified as biomimetic, racemic, or enantiomeric syntheses.



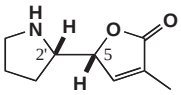
Pandamarilactonine-A (**7**)



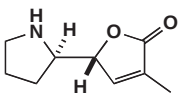
Pandamarilactonine-B (**8**)



Pandanamine (**13**)



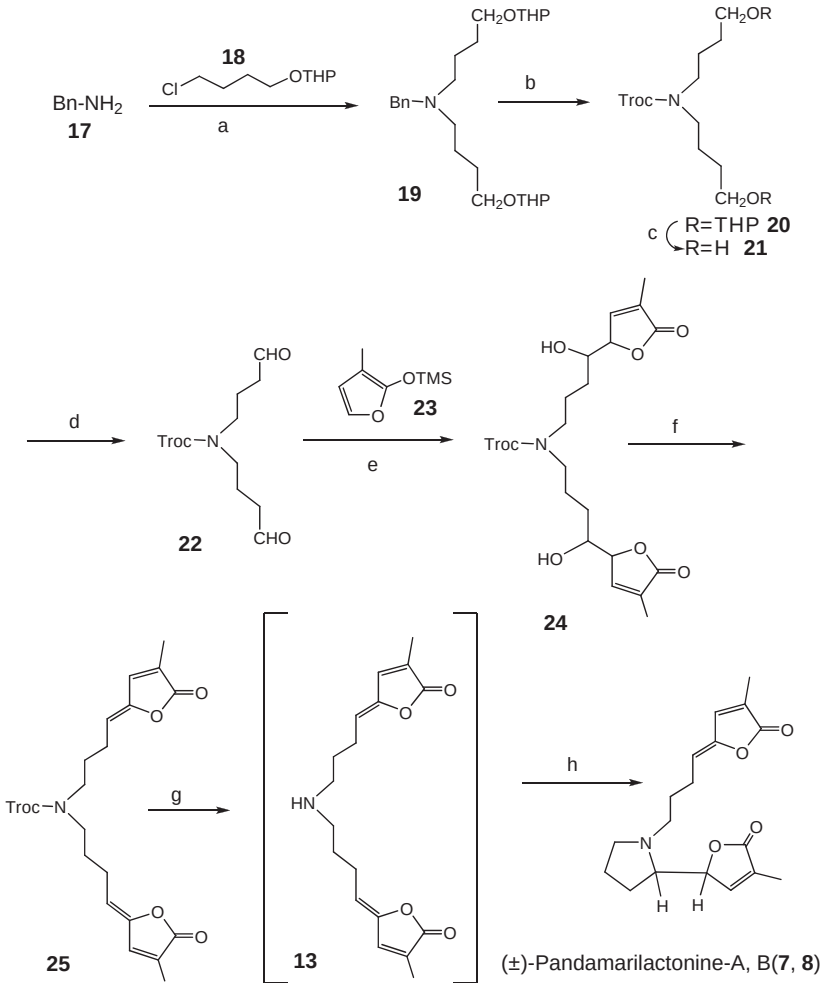
Norpandamarilactonine-A (**11**)



Norpandamarilactonine-B (**12**)

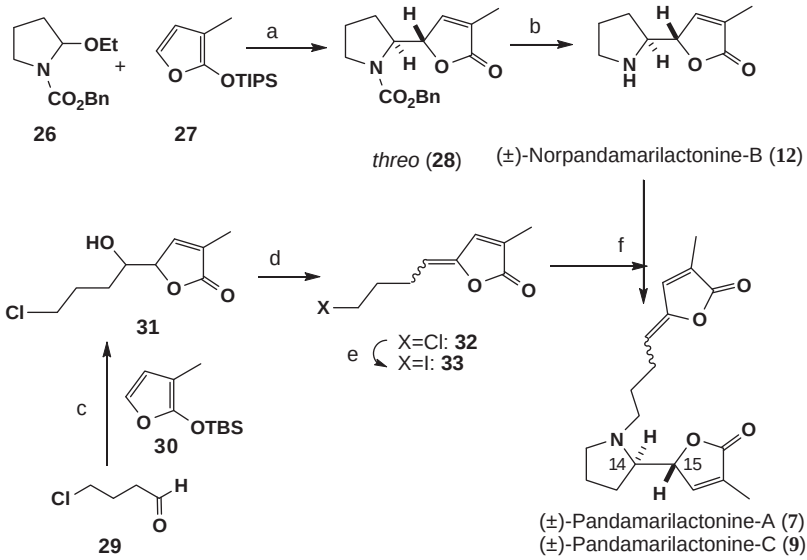
1. Biomimetic Synthesis

The total synthesis of **7** and **8** *via* a route that mimics the final step of the proposed biosynthesis (from **13** to **7**/**8**, see Section IV), was achieved as shown in Scheme 1 (36). The key secondary amine derivative **13**, which corresponds to the hypothetical biogenetic intermediate, and was isolated from *Pandanus* plants (45), was prepared as follows. *N*-Dialkylation of benzylamine (**17**) with *O*-tetrahydropyranyl (THP) 4-chlorobutanol (**18**) was carried out using potassium carbonate in the presence of a catalytic amount of sodium iodide to obtain tertiary amine **19** in 61% yield. Then, the benzyl group was converted into a β,β,β-trichloroethoxycarbonyl group to give carbamate **20**. After removal of the THP ether in **20**, the free alcohol groups in **21** were converted into the dialdehyde **22** in 72% yield *via* Swern oxidation. Aldol reaction of dialdehyde **22** with siloxyfuran **23** using boron trifluoride etherate ($BF_3 \cdot Et_2O$) gave a mixture of stereoisomeric adducts **24** in quantitative yield. Next, installation of the *exo*-double bond was accomplished by treating the mixed adducts **24** with a combination of TMSCl and DBU to give the γ-alkylidenebutenolide **25** in 41% yield, together with the *Z*, *E*-isomer in 25% yield. Finally, the protecting group on



Scheme 1 Reagents and conditions: a, K_2CO_3, NaI, CH_3CN, 61%; b, β,β,β-trichloroethoxycarbonylchloride, CH_3CN, 76%; c, p-TsOH · H_2O, aq. CH_3CN, 80%; d, Swern oxidation, 72%; e, $BF_3 \cdot Et_2O$, CH_2Cl_2, quant; f, TMSCl, DBU, CH_2Cl_2, **25**, 41%, *Z*, *E*-isomer, 25%; g, Zn, AcOH; h, cat. TFA, CH_3CN, **7**, 9%, **8**, 9%.

nitrogen in **25** was removed by reacting with Zn in AcOH. Crude amine **13** was directly treated with a catalytic amount of trifluoroacetic acid in CH_3CN. By careful separation of the crude products, (±)-pandamarilactonine-A (**7**) and -B (**8**) were obtained in 9% yield each. Direct comparison revealed that they were identical with their respective natural products. Although the chemical yield at the final step was low, the first biomimetic total synthesis of the alkaloids was accomplished, providing chemical support for the suggested biogenetic route of **7** and **8**, as well as proof of the chemical structures deduced by spectroscopic analysis.



Scheme 2 Reagents and conditions: a, $BF_3 \cdot Et_2O$, CH_2Cl_2, −78°C, 91%(*threo*:*erythro* = 4:1); b, TMSI, CH_3CN, r.t., 94%; c, $BF_3 \cdot Et_2O$, CH_2Cl_2; d, Tf_2O, Py, CH_2Cl_2, 48%(2 steps); e, NaI, acetone, 47%; f, K_2CO_3, CH_3CN, **7**, 33%, **9**, 7%.

2. Syntheses of the Racemic Form

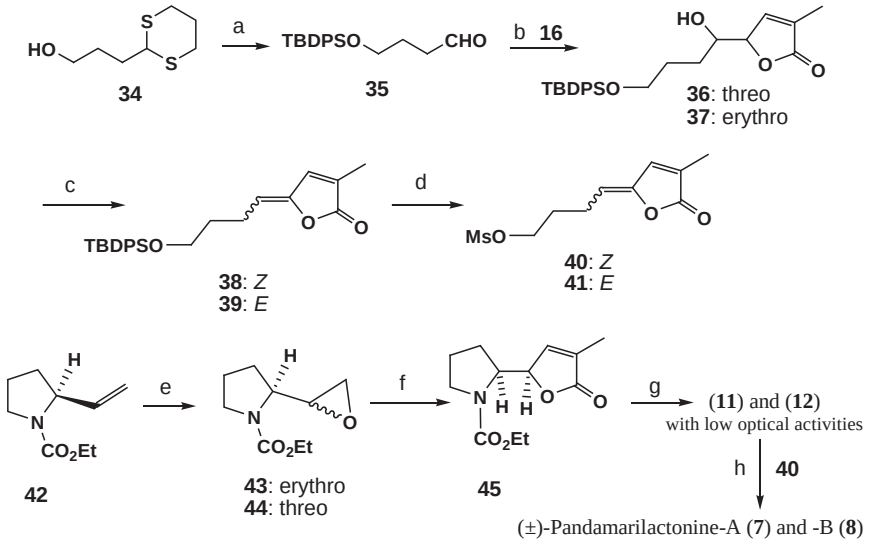
The lower part of the alkaloids, i.e., the pyrrolidinyl-α-methyl-α,β-unsaturated-γ-lactone residue, was synthesized according to the procedure of Martin *et al*. (37) as follows (Scheme 2) (43). Compound **28**, whose stereochemistry at the vicinal positions was established to be *threo* by X-ray analysis, was prepared by vinylogous Mannich coupling reaction of **26** and **27**. The protecting group on the nitrogen in **28** was removed by treatment with TMSI in CH_3CN to give (±)-norpandamarilactonine-B (**12**) in 94% yield. Iodide **33** corresponding to the upper part of the pandamarilactonines was prepared in three steps: (i) condensation of aldehyde **29** with siloxyfuran **30** in the presence of $BF_3 \cdot Et_2O$; (ii) dehydration of resultant aldol adduct **31** with trifluoromethanesulfonic anhydride and pyridine (48% overall yield); and (iii) halogen exchange of **32** with NaI in acetone (47% yield). The resulting nine-carbon unit **33**, which consisted of the *Z*- and *E*-isomers in the ratio of 4.6:1, was condensed with the secondary amine **12** in acetonitrile in the presence of K_2CO_3 to give two pandamarilactonines in 33% and 7% yields, both of which possessed the *threo* form at the C-14 and C-15 positions.

Based on the results of this synthetic study, the relative configuration at the vicinal positions of pandamarilactonines was unambiguously established. Thus, pandamarilactonine-A and -B were respectively the *threo* and *erythro* isomers.

A synthesis by Figueredo's group (45,49) starting from (*S*)-prolinol also provided racemic samples of these alkaloids and consequently provided a plausible mechanistic explanation for the configurational instability of the pandamarilactonines. The work involved the transformation of an oxirane to

an α-methylbutenolide using the dianion of 2-phenylselenopropionic acid (Scheme 3). Initially, the γ-butylidene-α-methyl-α,β-unsaturated-γ-lactone moiety was prepared in the following manner. Starting from hydroxydithiane **34**, aldehyde **35** was prepared in 88% yield. The vinylogous Mukaiyama reaction of **35** with silyloxyfuran **27** afforded a 7:1 mixture of *threo* and *erythro* alcohols **36** and **37** in 82% yield. Treatment of the diastereomeric alcohols with TMSCl/DBU in chloroform gave a mixture of olefins **38** and **39** in 94% yield after purification by silica gel chromatography in an approximately 3:1 ratio, and the olefins were respectively assigned as the *Z*- and *E*-isomers. Desilylation was accomplished in 86% yield to produce the free alcohols, and these were converted into sulfonates without intermediate purification. Isomeric sulfonates **40** and **41** were chromatographically separated (74% total yield). Synthesis of the pyrrolidine fragment was accomplished starting from carbamate **42**, which was prepared from (*S*)-prolinol. Oxidation of **42** with MCPBA furnished two oxiranes **43** and **44** in 77% total yield and an *erythro*/*threo* ratio of 1.5:1.

The oxiranes were separated and major isomer **43** was converted into *erythro*-methyl butenolide **45** *via* a three-step protocol consisting of addition of the dianion of 2-phenylselenopropionic acid to **43**, followed by acid-induced lactonization and oxidation of the selenide function with subsequent thermal elimination, giving an overall yield of 61%. Treatment of *erythro*-methyl butenolide **45** with TMSI in chloroform at reflux resulted in cleavage of the carbamate with concomitant epimerization of the stereogenic center of the



Scheme 3 Reagents and conditions: a, TBDPSCl, Im, DMF, 96%, then $CaCO_3$, MeI, aq. CH_3CN, 92%; b, $BF_3 \cdot Et_2O$, CH_2Cl_2, −78°C, 82% (**36**:**37** = 7:1); c, TMSCl, DBU, $CHCl_3$, reflux, 94%, (**38**:**39** = 3:1); d, Bu_4NF, THF, 86%, then MsCl, Py, CH_2Cl_2, 74%; e, MCPBA, $CHCl_3$, 77% (**43**:**44** = 1.5:1); f, (i) separation of diastereomers, (ii) $PhSeCH(CH_3)CO_2H$, LDA (2 equiv.), THF, (iii) AcOH, THF, reflux, (iv) H_2O_2, AcOH, 0°C, 61% (4 steps); g, TMSI, $CHCl_3$, reflux, 84%; h, Py, DMF, 60°C, 44%.

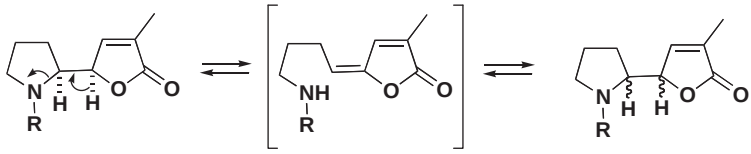


Figure 4 A mechanism that explains the configurational instability of the *Pandanus* alkaloids (45).

lactone moiety, furnishing an approximately 1:1 mixture of two norpandamarilactonines (**11** and **12**), which were separated by silica gel chromatography. The specific rotation measured for **11** was $[\alpha]_D^{20}$ −7 (*c*. 1.5, $CHCl_3$) and that for **12** was $[\alpha]_D^{20}$ −3 (*c*. 2.6, $CHCl_3$). The authors suspected that the reason for such low optical activity values could be that, besides epimerization, racemization also occurred to some extent. They proposed ring opening of the pyrrolidinyl ring under neutral or basic conditions, followed by ring closure, as shown in Figure 4. This sequence explains the loss of stereochemistry in the pandamarilactonines at both C-14 and at C-15. The facile epimerization of *erythro* intermediate (**45**) under reflux in $CHCl_3$ contrasts with the configurational stability of *threo*-(**28**) in CH_3CN at room temperature when each were exposed to TMSI.

When the synthetic norpandamarilactonine mixture (**11**/**12**) was treated with an equimolar amount of freshly prepared mesylate **40** in DMF in the presence of pyridine at 60°C, pandamarilactonines **7** and **8** were slowly formed in an approximate 1:1 ratio. After 3 days, the reaction mixture was purified by normal phase flash chromatography over silica gel to give pandamarilactonine-A (**7**) and pandamarilactonine-B (**8**) in an overall isolated yield of 44%. Neither diastereomer, when purified, showed any optical activity. However, it should be noted that the starting materials for this reaction themselves showed low optical activity.

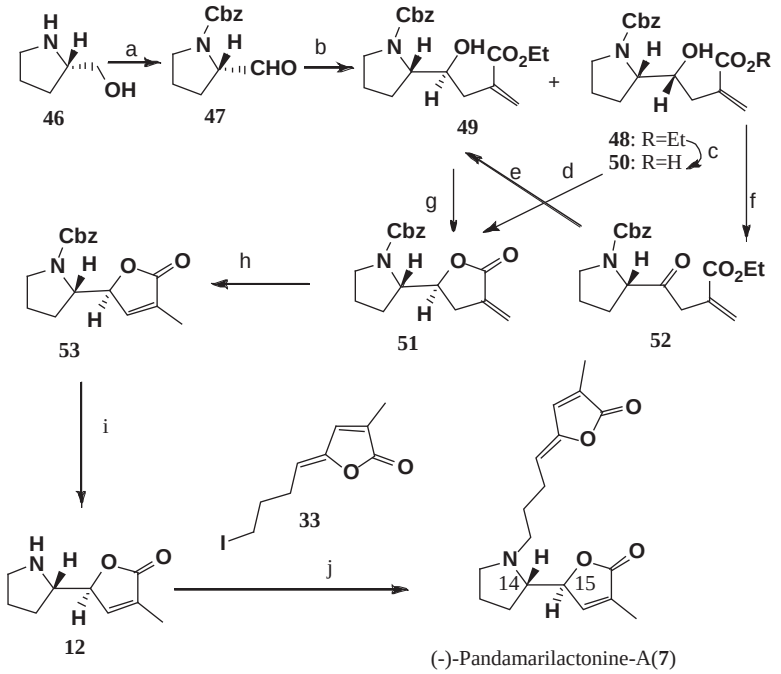
Figueredo *et al*. (45) made stereochemical assignments of oxiranes **43** and **44** and butenolide **45** on the basis of the NMR trends of pyrrolidinyl-α,β-unsaturated-γ-lactones that had been noted earlier by Martin *et al*. (37). With issues of relative configuration resolved, it is now apparent the same trends apply to both the norpandamarilactonines and pandamarilactonines series. The proton signal corresponding to H-14 of the pandamarilactonines (Tables IV and V) or to H-2′ of the norpandamarilactonines (Table VI) is upfield in the *erythro* series of compounds, while the corresponding carbon signal is downfield.

3. Syntheses of the Chiral Form

Two papers have described the chiral syntheses of pandamarilactonine alkaloids. In 2005, Takayama *et al*. described an approach to pandamarilactonine-A (**7**) which established the absolute configuration of the (+)-isomer (44), while in 2006, Honda *et al*. (50) reported the synthesis of a norpandamarilactonine precursor using an alternative synthetic approach, and converted this to a mixture of pandamarilactonines-A and -B (**7**/**8**).

In order to construct the chiral norpandamarilactonine moiety, a Reformatsky-type condensation using l-prolinal and 2-(bromomethyl)acrylate

was employed by Takayama *et al*. (44). l-Prolinol (**46**) was converted into aldehyde (**47**, $[\alpha]_D^{26}$ –63.1 (*c*. 1.14, MeOH)) in two steps, and then the Reformatsky-type condensation with ethyl 2-(bromomethyl)acrylate was carried out (Scheme 4). When Zn metal was used, the adducts were obtained in 52% yield, which contained the *erythro* (more polar, **48**) and the *threo* (less polar, **49**) isomers in the ratio of 4:1, the stereochemistry of which was determined in the later stage of the synthesis, as described below. On the other hand, indium-mediated coupling gave the same adducts in 80% yield, the diastereoselectivity of which was 2:1. The e*rythro* isomer **48**, which had an undesirable stereochemistry, was transformed into the *threo* isomer **49** by inversion of the secondary alcohol. Initially, the intramolecular Mitsunobu reaction was applied to carboxylic acid **50** that was prepared by the alkaline hydrolysis of **48**. Treatment of **50** with di-*tert*-butyl azodicarboxylate (DTAD) and PPh_3 in THF at room temperature gave the lactone derivative in quantitative yield, which contained the *threo* isomer **51** and its *erythro* isomer in the ratio of 7.3:1. The unexpected minor *erythro* lactone, which showed retention of the configuration of the alcohol, was formed *via* an acyloxyphosphonium ion intermediate. Application of the conventional intermolecular Mitsunobu reaction to ester **48** resulted in the recovery of the starting material.

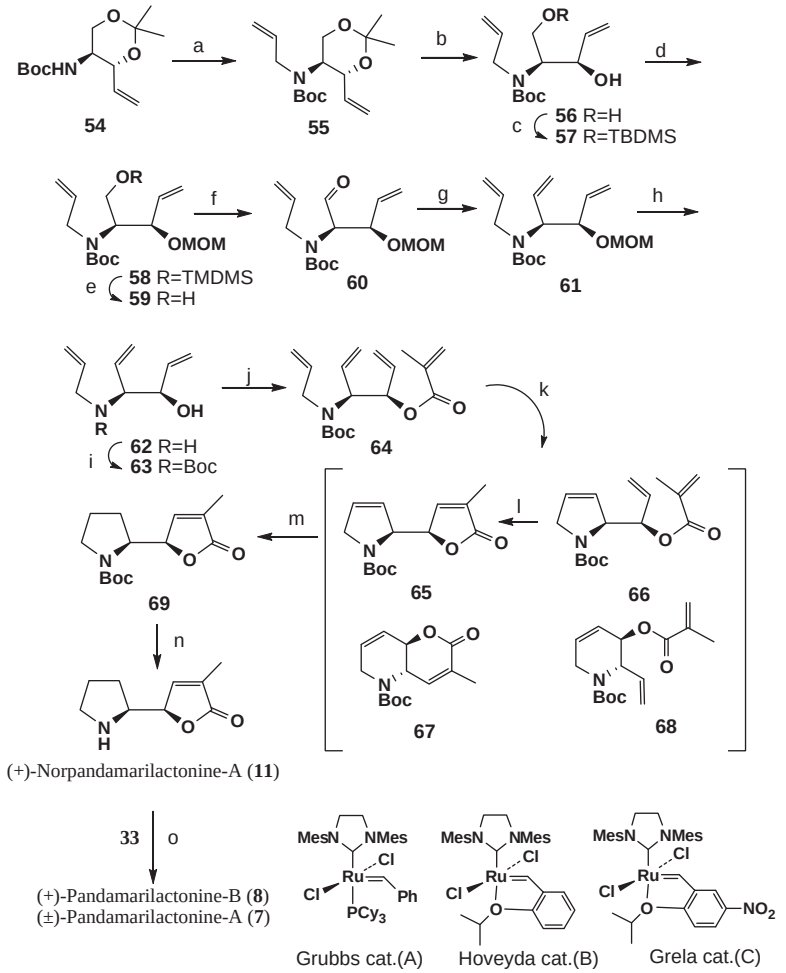


Scheme 4 Reagents and conditions: a, Cbz-Cl, K_2CO_3, CH_3CN, then Swern oxidation, 91%; b, ethyl 2-(bromomethyl)acrylate, 2 equiv Zn, THF-aq. sat. NH_4Cl or 1.1 equiv indium, aq. EtOH; c, LiOH, aq. THF, quant; d, DTAD, PPh_3, THF, r.t.; e, DMP, CH_2Cl_2, r.t., quant; f, $NaBH_4$, MeOH, −20°C, 86%; g, TFA, CH_2Cl_2, r.t., 90%; h, 5 mol% Et_3SiH, 10 mol% $Rh(PPh_3)_3Cl$, toluene, reflux, 86%; i, TMSI, CH_3CN, −15°C, quant; j, Ag_2CO_3, CH_3CN, r.t.

For the inversion of the alcohol in **48**, an oxidation–reduction sequence was attempted. Ketone derivative **52**, prepared from **48** by oxidation with Dess–Martin periodinane, was reduced with $NaBH_4$ in MeOH at –20°C to afford *threo* **49** and *erythro* **48** alcohols in the ratio of 2.6:1. The major alcohol **49** thus obtained was treated with trifluoroacetic acid in CH_2Cl_2 to give lactone **51** in 90% yield, which was identical with the major product obtained *via* the intramolecular Mitsunobu reaction described above. Next, the isomerization of the double bond in **51** from the *exo* to the *endo* position was performed using Et_3SiH (5 mol%) and tris(triphenylphosphine)rhodium chloride (10 mol%) in refluxing toluene to afford the α-methyl butenolide **53** in 86% yield. The ^1H- and ^{13}C NMR spectra of **53** ($[\alpha]_D^{25}$ −183 (*c*. 0.49, $CHCl_3$), 100% *ee* based on chiral HPLC analysis) were identical with those of racemic material prepared previously by Martin *et al*. (37), the relative stereochemistry at C-14 and C-15 of which was established to be *threo* by X-ray analysis. Careful removal of the Cbz group (TMSI in CH_3CN at –15°C for 30 min) gave the secondary amine, norpandamarilactonine-B (**12**) ($[\alpha]_D^{24}$ –49.4 (*c*. 1.22, $CHCl_3$)) in quantitative yield.

The final stage of the total synthesis of **7** was the coupling of optically active amine **12** with the nine-carbon unit containing a γ-alkylidene butenolide moiety. Iodide **22**, which consisted of the *Z*- and *E*-isomers in the ratio of 5:1, was condensed with freshly prepared **12** in CH_3CN in the presence of Ag_2CO_3 at room temperature to furnish the adducts in 66% yield. After repeated column chromatography, pure pandamarilactonine-A (**7**) was obtained in 48% yield. The synthetic compound, with 14*S* and 15*S* configurations, displayed spectroscopic data, including ^1H- and ^{13}C NMR, UV, IR, MS, and HR-MS, completely identical with those of the natural product, and exhibited $[\alpha]_D^{23}$ −94.0 (*c*. 0.12, $CHCl_3$). This demonstrated that the absolute configuration of the major enantiomer in ''natural'' pandamarilactonine-A (**7**) was 14*R* and 15*R*.

Honda *et al*. have prepared enantiopure *N*-Boc-norpandamarilactonine-A by a double ring-closing metathesis (RCM) reaction of tetraene derivative **64** that yields a dehydro analogue (Scheme 5) (50). Initially, L-serine methyl ester hydrochloride was converted into the known compound **54** which, on allylation with allyl iodide and NaH in DMF, gave the *N*-allyl compound **55**. Removal of the acetonide in **55** on treatment with *p*-toluenesulfonic acid gave diol **56**. After selective protection of the primary alcohol of **56** with *tert*-butyldimethylsilyl chloride, the resulting silyl ether **57** was protected as its MOM ether **58**, which was further converted into primary alcohol **59** by treatment with TBAF. Oxidation of **59** with Dess–Martin periodinane proceeded smoothly to give aldehyde **60** which, on methylenation with methyltriphenylphosphonium bromide and *n*-BuLi in the usual manner, afforded the desired triene **61** in good yield. After the two-step manipulation of the protecting groups in **61**, which involved removal of the Boc and MOM groups by acid hydrolysis, followed by protection of the resulting amino group of **62** with Boc_2O, the resulting secondary alcohol **63** was esterified with methacrylic acid in the presence of DCC and DMAP to provide the desired tetraene derivative **64**. With this tetraene available, a study was conducted to determine the best conditions for the double RCM reaction.



Scheme 5 Reagents and conditions: a, allyl iodide, NaH, DMF, 63%; b, p-TsOH, MeOH, 92%; c, TBDMSCl, Im, DMF, 93%; d, MOMCl, *i*Pr_2NEt, DMAP, CH_2Cl_2, 77%; e, TBAF, THF, 95%; f, Dess–Martin periodinane, CH_2Cl_2, 96%; g, $CH_3P^+Ph_3Br^-$, *n*-BuLi, THF, 85%; h, 10% HCl, MeOH, 73%; i, Boc_2O, Et_3N, THF, 76%; j, methacrylic acid, DCC, DMAP, CH_2Cl_2, 86%; k, (i) 10% 2nd Grubbs cat., benzene, 80°C, **65** 2%, **66** 25%, **67** 7%, **68** 25%, (ii) 10% Hoveyda cat., benzene, 80°C, **65** 6%, **66** 2%, **67** 28%, **68** 24%, (iii) 10% Hoveyda cat., benzene, 60°C, **65** 73%, **66** 3%, **67** 0%, **68** 23%, (iv) 10% Grela cat., benzene, 60°C, **65** 76%, **66** 2%, **67** 0%, **68** 22%; l, 10% Hoveyda cat., benzene, 60°C, 76%; m, Wilkinson cat., H_2 (5 atm), CH_2Cl_2, 95%; n, TMSOTf, 2,6-lutidine, CH_2Cl_2, 43%; o, Ag_2CO_3, CH_3CN, (+)-(**8**) 62%, (±)-(**7**) 15%.

First, the authors attempted the double RCM of **64** using 10 mol% Grubbs's 2nd-generation ruthenium catalyst (A) in benzene at 80°C for 20 h; however, desired compound **65** was isolated in only 2% yield. The major products were mono-cyclized **66** and **68** in 25% yield each. When this reaction was carried out using 10 mol% Hoveyda catalyst (B) in benzene at 80°C for 20 h, bicyclic tetrahydropyridine derivative **67** was isolated as the major product in 28% yield,

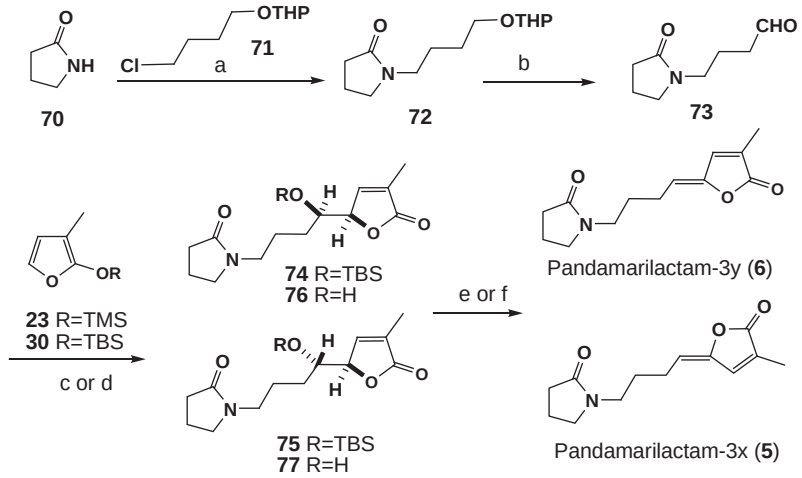
together with the desired compound **65** (6%), pyrrolidine derivative **66** (2%), and tetrahydropyridine derivative **68** (24%). Interestingly, a similar reaction of **64** with 10 mol% Hoveyda catalyst at 60°C gave the desired compound **65** in 73% yield, together with **68** (23%). On screening a variety of reaction conditions for RCM of **64**, the authors found that the use of 10 mol% Grela catalyst (C) in benzene at 60°C for 20 h afforded the desired compound **65** in 76% yield. Mono-cyclized pyrrolidine **66** could also be converted into **65** in 84% yield by further reaction with 10 mol% Hoveyda catalyst in benzene at 60°C for 20 h. The structure of **65** was unambiguously determined by X-ray crystallography. The fact that the cyclization product ratios depended on the reaction conditions, particularly the reaction temperature, implied that the bis-five-membered compound **65** is a kinetically controlled product.

Selective reduction of the double bond in the pyrrolidine ring of **65** was successfully achieved by catalytic hydrogenation with the Wilkinson catalyst under 5 atm hydrogen to afford enantiopure *N*-Boc-norpandamarilactonine-A (**69**) (m.p. 75–77°C, $[\alpha]_D$ −51.1 (*c*. 1.0, $CHCl_3$)) in 95% yield. Treatment of **69** with trimethylsilyl triflate provided (+)-norpandamarilactonine-A (**11**), $[\alpha]_D$ +55.0 (*c*. 0.82, $CHCl_3$) [lit. (50), +80.2 (*c*. 0.79, $CHCl_3$)], as the sole product. Compound **11**, however, gradually became a mixture with norpandamarilactonine-B (**12**), due to rapid epimerization. The conversion of (+)-**11** into pandamarilactonine-B **2** by coupling with iodide **22** was attempted according to Takayama's procedure, and gave racemic pandamarilactonine-A (**7**) and optically active pandamarilactonine-B (**8**) in 15% and 62% yields, respectively (50).

B. Pandamarilactams

Pandamarilactam-3y (**6**) and -3x (**5**) (35) are the members with the simplest structure among the known *Pandanus* alkaloids. Their synthesis was accomplished by sequential coupling of three components: 2-pyrrolidinone (**70**), the propane fragment **71**, and siloxyfuran **30** (Scheme 6) (51). *N*-Alkylation of 2-pyrrolidinone (**70**) with THP 4-chlorobutanol (**71**) was efficiently carried out using potassium fluoride on alumina in the presence of a catalytic amount of sodium iodide to produce tertiary amide **72** in 76% yield. After removal of the THP ether in **72**, the resultant free alcohol was converted into aldehyde **73** in 85% yield by Swern oxidation. The aldol reaction of aldehyde **73** with siloxyfuran **30** using tetrabutylammonium fluoride (TBAF) in the presence of TBSOTf gave a mixture of adducts in 37% yield [*syn* (**74**): *anti* (**75**)=3:7] along with desilylated alcohols (**76** and **77**) (3:7) in 11% yield.

Condensation of aldehyde **73** with 2-trimethylsiloxy-3-methylfuran (**23**) in the presence of $BF_3 \cdot Et_2O$ gave the adducts as a mixture of *syn* (**76**) and *anti* (**77**) isomers (ratio 88:12) in 80% yield. Treatment of a mixture of the *O*-silylated adduct [(**74** and **75**) (3:7 ratio)] with DBU gave γ-alkylidenebutenolides in 92% yield in a 9:1 ratio of the *Z*- and *E*-mixture. This result was ascribable to the E1cb mechanism that favored the formation of the thermodynamically more stable isomer. Dehydration of the major aldol adduct **76** with excess DEAD/PPh_3 resulted in *anti*-elimination to form exclusively the *Z*-isomer **6** in 82% yield.



Scheme 6 Reagents and conditions: a, KF-alumina, cat. NaI, DMF, 76%; b, (i) p-TsOH, MeOH, (ii) Swern oxidation, 85%; c, TBAF, TBSOTf, CH_2Cl_2, 48%; d, $BF_3 \cdot Et_2O$, CH_2Cl_2, 80%; e, DBU, $CHCl_3$, 70°C, 92%; f, DEAD, PPh_3, THF, −40°C, 82%.

The geometric mixture obtained above by the elimination reaction with DBU was purified by HPLC with a C18-silica column using $MeCN/H_2O$ as eluant. The major and minor isomers were identical with the natural products, pandamarilactam-3y (**6**) and -3x (**5**), respectively (35).

IV. BIOGENESIS

The *Pandanus* alkaloids represent a new structural class of alkaloids with a C9-N-C9 skeletal structure possessing a γ-alkylidene-α,β-unsaturated-γ-lactone or a γ-alkylidene-α,β-unsaturated-γ-lactam moiety. When considering how these alkaloids might be formed in the plant, the existence of a symmetrical secondary amine as a precursor intermediate can be postulated, which then undergoes intramolecular cyclization to give the isolated alkaloids.

It is likely that the five-membered lactone ring of these alkaloids is derived from 4-hydroxy-4-methylglutamic acid (**78**), which has been identified in some higher plants, and was found to occur in the related species *P. veitchii* (33). A biosynthetic study conducted by Peterson and Fowden suggested that leucine could be the biogenetic origin of 4-methylglutamic acid, which was then converted to 4-hydroxy-4-methylglutamic acid (34). 4-Hydroxy-4-methylglutamic acid, which is a suitable precursor for the five-membered ring structure can cyclize to either a lactone or a lactam ring product. The carbon chain C-6/C-9 and the nitrogen of the *Pandanus* alkaloids may derive from glutamic acid. The pandamarilactams (**5**, **6**) could then derive from combination of one unit of **78** derived from leucine with the C_4-N-C_4 unit **79** while the norpandamarilactonines (**11**, **12**) could derive from **78** and glutamate (Figure 5).

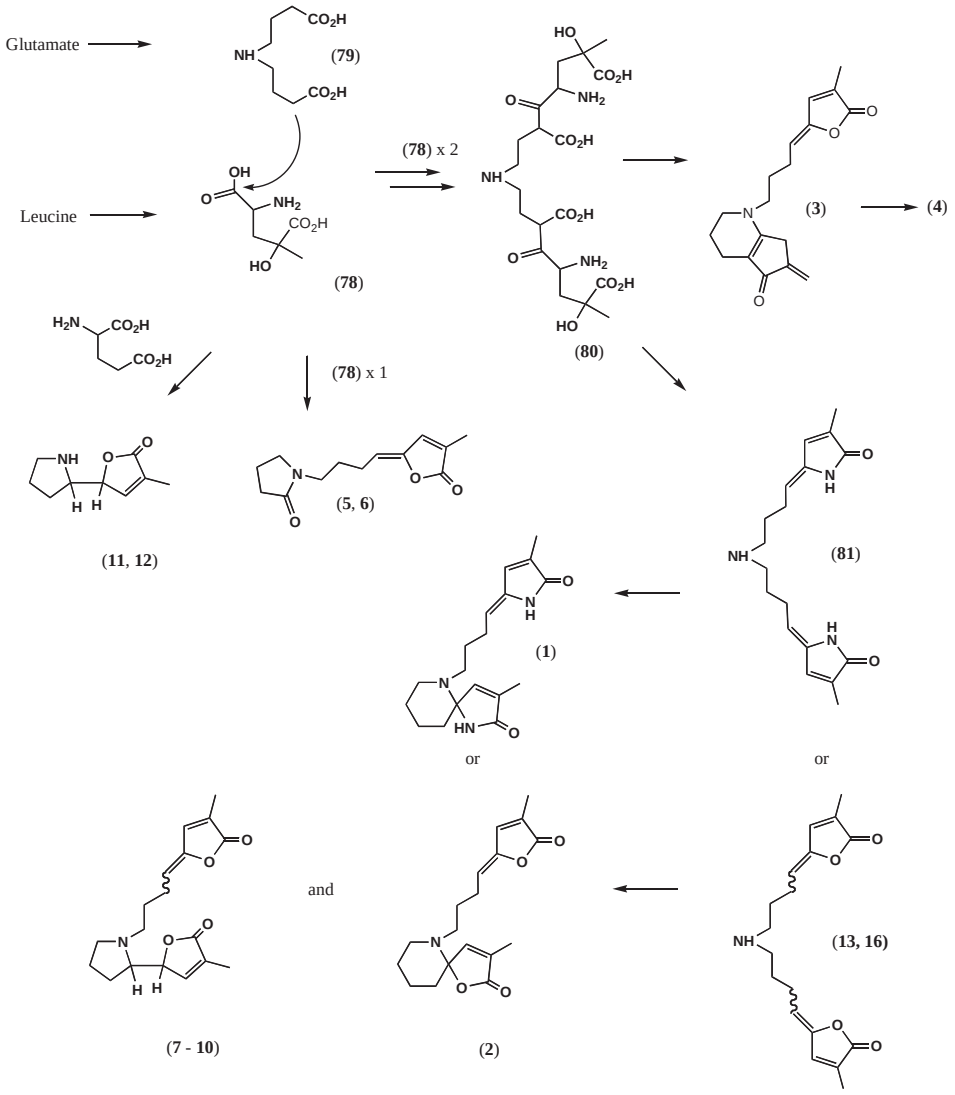


Figure 5 Biogenetic pathway of the *Pandanus* alkaloids.

The majority of *Pandanus* alkaloids derived from combination of two 4-hydroxy-4-methylglutamic acid units with the glutamate-derived C_4-N-C_4 unit *via* an intermediate equivalent to **80** that may undergo decarboxylation, dehydration, and transamination to generate the requisite γ-alkylidene-α,β-unsaturated lactone moieties. The pathway from precursor **80** to alkaloids **3** and **4** could involve the formation of a double bond between C-14 and C-15 followed by a ''domino''-like cyclization triggered by attack of an imine or amine nitrogen on this double bond, with eventual C–C bond formation between C-14 and the carboxyl group.

The isolation of the symmetrical pandanamine (**13**) in Nature (47) strongly supports the final stages of the proposed biogenetic pathways (Figure 5) leading to the diverse *Pandanus* alkaloid structures (**2**, **7–10**, **14**, **15**) described earlier in this Chapter. The involvement of the lactam equivalent **81** of pandanamine (**13**) in *Pandanus* chemistry was first discussed by Byrne *et al.* (29) when this compound was proposed as the precursor to pandamarine (**1**), and later by Takayama *et al.* (36). It is intriguing that **1**, whose structure was elucidated using X-ray diffraction (29), and **2** (30) differ in structure only by the replacement of a NH by an O in the two heterocyclic rings. This is especially interesting since the extract from which **2** was isolated was supposedly obtained from the same *Pandanus* species, but collected at a different time and place. No pandamarine (**1**) was observed in the plant extract from which **2** was obtained.

V. PHARMACOLOGY

The spiropiperidine structural unit present in pandamarine (**1**) and pandamarilactone-1 (**2**) has received considerable attention from several synthetic organic chemists (52,53). Natural alkaloids with spiropiperidine units are said to display interesting biological properties, such as ion transport inhibition at the cholinergic receptor and phospholipase A2 (PLA_2) activity inhibition, and thus are potential anti-inflammatory agents (54,55). To date, there are no reports of any biological activity of the *Pandanus* plants that is directly associated with the alkaloids.

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