Pyrrolizidine Alkaloids from *Amphorogyne spicata*

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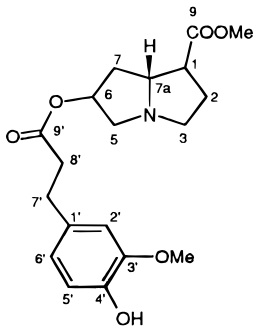
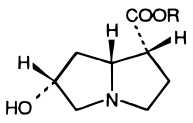
Four pyrrolizidine alkaloids, amphorogynines A−D (**1**−**4**), which belong to a new type having substituents at C-1 and C-6, were isolated from *Amphorogyne spicata* (Santalaceae). Their structures were elucidated by spectroscopic methods. The absolute stereochemistry (7a*R*) of the two main alkaloids, amphorogynines A and D, was determined using chemical correlations.

In a systematic search for alkaloids in New Caledonian plants,[1] we have isolated four pyrrolizidine-type alkaloids, amphorogynines A−D (**1**−**4**), from the leaves of *Amphorogyne spicata* Stauffer & Hürlimann (Santalaceae). The genus *Amphorogyne* has never been studied before for its chemical content. However, pyrrolizidine alkaloids were decribed from a species of another genus belonging to the family Santalaceae, *Thesium minkwitzianum*.[2]



1 H-1β, H-6β
2 H-1α, H-6α
3 H-1β, H-6α



4 R = Me
5 R = H
6 R = Et

The alkaloids were obtained from the ground leaves using the usual extraction method and purified by repeated chromatography on silica gel.

The major alkaloid, amphorogynine A (**1**), gave an MH^+ peak in the HRCIMS at *m*/*z* 364.1747, which corresponded to the molecular formula of $C_{19}H_{25}NO_6$. The IR exhibited an intense ester band at 1730 cm^{-1}. The ^1H NMR (Table 1) showed signals of a trisubstituted benzene ring at δ 6.64 (d, $J = 2$ Hz), 6.74 (d, $J = 8$ Hz), 6.62 (dd, $J = 8, 2$ Hz), two methoxy singlets at δ 3.64 and 3.81, an oxymethine group at δ 5.15, and a series of methine or methylene groups between δ 3.8 and 1.4. The ^{13}C NMR showed a pattern characteristic of an aromatic ring substitued by two ortho OH and OMe (δ 55.8) groups, while the third substituent para to the OH or to the OMe functions was an alkyl group. Two ester carbonyls were observed at δ 172.6 and 173.3; one of them was part of a COOMe group with the OMe appearing at δ 51.7. The number and chemical shifts of the three methine and the six methylene groups between δ 64.5 and 26.5, together with the interpretation of the COSY and HETCOR experiments, suggested structure **1** with four methylenes belonging to the pyrrolizidine ring; the two others were assigned to the phenylpropionic acid ester chain at position 6, while the COOMe group was attached to C-1. The location of the aromatic 3′-OMe group was deduced[3,4] from the ^{13}C resonances of H-2′ (111.2) and H-5′ (114.6) and further from the NOESY cross-peak OMe-3′/H-2′. The HMBC spectrum (Table 1) confirmed the structure of **1**, showing numerous correlations (Table 1) including the diagnostic cross-peaks H-6/C-7a,C-9′ and H-1/C-2,C-3,C-7,C-7a,C-9. The 1β,6β,7aβ relative stereochemistry of the pyrrolizidine moiety was deduced from the NOESY spectrum (Table 1), especially the correlation H-6/H-7a and the strong correlations H-1/H-7a and H-6/H-7β.

The minor alkaloids, amphorogynines B (**2**) and C (**3**), were isomers of amphorogynine A (**1**). Both **2** and **3** exhibited the same MH^+ peak as **1** in the CIMS at *m*/*z* 364 corresponding to the same molecular formula $C_{19}H_{25}NO_6$. They differed from alkaloid **1** by the configuration at C-1 and C-6 (compound **2**) or C-6 only (compound **3**). The NMR spectra (Table 1) were very similar to those of compound **1**. Relative stereochemistry was established using NOESY experiments (Table 1). Thus, alkaloid **2** showed very strong correlations H-7β/H-7a and H-6/H-7α, which proved the H-6α configuration and a correlation H-1/H-7α diagnostic of the H-1α configuration. Alkaloid **3** showed intense cross-peaks H-1/H-7a and H-7β/H-7a, indicating an H-1β configuration and in addition the correlation H-6/H-7α, which proved the 6α configuration.

Amphorogynine D (**4**) revealed an MH^+ peak in the HRFABMS at *m*/*z* 172.0965 corresponding to the molecular formula $C_8H_{13}NO_3$. The IR spectrum exhibited an OH absorbance at 3440 cm^{-1} and a C=O band at 1730 cm^{-1}, which corresponded to an acid, since the NMR spectra clearly showed no aromatic ester and no carboxymethyl resonances. The ^1H NMR spectrum exhibited only aliphatic signals with one oxymethine at δ 4.74. The ^{13}C, COSY, and HETCOR and NOESY spectra were in accordance with structure **4**, that is the acid corresponding to **1** with a free alcohol function at position 6. The H-1β configuration was deduced from the NOESY correlation H-1/H-7a. As for the H-6 configuration, the correlation H-6/H-7a could not be observed in $CDCl_3$ since the signals of H-7a and H-5 overlapped at δ 3.50. In CD_3OD, H-7a (at δ 3.72) was well separated from H-5 at δ 3.62, but there was no NOESY cross-peak C-6/C-7a; however, in the COSY (CD_3OD) a long-range correlation was observed between H-6 and H-7a, indicating an H-6β configuration. Finally, **4** was correlated to **1** through the carboxymethyl derivative **5**. The latter was obtained by esterification of **4** as well as by acid hydrolysis (MeOH−HCl) of **1**.

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Table 1. ^{13}C NMR (75 MHz) and ^{1}H NMRData for Compounds **1**−**4** in $CDCl_3$[a]

	1				**2**			**3**			**4**		
position	δ C	δ H (*J* Hz)	HMBC	NOESY	δ C	δ (*J* Hz)	NOESY	δ C	δ (*J* Hz)	NOESY	δ C	δ (*J* Hz)	NOESY
1	47.2	3.18 ddd (8)	2, 3, 7, 7a, 9	2*β*, 7a	50.0	2.63 m	2, 7α, 7a	46.9	3.14 ddd (8)	2*β*, 7a	48.5	3.09 m	2, 3a, 3b, 7a
2	26.3	α 2.22 m	1, 3, 9	2*β*, 3α	30.9	2.20 m	3α, 3*β*	27.4	α 2.09 m	2*β*, 3α, 7α	33.8	2.34 m	
		β 1.90 m	1, 3, 7a, 9	3α, 3*β*					*β* 1.95 m	3α, 3*β*			
3	55.1	α 2.78 *μ*		3*β*, 5α	54.5	α 2.65 m	3*β*	54.1	α 2.79 m	3*β*, 5α	55.1	a 2.96 m	3b
		β 3.05 ddd (11, 8, 6)	1, 2, 5, 7a			*β* 3.28 ddd (6)			*β* 3.06 m			b 3.23 m	
5	59.4	α 2.72 dd (12,6)	3, 6, 7, 7a	5*β*, 7α	59.9	α 2.77 dd (12, 4, 5)	5*β*, 6, 6α	60.7	α 2.86 m	5*β*, 6, 7α	64.7	α 2.85 dd (13, 3)	5*β*, 7a
		β 3.26 dd (12, 5)	3, 6, 7, 7a	6, 7a		*β* 3.16 dd (12)			*β* 3.21 dd (12)			*β* 3.48 dd (13)	6, 7a
6	75.3	5.15 dddd (6)	8, 9′	7*β*, 7a	76.6	5.36 m	7α	76.6	5.32 m	7α	78.8	4.74 br s	7
7	34.4	α 1.48 ddd (14, 8.5)	1, 5, 6, 7a	7*β*	37.7	α 1.88 ddd (13, 7.5)	7*β*, 7a	34.6	α 1.65 ddd (14, 7, 5)	7*β*, 7a	33.5	2.18 m	
		β 2.18 ddd (14, 8)	1, 5, 6, 7a	7a		*β* 2.16 m	7a		*β* 1.86 ddd (14, 7)	7a			
7a	64.5	3.75 ddd (8)	2, 3, 5, 6		66.9	3.85 m		64.8	3.86 ddd (6)		63.9	3.50 m	
9	173.3				173.9			173.8			173.5		
9-OMe	51.7	3.64 s	9		56.0	3.68 s		52.1	3.66 s				
1′	131.9				132.3			132.4					
2′	111.2	6.64 d(2)	1, 2′,3′, 5′, 6′, 7′	7′, 8′,3′-OMe	111.3	6.67 d (2)	7′, 8′, 3′-OMe	111.4	6.65 d (2)	7′, 8′, 3′-OMe			
3′	147.0				146.8			147.0					
4′	144.6				144.4			144.6					
5′	114.6	6.74 d (8)		6′	114.8	6.78 d (8)	6′	114.8	6.77 d (8)	6′			
6′	120.7	6.62 dd (8, 2)	1′, 2′, 4′, 7′	7′, 8′	120.9	6.63 dd (8, 2)	7′, 8′	121.0	6.64 dd (8)	7′, 8′			
7′	30.6	2.81 t (7)	1′, 2′, 6′, 8′, 9′	8′	30.7	2.83 t (7.5)	8′	30.9	2.18 t (7.5)				
8′	36.3	2.53 t (7)	1′, 7′, 9′		36.3	2.55 t (7.5)		36.6	2.53 t (7.5)				
9′	172.6				172.9			173.0					
3′-OMe	55.6	3.81 s	3′		56.4	3.83 s		56.1	3.83s				

[a] Assignments based on 2D experiments.

The amphorogynines represent a new class of pyrrolizidine alkaloids since alkaloids showing substituents at both C-1and C-6 only have not been reported previously.[5] However, derivative **6**, i.e., the ethyl ester corresponding to **5**, had been prepared in an optically active form (7a*R*) as an intermediate in the synthesis of simple pyrrolizidine alkaloids.[6] Esterification of alkaloid **4** with EtOH−HCl afforded compound **6** having the same positive optical rotation as the synthetic derivative. Thus, the absolute chemistry of the major alkaloids **1** and **4** was determined as depicted (7a*R*).

Experimental Section

General Experimental Procedures. Optical rotations at 20 °C were taken on a Perkin-Elmer 241 polarimeter. Spectra were recorded as follows: IR, Nicolet 205 FT-IR spectrometer; HRCIMS (reagent gas: CH_4), Kratos MS 9; FABMS, Kratos MS 80; HRFABMS, VG-Zab-Seq spectrometer; NMR, Bruker AC 300 (^{1}H and ^{13}C NMR spectra) and AM 400 (2D NMR spectra). Column chromatography was performed using Si gel Merck H60. The solvent used for TLC (Si gel Merck 60 F_{254}) was EtOAc−MeOH 50:50 (visualization: Dragendorff spray reagent).

Plant Material. Leaves of *A. spicata* Stauffer & Hürlimann were collected in Aoupinié Forest, New Caledonia East Coast, in May 1997. The identification was made by one of us (M.L.) and Dr. M. Schmid (Muséum National d'Histoire Naturelle, Paris). Voucher specimens (LIT 00292) are deposited in the Herbarium of the Centre ORSTOM, Noumea, New Caledonia.

Extraction and Isolation. The dried ground leaves of *A. spicata* (1 kg), after basification with NH_4OH 40%, were Soxhlet extracted with CH_2Cl_2. The solution was concentrated and diluted with ether. The organic layer was further extracted with 5% HCl. The acid aqueous layer was washed with ether, basified with NH_4OH, and extracted with CH_2Cl_2. Repeated column chromatography of the organic extract (2.27 g) on Si gel with EtOAc/MeOH mixtures afforded compounds **2** (74 mg, EtOAc/MeOH 8:2; TLC R_f 0.60), **3** (6.5 mg, EtOAc/MeOH 8:2; TLC R_f 0.52), **1** (1.23 g, EtOAc/MeOH 8:2; TLC R_f 0.51), and **4** (152 mg, EtOAc/MeOH 6:4; TLC R_f 0.2).

Amphorogynine A (**1**): small white crystals from MeOH/heptane; mp 108 °C; $[\alpha]_D$ +53° ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$) 1730 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRCIMS *m*/*z* 364.1747 ($C_{19}H_{26}NO_6$, Δ −1.3 mmu).

Amphorogynine B (2): amorphous gum; $[\alpha]_D$ −7° ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$) 1730 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRCIMS *m*/*z* 364.1748 ($C_{19}H_{26}NO_6$, Δ −1.4 mmu).

Amphorogynine C (3): small white crystals from MeOH; mp 130 °C; $[\alpha]_D$ −2° ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$) 1730 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRCIMS *m*/*z* 364.1751 ($C_{19}H_{26}NO_6$, Δ −1.7 mmu).

Amphorogynine D (4): amorphous gum; $[\alpha]_D$ +17° ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$) 3440, 1730 cm^{-1}; ^{1}H and ^{13}C NMR data in $CDCl_3$, see Table 1; ^{1}H NMR (CD_3OD) δ 3.31 (1H, m, H-1), 2.44 (1H, m, H-2α), 2.54 (1H, m, H-2*β*), 3.23 (1H, m, 3a), 3.37 (1H, m, H-3b), 3.08 (1H, dd, *J* = 13, 2 Hz, H-5α), 3.62 (1H, dd, *J* = 13, 3 Hz, H-5*β*), 4.95 (1H, m, H-6), 2.44 (1H, m, H-7α), 2.30 (1H, m, H-7*β*), 3.72 (1H, m, H-7a); HRFABMS *m*/*z* 172.0965 ($C_8H_{13}NO_3$, Δ −1.1 mmu); NOESY correlations H-1/H-2*β*, H-1/H-7α, H-2α/H-2*β*, H-5α/H5*β*, H-5*β*/H-6, H-6/H-7α, H-6/H-7*β*, H-7α/H-7*β*, H-7*β*/H-7a.

Methyl 6α-Hydroxy-7a*R*-pyrrolizidine-1α-carboxylate (5). (a) By Acid Hydrolysis of Alkaloid 1. To a solution of alkaloid **1** (200 mg) in MeOH (2 mL) was added a mixture of MeOH (1.2 mL) and concentrated HCl (0.8 mL). The mixture was refluxed for 1 h, evaporated to dryness, and dissolved in water. The aqueous solution was extracted with ether. The organic layer was dried and evaporated, yielding the known dihydroferulic acid methyl ester[7] (103 mg). The aqueous layer was evaporated under reduced pressure, yielding the hydrochloride of **5** (100 mg), which crystallized from Me_2CO/MeOH: mp 147−148 °C; $[\alpha]_D$ +32° (MeOH, *c* 1); IR ν_{max} ($CHCl_3$) 3350, 2450, 1735 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.50 (1H, m, H-1), 2.25 (1H, m, H-2a), 2.65 (1H, m, H-2b), 3.50 (1H, m, 3a), 3.57 (1H, m, H-3b), 3.15 (1H, dd, *J* = 13, 5, Hz, H-5α), 3.70 (1H, dd, *J* = 13, 6 Hz, H-5*β*), 4.50 (1H, m, H-6), 2.45 (1H, m, H-7α), 1.72 (1H, m, H-7*β*), 3.50 (1H, ddd, H-7a), 3.74 (3H, s, 9-OMe); ^{13}C NMR (CD_3OD) δ 45.9 (C-1), 25.7 (C-2), 54.6 (C-3), 60.4 (C-5), 69.5 (C-6), 35.6 (C-7), 66.5 (C-7a), 170.4 (C-9), 51.6 (9-OMe); *anal.* C 48.45%, H 7.08%, N 6.27%, O 21.64%, Cl 16.35%, calcd for $C_9H_{16}ClNO_3$, C 48.76%, H 7.28%, N 6.32%, O 21.65%, Cl 15.99%. **(b) From Alkaloid 4.** To a solution of alkaloid **4** (16 mg) in MeOH (1 mL) was added a mixture of MeOH (0.6 mL) and concentrated HCl (0.4 mL). The mixture was refluxed for 1 h and evaporated to dryness, yielding **5** (hydrochloride, 20 mg) and physical and spectral data identical to those of the compound obtained from **1**.

Ethyl 6α-Hydroxy-7a(*R*)-pyrrolizidine-1α-carboxylate (6). To a solution of alkaloid **4** (63 mg) in EtOH (1 mL) was added a mixture of EtOH (0.6 mL) and concentrated HCl (0.4 mL). The mixture was refluxed for 1 h and evaporated to dryness. The residue was partitioned between aqueous saturated $NaHCO_3$ and CH_2Cl_2. The organic layer was evaporated, yielding **6** (35 mg), which was crystallized from EtOAc: mp 110 °C; $[\alpha]_D$ +68° ($CHCl_3$, *c* 1) [lit.[6] mp 109−110 °C, $[\alpha]_D$ +73.4°]; ^{1}H NMR ($CDCl_3$) δ 3.10 (1H, m, H-1), 1.90 (1H, m, H-2a), 2.20 (1H, m, H-2b), 3.90 (2H, m, 3), 2.52 (1H, dd, *J* = 13, 5 Hz), 3.22 (1H, dd, *J* = 13, 6 Hz, H-5*β*), 4.32 (1H, m, H-6), 1.40 (1H, m, H-7α), 2.53 (1H, m, H-7*β*), 3.75 (1H, m, H-7a), 4.18 (2H, m, COO*CH*$_2$Me), 1.23 (3H, t, *J* = 7 Hz, COOCH$_2$*Me*); ^{13}C NMR ($CDCl_3$): δ 47.8 (C-1), 27.1 (C-2), 54.0 (C-3), 62.4 (C-5), 72.3 (C-6), 37.6 (C-7), 64.9 (C-7a), 174.0 (C-9), 60.8 (COO*CH*$_2$Me), 14.4 (COOCH$_2$*Me*).

References and Notes

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