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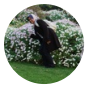

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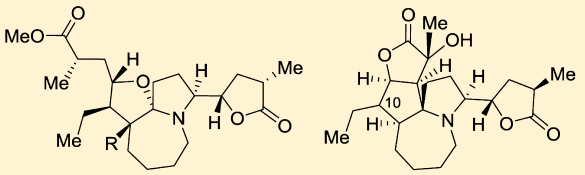
Alkaloids from the Roots of *Stichoneuron caudatum* and Their Acetylcholinesterase Inhibitory Activities

Rosdayati A. Ramli, Wilford Lie, and Stephen G. Pyne*

[†]School of Chemistry, University of Wollongong, Wollongong, New South Wales 2522, Australia

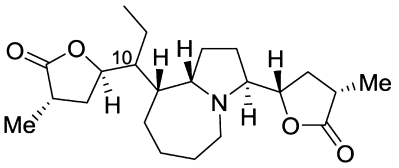
Supporting Information

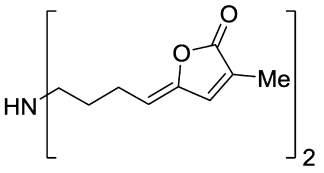
ABSTRACT: Four new stichoneurine-type alkaloids, stichoneurines F and G (**1**–**2**) and sessilistemonamines E and F (**3**–**4**), have been isolated from the root extracts of *Stichoneuron caudatum*. The structures and relative configurations of these alkaloids have been determined by spectroscopic methods and molecular modeling experiments. Compounds **1**–**4** were tested for their acetylcholinesterase (AChE) inhibitory activities against human AChE. Compound **3** showed significant inhibitory activity with an IC_{50} value of 9.1 ± 0.15 μM.



Stemonaceae is a small monocotyledonous family comprising three genera, *Stemona*, *Croomia*, and *Stichoneuron*.[1] *Stemona* is the largest genus of this family, and extracts of the roots of this plant species have been used in traditional medicine to treat the symptoms of bronchitis, pertussis, and tuberculosis and have been used as antiparasitics on humans and animals.[2] More than 130 unique alkaloids (collectively named as *Stemona* alkaloids) have been isolated from the root extracts, and sometimes from the leaf, of *Stemona* species. Some of these alkaloids have significant antitussive activity[3] as well as insect toxicity, antifeedant, and repellent activities.[4] These latter properties are most likely associated with the ability of these alkaloids to inhibit insect acetylcholinesterase (AChE).[4,5] Others have shown oxytocin antagonism,[6] nitric oxide inhibition,[7] and the ability to inhibit P-glycoprotein in multidrug-resistant cancer cell lines.[8]

The *Stemona* group of alkaloids have been structurally classified into seven different groups by Pilli[2a,c] and three skeletal types by Greger.[2b,d] The pyrrolo[1,2-*a*]azepine nucleus is common to the majority of compounds in these groups; however, several with a pyrido[1,2-*a*]azepine system,[9] two with a pyrido[1,2-*a*]azonine,[10] and one with an indolizidine nucleus[11] have also been reported. In contrast to *Stemona* species, only a few studies have been made on species of the genera *Croomine* and *Stichoneuron*.[2] Four species from the genus *Stichoneuron* have been documented from Peninsular Thailand and Malaysia; these are *S. bognerianum*, *S. calcicola*, *S. caudatum*, and *S. halabalensis*.[1] A study of the root extracts of *S. caudatum* from southern Thailand resulted in the isolation and identification of stichoneurines A and B (Figure 1).[12] A similar analysis of the root extracts of *S. calcicola* from southern Thailand surprisingly revealed the isolation of geometric isomers of pandanamine (Figure 1),[13] which suggested a close relationship between the plant families Pandanaceae and Stemonaceae. More recently, we reported the isolation of three new *Stemona* alkaloids, stichoneurines C–E (Figure 1), from the root and leaf extracts of *Stichoneuron halabalensis* collected from plants growing in the eastern part of Peninsular Malaysia.[14] Structurally, these alkaloids, along with the known stemoninine and bisdehydrooxystemoninine A, were, like





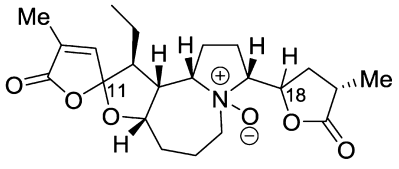


Figure 1. Alkaloids previously isolated from *Stichoneuron* species.

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stichoneurines A and B, all stichoneurine-type *Stemona* alkaloids.

In this paper, the phytochemical studies of the root extracts of *Stichoneuron caudatum* Ridley collected from plants growing in the eastern part of Peninsular Malaysia are described. The inhibitory activities of some of these alkaloids against human acetylcholinesterase (hAChE) are also reported.

RESULTS AND DISCUSSION

The roots of *S. caudatum* were collected near Lojing River, Gua Musang, Kelantan, Malaysia, in December 2011. Successive purification of the crude MeOH extract (56.0 g) of the roots by column chromatography gave pure samples of stichoneurines F (**1**) and G (**2**) and sessilistemonamines E (**3**) and F (**4**) (Figure 2).

Compounds **1**–**4** are new compounds; alkaloids **1** and **2** have been named based on their botanical origins and **3** and **4** on their structural relationship with the known sessilistemonamines A–C (Figure 1). Compound **1** was obtained as a pale yellow gum. Its molecular formula was confirmed as $C_{23}H_{37}NO_5$ from its HRESIMS (*m/z* 408.2750 $[M + H]^+$, calc for 408.2753) and ^{13}C NMR data. The IR spectrum showed characteristic ester and γ-lactone carbonyl absorptions at 1734 and 1771 cm^{-1}, respectively. The ^{13}C/DEPT NMR spectrum displayed resonances for three methyl [δ 10.4 (C-17), 13.6 (C-22) and 16.6 (C-15)], nine methylene [δ 45.0 (C-5), 37.2 (C-12), 32.8 (C-19), 31.1 (C-7), 30.7 (C-1), 26.3 (C-8), 23.3 (C-16), 23.3 (C-2), and 23.0 (C-6)], five methine [δ 66.4 (C-3), 49.4 (C-9), 46.3 (C-10), 36.7 (C-13), and 34.7 (C-20)], two oxymethine [δ 82.1 (C-18) and 76.6 (C-11)], a methoxy group [δ 50.3], and three quaternary carbons [δ 180.2 (C-21), 176.9 (C-14), and 104.5 (C-9a)] (Table 1). The correlations in the COSY spectrum indicated the spin system H-1–H-2–H-3–H-18–H-19–H-20–H-22, typical of the pyrrolidine ring of the *Stemona* alkaloids with a γ-lactone substituent at C-3 (Figure 3). However, unlike many of these alkaloids,[2] C-9a in **1** was a quaternary carbon, and not a methine, since H-1 showed only a COSY correlation to H-2. COSY correlations were also observed between the vicinal pairs of contiguous protons along the C-5–C-13 backbone, with vicinal correlations also observed between H-10 and H-16; H-16 and H-17; and H-13 and H-15 (Figure 2). The ^1H NMR/COSY spectra indicated the presence of two different methyl groups from the resonances at δ 1.16 (d, *J* = 6.5 Hz, 3H, H-22) and δ 1.12 (d, *J* = 7.0 Hz, 3H, H-15) that are coupled to methines at δ 2.73–2.63 (m, 1H, H-20) and δ 2.72–2.63 (m, 1H, H-13), respectively, and a methyl group at δ 0.90 (t, *J* = 7.5 Hz, 3H, H-17) which is vicinally coupled to the methylene protons at δ 1.46–1.26 (m, 2H, H-16).

Key HMBC correlations for **1** are shown in Figure 3, while full details are provided in Table 2. HSQC and HMBC experiments identified the protons and carbons (C-18–C-22) of the γ-butyrolactone moiety (Figure 3), which was linked to C-3 based on the HMBC correlation between H-3 (δ 3.06 (app t, *J* = 7.0 Hz, 1H)) and C-18 (δ 82.1). The carbonyl carbon resonance at δ 176.9 (C-14) and the methoxy proton resonance at δ 3.63 suggested a methyl ester. This was confirmed from their mutual correlation in the HMBC spectrum. The HMBC correlation between C-14 and the Me-15 protons [δ 1.12 (d, *J* = 7.0 Hz, 3H)] indicated that the Me-15 was in a position α to the ester group. The HMBC correlations from H-1 and H-10 to the quaternary carbon resonance at δ 104.5 identified the latter as that of C-9a. The deshielded ^{13}C NMR chemical shift of C-9a indicated that this carbon was substituted by two heteroatoms, in this case a nitrogen and an oxygen atom.

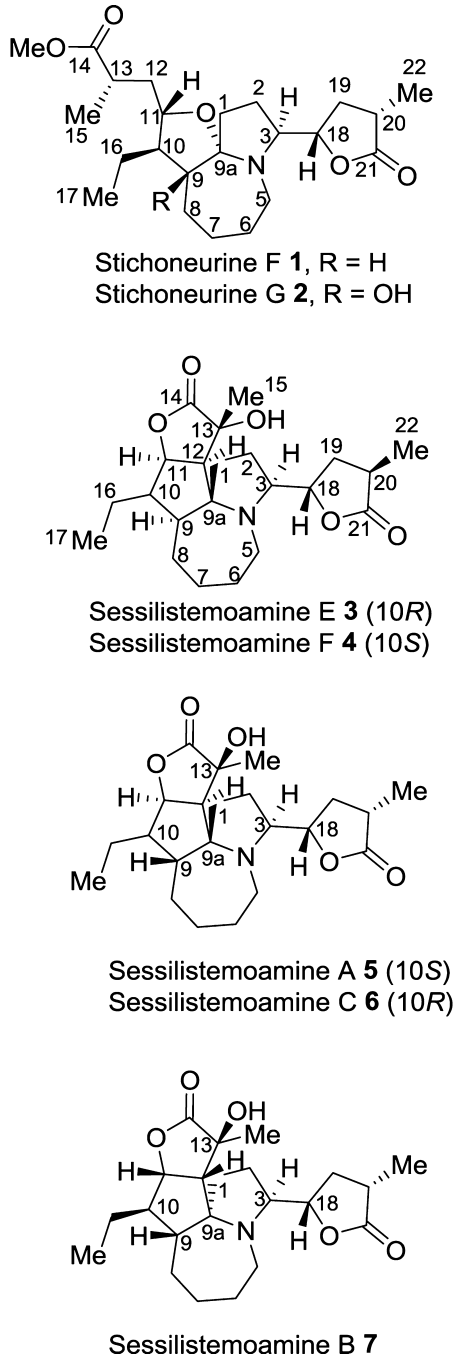

Figure 2. New *Stemona* alkaloids **1**–**4** and the known sessilistemoamines **5**–**7**.

The HMBC correlations of H-9 to C-10 and of H-10 to C-9a, C-11, C-16, and C-17 identified the connectivity and position of the C-10 ethyl substituent in the tetrahydrofuran ring of **1**. The HMBC spectrum showed that C-12 (δ 37.2) correlated to H-11 (δ 3.17) and H-13 (δ 2.72–2.63); C-14 (δ 176.9) correlated to H-12 (δ 2.05–1.97) and H-15 (δ 1.12); and C-11 (δ 76.6) correlated to H-12 (δ 2.05–1.97). These data and correlations indicated that a methyl 2-methylpropanoate moiety (C-12–C-14) was attached via C-12 to C-11 of the tetrahydrofuran ring. The relative configuration of **1** was determined from ROESY and molecular modeling experiments.

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 Hz) NMR Data for Compounds 1−4 in Methanol-d_4 (δ in ppm)

	δ_H (J in Hz)				δ_C			
position	1	2	3	4	1[a]	2	3	4
1α	1.77−1.68, m	1.70−1.64, m	1.91−1.77, m	1.27−1.17, m	30.7	33.8	43.2	34.6
β	1.67−1.59, m	1.95−1.89, m	1.91−1.77, m	1.95−1.83, m				
2α	2.03−1.93, m	2.05−1.98, m	1.90−1.80, m	2.06−1.94, dt (7.0, 13.0)	23.3	22.5	24.9	23.9
β	1.36−1.26, m	1.47−1.41, m	1.56−1.47, m	1.51−1.35, m				
3	3.06, app t (7.0)	3.08, app t (9.5)	3.28−3.25, m	3.21, ddd (8.5, 8.5, 8.5)	66.4	67.9	69.5	67.0
5 α	2.96, app t (13.0)	3.00−2.95, m	2.48−2.41, m	2.81−2.76, dd, (10.5, 16.0)	45.0	45.6	48.5	51.2
β	2.69−2.61, m	2.67, br d (15.0)	2.88−2.83, m	3.32−3.20, m				
6	1.57−1.37, m	1.71−1.62, m	1.57−1.48, m	1.40−1.31, m	23.0	23.9	31.0	26.1
	1.57−1.37, m	1.60−1.51, m	1.78−1.71, m	1.60−1.50, m				
7α	1.55−1.47, m	1.72−1.66, m	1.21−1.13, m	1.74−1.65, m	31.1	36.2	32.1	26.5
β	1.47−1.40, m	1.91−1.85, m	1.86−1.79, m	1.49−1.41, m				
8α	1.69−1.60, m	1.55−1.49, m	0.93−0.81, m	1.26−1.14, m	26.3	30.6	27.3	29.3
β	1.69−1.60, m	1.55−1.49, m	1.64−1.59, m	1.86−1.76, m				
9	2.01−1.93, m		2.04−1.99, m	1.77−1.65, m	49.4	83.7	53.9	48.0
9a					104.5	109.1	81.9	76.9
10	1.69−1.60, m	1.77−1.71, m	2.06−1.98, m	1.57−1.45, m	46.3	51.1	48.6	47.3
11	3.17, t (8.5)	3.40, br t (9.5)	4.95, dd (5.0, 6.3)	4.93, t (7.3)	76.6	76.1	85.5	85.1
12 a	1.60−1.52, m	1.63−1.56, m	2.60, d (6.3)	2.40, d (7.3)	37.2	38.1	60.2	58.1
b	2.05−1.97, m	2.11−2.03, m						
13	2.72−2.63, m	2.78−2.70, m			36.7	35.4	77.8	75.5
14					176.9	177.8	180.5	181.0
15	1.12, d (7.0)	1.18, d (7.0)	1.76, s	1.66, s	16.6	17.1	20.5	20.4
16	1.46−1.26, m	1.58−1.49, m	1.68−1.58, m	1.58−1.49, m	23.3	17.9	20.0	20.7
	1.46−1.26, m	1.28−1.19, m	1.56−1.46, m	1.36−1.49, m				
17	0.90, t (7.5)	0.99, t (7.5)	0.98, t (7.0)	1.00, t (7.0)	10.4	12.3	12.7	11.3
18	4.29−4.26 (m)	4.34−4.30, m	4.54, ddd (5.5, 10.0, 10.0)	4.33, ddd (5.5, 8.5, 11.0)	82.1	82.9	79.6	81.4
19 α	1.53−1.42, m	1.57−1.50, m	1.61−1.56, m	1.56−1.41, m	32.8	33.5	36.7	33.8
β	2.46−2.37, m	2.52−2.44, m	2.53−2.46, m	2.47−2.32, m				
20	2.73−2.63, m	2.78−2.71, m	2.81−2.72, m	2.73−2.65, m	34.7	37.2	36.6	35.0
21					180.2	181.2	182.2	180.9
22	1.16, d (6.5)	1.22, d (7.0)	1.21, d (7.0)	1.19, d (7.0)	13.6	14.1	15.2	13.8
OMe	3.63 (s)	3.65 (s)			50.3	50.9		

[a]In some cases, the ^{13}C NMR resonances of **1** were determined from the HSQC and HMBC spectra.

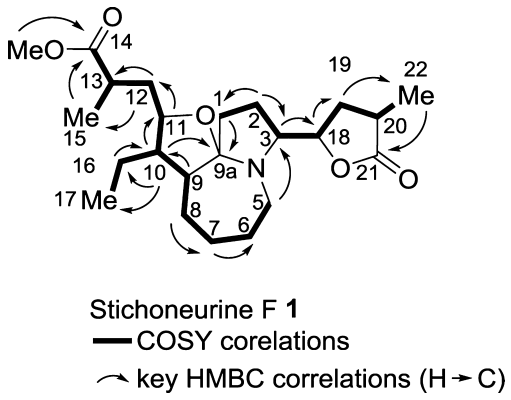

Figure 3. Key COSY and HMBC correlations for compound **1**.

The ROESY spectrum showed correlations between H-9 and H-11; H-11 and H-1 α and H-16; H-1α and H-2α; and H-2α and H-3. These correlations, along with molecular modeling studies (Figure 4), indicated that the tetrahydrofuran ring in **1** was *cis*-fused to the azepine ring at C-9 and C-9a and that the C-3 proton was on the opposite face of the pyrrolo[1,2-*a*]azepine nucleus to H-9, H-11, and H-16, which had a mutual *syn*-stereochemical relationship. Figure 4 shows the lowest energy conformer of **1**. Other low energy conformers (the next 20 higher energy ones, ranging from ca. 1−12 kcal/mol higher in energy) were also examined; however, in these conformers the conformation of the basic tricyclic structure remained essentially the same. The relative configurations proposed for the pyrrolo[1,2-*a*]azepine and tetrahydrofuran rings in this structure are consistent with the calculated interproton distances (all less than 3 Å) for the aforementioned correlated protons. Since the majority of *Stemona* alkaloids have the 3*S* configuration, including stichoneurines A and B (Figure 1), we have tentatively assigned this absolute configuration at C-3 for compound **1**. The ROESY correlations between H-18 and H-19β; H-19β and H-20; and H-19α and H-22 indicated the relative *syn*-configuration between H-18 and H-20 of the γ-lactone moiety (Figure 4). Because of the relatively free rotation around the C-3−C-18 bond in both possible diastereomeric structures for **1** ((3*S*, 18*S*, 20*S*)-**1** or (3*S*, 18*R*, 20*R*)-**1**), it was not possible to confidently assign the relative configurations of these two vicinal (C-3 and C-18) stereocenters from molecular modeling and ROESY NMR studies. However, we have tentatively assigned the 3*S*,18*S*,20*S* configuration to **1** since this is the most commonly found absolute configuration of the *Stemona* alkaloids.[2] For similar conformational reasons we cannot unambiguously assign the configuration at C-13 relative to that of C-11; however, we have assigned a 13*S*-configuration, based on this configuration in

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Table 2. HMBC, ROESY, and COSY Correlations of Compounds 1 and 2

	1				2			
position	δC	HMBC	ROESY	COSY	δC	HMBC	ROESY	COSY
1α	30.7	2, 3	1β, 2α, 3, 11	2	33.8	2	1β, 2α, 11	2
β			1α, 2β				1α, 2β	
2α	23.3	1, 18	1α, 2β, 3, 11, 15, 18	1, 3	22.5	1, 3, 18	1α, 3, 2β, 15	1, 3
β			1β, 2α, 15				1β, 2α, 15, 18, 19β	
3	66.4	1, 5, 18, 19	1α, 2α, 5β, 10, 18, 19α	2, 18	67.9	1, 2, 5, 18, 19	2α, 5α, 5β, 18, 19α	2, 18
5α	45.0	3, 7	5β, 7α, 8α, 12a	6	45.6	6	3, 5β, 7α, 8α, 12a	6
β			3, 5α, 18, 19α, 22				3, 5α, 18, 19α, 22	
6α	23.0	7		5, 7	23.9	7	19α	5, 7
β			18, 19β					
7α	31.1	5, 6	5α	6, 8	36.2	5, 8	5α	6, 8
β								
8α	26.3	7	5α, 9, 16a	7, 9	30.6	5α, 7, 10	5α	7
β								
9	49.4	10	8α, 11	8, 10	83.7	1, 8, 10, 11, 16		
9a	104.5	1, 2, 5, 10			109.1	1, 2, 5		
10	46.3	8, 16, 17	3, 12a, 16a, 16b	9, 11	51.1	7, 8, 11, 16, 17	12a, 16a, 16b, 17	11
11	76.6	10, 12, 16	1α, 2α, 9, 12b, 13, 15, 16b	10, 12	76.1	1, 10, 12, 13, 16	1α, 12b, 13, 15	10, 12
12a	37.2	15, 16	5α, 10, 12b, 15, 16b	11, 13	38.1	10, 11	5α, 10, 12b, 15, 16b	11, 13
b			11, 12a, 13, 15, 17				11, 12a, 13, 15, 17	
13	36.7	12	11, 12b, 15, 17	12	35.4	10, 12	11, 12b, 15	12
14	176.9	12, 15, OMe			177.8	12, 15, OMe		
15	16.6	12, OMe	2α, 2β, 11, 12a, 12b, 13	13	17.1	11, 12	2α, 2β, 11, 12a, 12b, 13	13
16a	23.3	11, 17	10, 8α	10, 17	17.9	10, 17	10	10, 17
b			10, 11, 12a, 17				10, 12a, 17	
17	10.4	16	12b, 13, 16b	16	12.3	10, 16	10, 12b, 16b	16
18	82.1	2, 3, 19β, 20	2α, 3, 5β, 6β, 19β	3, 19	82.9	2, 3	2β, 3, 5β, 19β, 20	3, 19
19α	32.8	20, 22	3, 5β, 19β, 22	18, 20	33.5	20	3, 6α, 5β, 19β, 22	18, 20
β			6β, 18, 19α, 20				2β, 18, 19α, 20	
20	34.7	19, 22,	19β, 22	19, 22	37.2	19, 22	18, 19β, 22	19, 22
21	180.2	19, 20, 22,			181.2	19, 20, 22		
22	13.6	19, 20	5β, 19α, 20	20	14.1	19, 20	5β, 19α, 20	20
OMe	50.3				50.9	13		

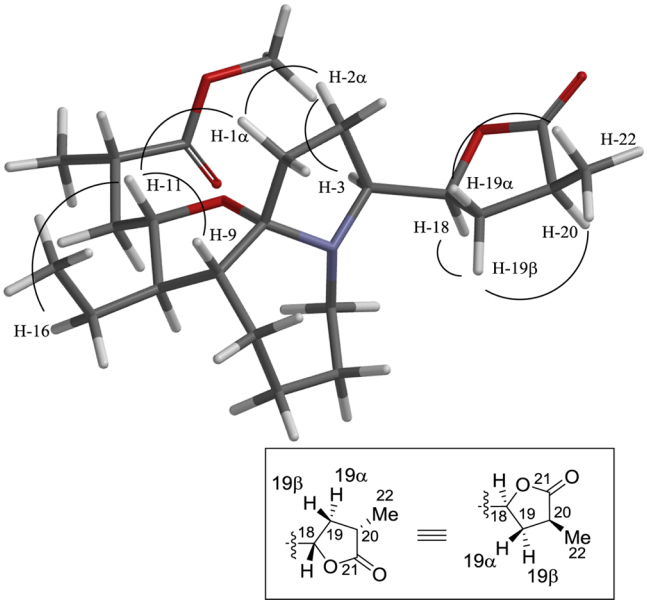

Figure 4. Spartan '10 generated lowest energy conformation of **1** showing key ROESY cross-peaks. (The structures shown in here and in figure 6 were generated using Spartan '10 and conformational searching (MMFF) to find the lowest energy conformers).

stichoneurines A and B. Indeed, it is conceivable that **1** arises biosynthetically from stichoneurine B via cyclization of the free 11-hydroxy group onto an N-4−C-9a iminium ion intermediate to produce the tetrahydrofuran ring of **1**.

Analysis of the ^{1}H and ^{13}C NMR spectra of **1** (Table 1), as well as 2D NMR analyses (COSY, HMBC, and ROESY, Table 2), established the complete structure and relative configuration of **1**.

Compound **2** was obtained as a pale yellow gum. The molecular formula of **2**, $C_{23}H_{37}NO_6$, was confirmed from its HRESIMS (*m/z* 424.2699 $[M + H]^+$, calcd for $C_{23}H_{38}NO_6$ 424.2682) and ^{13}C NMR data. These data suggested that compound **2** had one more oxygen atom than compound **1**. The IR spectrum of **2** showed characteristic hydroxy, ester, and γ-lactone carbonyl absorptions at 3520, 1730, and 1761 cm^{-1}, respectively. The ^{13}C/DEPT NMR spectrum of **2** was similar to that of **1** and indicated that one methine carbon in compound **1** had become a quaternary carbon in compound **2**. The chemical shift of the resonance for the new quaternary carbon at δ 83.7 (C-9) suggested that it was substituted by a hydroxy group. There were ^{13}C/DEPT NMR resonances for three methyl [δ 12.3 (C-17), 13.6 (C-22), and 17.1 (C-15)], nine methylene [δ 45.6 (C-5), 38.1 (C-12), 33.5 (C-19), 36.2 (C-7), 33.8 (C-1), 30.6 (C-8), 23.9 (C-6), 22.5 (C-2), and 17.9 (C-16)], four methine [δ 67.9 (C-3), 51.1 (C-10), 37.2 (C-20), and 35.4 (C-13)], two oxymethine [δ 82.9 (C-18) and 76.1 (C-

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11)], a methoxy group [δ 50.9], and four quaternary carbons [δ 181.2 (C-21), 177.8 (C-14), 109.1 (C-9a), and 83.7 (C-9)]. The COSY and HMBC spectra of **2** showed analogous correlations to those observed in **1**, except that the correlations observed from H-9 in the spectrum of **1** were absent. The ROESY spectrum of **2** showed correlations between H-11 and H-1α and H-16; H-1α and H-2α; and H-2α and H-3, which were also found in the ROESY spectrum of compound **1**, and correlations between H-18 and H-19β; H-19β and H-20; and H-19α and H-22 which indicated the relative *syn*-configuration between H-18 and H-20 of the γ-lactone moiety. This data was thus consistent with **2** being the C-9 hydroxy derivative of compound **1**.

Compound **3** was obtained as a pale yellow gum. The molecular formula of **3**, $C_{22}H_{33}NO_5$, was confirmed by HRESIMS (m/z 392.2436 $[M + H]^+$, calcd for $C_{22}H_{34}NO_5$ 392.2376) and ^{13}C NMR data. The IR spectrum of **3** showed characteristic hydroxy and γ-lactone carbonyl absorptions at 3348 and 1761 cm^{-1}, respectively. The ^{13}C/DEPT NMR spectrum displayed resonances for three methyl [δ 20.5 (C-15), 15.2 (C-22), and 12.7 (C-17)], eight methylene [δ 48.5 (C-5), 43.2 (C-1), 36.7 (C-19), 32.1 (C-7), 31.0 (C-6), 27.3 (C-8), 24.9 (C-2), and 20.0 (C-16)], two oxymethine [δ 79.6 (C-18) and 85.5 (C-11)], five methine [δ 69.5 (C-3), 60.2 (C-12), 53.9 (C-9), 48.9 (C-10), and 36.6 (C-20)], and four quaternary carbons [δ 182.2 (C-21), 180.5 (C-14), 81.9 (C-9a), and 77.8 (C-13)]. The quaternary carbon resonances at δ 180.5 (C-14) and 182.2 (C-21) and the oxymethine carbon resonances at δ 85.5 (C-11) and 79.6 (C-18), with their corresponding deshielded ^1H NMR resonances at δ 4.95 (dd, J = 5.0, 6.3, 1H, H-11) and δ 4.54 (ddd, J = 5.5, 10.0, 10.0, 1H, H-18), respectively, indicated the presence of two γ-butyrolactone rings.

The COSY correlations in **3** indicated the spin system H-1−H-2−H-3−H-18−H-19−H-20−H-22, typical of the pyrrolidine ring of the *Stemona* alkaloids with a γ-lactone substituent at C-3 (Figure 5). Similar to compounds **1** and **2**, C-9a in **3** was a

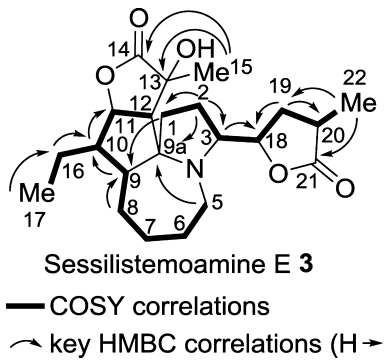

Figure 5. Key COSY and HMBC correlations for compound **3**.

quaternary carbon since H-1 showed only a COSY correlation to H-2. COSY correlations were also observed between the vicinal pairs of contiguous protons along the C-5−C-12 backbone, with correlations indicating the vicinal relationships between H-10 and H-16 and H-16 and H-17 (Figure 5). The ^1H NMR/COSY spectra indicated the presence of a methyl group [δ 1.76 (s, 3H, H-15)] attached to a quaternary carbon (C-13), a methyl group [δ 1.21 (d, J = 7.0 Hz, 3H, H-22)] coupled to a methine at δ 2.81−2.72 (m, 1H, H-20), and a methyl group [δ 0.98 (t, J = 7.0 Hz, 3H, H-17)] which was vicinally coupled to the methylene resonance at δ 1.56−1.46 (m, 2H, H-16).

Key HMBC correlations are shown in Figure 5, while full details are provided in Table 3. HSQC and HMBC experiments identified the protons and carbons (C-18−C-22) of the appended γ-butyrolactone moiety (Figure 5), which was clearly attached to C-3 from the HMBC correlation between H-3 [δ 3.21 (ddd, J = 8.5, 8.5, 8.5 Hz, 1H)] and C-18 (δ 79.6). HSQC and HMBC experiments facilitated identification of the protons and carbons (C-11−C-15) associated with the fused γ-butyrolactone moiety of **3** (Figure 5).

These data suggested that **3** was a stereoisomer of the sessilistemonamines A−C (Figure 2), which were isolated from the root extracts of *Stemona sessilifolia*.[15] Compound **3** showed ROESY correlations between H-9 and H-10; H-10 and H-11; H-11 and H-12 (Figure 6 and Table 3), indicating the mutal *syn*-stereochemical relationships between all these hydrogens and that **3** was a different stereoisomer to sessilistemonamines A−C. ROSEY correlations were also observed between H-1α and H-3; H-2α and H-3; H-3 and 15-Me; H-2β and H-18; H-18 and H-5β and H-19β; H-5α and H-9; and H-19β and 22-Me (Figure 6). These data were consistent with the structure assigned to compound **3** which has the opposite configurations at C-9, C-13, and C-20 to sessilistemonamines A−C (**5**−**7**), the opposite configuration at C-10 to sessilistemonamine A (**5**), and the opposite configuration at C-9a to sessilistemonamine B (**7**) (Figure 2).

For conformational mobility reasons the relative configurations between the rigid tetracyclic framework and that of the appended γ-butyrolactone in **3** could not be unequivocally assigned, although the ROESY correlations observed were consistent with the structure and the relative configuration proposed for **3** (Figure 6).

Compound **4** was obtained as a pale yellow gum. The molecular formula of **4**, $C_{22}H_{33}NO_5$, was confirmed by HRESIMS (m/z 392.2437 $[M + H]^+$, calcd for $C_{22}H_{34}NO_5$ 392.2376) and ^{13}C NMR data. The IR spectrum of **4** showed characteristic hydroxy and γ-lactone carbonyl absorptions at 3454 and 1764 cm^{-1}, respectively. The ^{13}C/DEPT NMR spectrum of **4** displayed signals for three methyl [δ 20.4 (C-15), δ 13.8 (C-22), and δ 11.3 (C-17)], eight methylene [δ 51.2 (C-5), δ 34.6 (C-1), δ 33.8 (C-19), δ 29.3 (C-8), δ 26.5 (C-7), δ 26.1 (C-6), δ 23.9 (C-2), and δ 20.7 (C-16)], two oxymethine [δ 85.1 (C-11) and δ 81.4 (C-18)], five methine [δ 67.0 (C-3), δ 58.1 (C-12), δ 48.0 (C-9), δ 47.3 (C-10), and δ 35.0 (C-20)], and four quaternary carbons [δ 181.0 (C-14), δ 180.9 (C-21), δ 76.9 (C-9a), and δ 75.5 (C-13)]. The quaternary carbon resonances at δ 180.9 (C-21) and 181.0 (C-14) and the methine resonances at δ 85.1 (C-11) and 81.4 (C-18), with their corresponding deshielded ^1H NMR resonances at δ 4.95 (dd, J = 5.0, 6.3, 1H, H-11) and δ 4.54 (ddd, J = 5.5, 10.0, 10.0, 1H, H-18), respectively, indicated the presence of two γ-butyrolactone rings. These data suggested that **4** was a stereoisomer of **3** and sessilistemonamines A−C. The ROESY spectrum of **4** showed correlations between H-1α and H-3; H-2α and H-3; H-3 and H-15; H-9 and H-16; H-11 and H-12; H-11 and H-16; and H-12 and H-15. These correlations indicated that **4** had the same configurations at C-9, C-9a, and C-11−C-13 as compound **3**. Howeve, the lack of ROESY correlations between H-9 and H-10 and H-10 and H-11, as were observed in the ROESY spectrum of **3**, and the correlations between H-16 and H-9 and H-10, indicated that **4** had the opposite configuration at C-10. Alkaloid **4** had ROESY correlations

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Table 3. HMBC, ROESY, and COSY Correlations of Compounds 3 and 4

	3				4			
position	δ_C	HMBC	ROESY	COSY	δ_C	HMBC	ROESY	COSY
1α	43.2	9, 12	1β, 2α, 3, 12, 16a, 19α	2	34.6		1β, 2α,3, 12, 19α	2
β			1α, 16b, 19β				1α, 2β, 10	
2α	24.9	1	1α, 2β, 3, 18, 19β	1	23.9	1, 3	1α, 2β, 3, 12, 18	1
β			2α, 19β				1β, 2α, 5β, 19β	
3	69.5	2	1α, 2α, 12, 15, 19α	2	67.0	1, 2, 5, 8, 19	1α, 2α, 15, 19α	2
5α	48.5		5β, 9	6	51.2	3	5β, 9	6
β			5α, 6β, 18				2β, 5α, 7β, 8β, 19β	
6α	31.0	8		5	26.1			5
β			5β					
7α	32.1	5, 9	9	6, 8	26.5			6, 8
β							5β, 17	
8	27.3	9, 10		7, 9	29.3	2, 5, 7, 12		7, 9
							5β	
9	53.9	1, 8, 10, 12	5α, 7α, 10, 12	8, 10	48.0	8, 12	5α, 16a	8, 10
9a	81.9	1, 2, 9, 5			76.9	1, 2, 5, 7, 8, 9, 15		
10	48.6	9, 12, 16, 17	9, 11, 17		47.3	9, 11, 12, 16, 17	1β	
11	85.5	10, 16	10, 12	10, 12	85.1	10, 12, 16	12, 16a, 17	10, 12
12	60.2	1, 9	1α, 3, 9, 11, 15	11	58.1	1, 8, 15	1α, 2α, 11, 15, 16	11
13	77.8	12, 15			75.5	12, 15		
14	180.5	12, 15			181.0	15, 11		
15	20.5		3, 12		20.4	12	3, 12, 19α	
16a	20.0	10, 17	1α	10, 17	20.7	17	9, 11, 12	10, 17
b			1β					
17	12.7	16	10	16	11.3	10, 16	7β, 11	16
18	79.6	3, 19	2α, 5β, 19β	3, 19	81.4	3, 19	2α, 19β	3, 19
19α	36.7	22	1α, 3, 5α, 19β, 20	18, 20	33.8	20, 22	1α, 3, 5α, 15, 20	18, 20
β			1β, 2α, 18, 19α, 22				2β, 5β, 18,22	
20	36.6	19, 22	19α, 22	19, 22	35.0	19, 22	19α, 22	19, 22
21	182.2	19, 20, 22			180.9	22, 19		
22	15.2	19, 20	19β, 20	20	13.8	20, 19	19β, 20	20

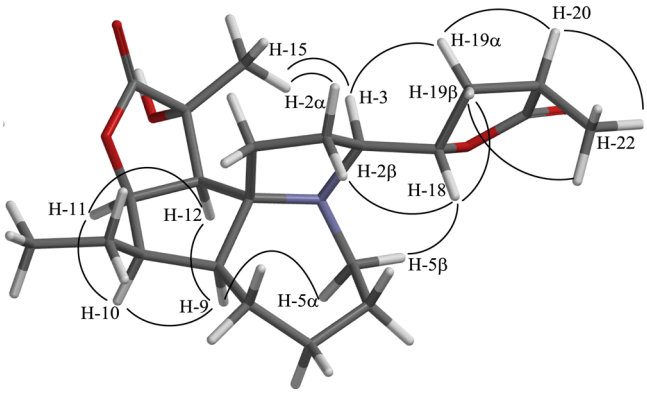

Figure 6. Spartan '10 generated lowest energy conformation of **3** showing key ROESY cross-peaks.

between the pyrrolidine ring protons and those on the C-3 γ-lactone ring similar to those observed in compound **3**, specifically between H-3 and H-19α; H-2β and H-18; H-18 and H-19β; and H-19β and 22-Me (Table 3). These ROESY correlations indicated that compounds **3** and **4** were epimeric at C-10 (see Tables 1 and 3).

Compounds **1**−**4** and the methanol root extract of *S. caudatum* were tested as inhibitors of human acetylcholinesterase (hAChE). These results are presented in Table 4 along with those of sessilistemonamines A−C, which were tested on an unspecified AChE.[15] The methanol root extract had a weak

Table 4. hAChE Inhibitory Activities of Isolated Compounds

compd	IC_{50} values μM (R^2)
1	71.5 ± 0.10 (0.9021)
2	>100
3	9.1 ± 0.15 (0.9444)
4	>100
methanol root extract	41.8 ± 0.05 μg/mL (0.9811)
galanthamine	0.55 ± 0.03 (0.9503)
sessilistemonamine A	68.8 ± 9.5[15]
sessilistemonamine B	17.1 ± 2.5[15]
sessilistemonamine C	>100[15]

activity against hAChE (IC_{50} 41.8 mg/mL), while alkaloid **1** showed modest activity (IC_{50} 71.5 μM) and alkaloids **2** and **4** were not active. Of the four new alkaloids, only alkaloid **3** showed a significant inhibitory activity with an IC_{50} value of 9.1 μM (Table 4). Compound **3** was significantly less active than the positive control, galanthamine (IC_{50} 0.55 μM). A comparison of the inhibitory activities of **1** and **2** indicated that the additional C-9 hydroxy group in **2** had an adverse effect on activity. The configuration at C-10 in the C-10 epimeric alkaloids **3** and **4** made a clear difference in their hAChE inhibitory activities. Sessilistemonamines A−C also showed a similar range of inhibitory activities as compounds **1**−**4** with sessilistemonamine B (IC_{50} 17.1 μM) showing similar activity to that of **3** (IC_{50} 9.1 μM). Interestingly these compounds have nearly mirror image structures of the core tetracyclic feature,

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except at C-3. Since the stereochemical assignments were only relative and not absolute, the above similar inhibitory activities may suggest that they have the same absolute configurations at C-9a and C-9−C-13.

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H (500 MHz), ^{13}C (125 MHz), and 2D NMR spectra were recorded on a Varian Unity 500 spectrometer in methanol-d_4 solution (referenced to residual solvent peaks at δ_H 3.31 and δ_C 49.0, respectively). HRESIMS were obtained with a Micromass QTOF 2 mass spectrometer using a cone voltage of 30 V and polyethylene glycol as an internal reference. TLC analyses were performed on aluminum-backed Merck 60 GF_{254} silica gel, and bands were detected by staining with Dragendorff's reagent. Column chromatography (CC) was performed using Merck GF_{254} flash silica gel (40−63 μm), and peparative TLC was performed using Merck TLC silica gel 60 GF_{254} (20 × 20 cm). Human acetylcholinesterase (906 U/mg) was generously donated by Dr. Moeava Tehei from the School of Chemistry, University of Wollongong.

Plant Materials. *S. caudatum* was collected near the Lojing River, Gua Musang, Kelantan, Malaysia, in December 2011. A voucher specimen (UKM29978) was deposited at the herbarium of the Department of Biology, National University of Malaysia. Plant material was identified by Prof. Latiff Mohammad from the Department of Biology, National University of Malaysia.

Extraction and Isolation. The dry, ground roots of *S. caudatum* (1.3 kg) were extracted with 95% MeOH (4 × 1000 mL) over 3 days at room temperature. The MeOH solution was evaporated to give a dark residue (56.0 g). The residue was chromatographed on silica gel (200 mL) using gradient elution from *n*-hexane/EtOAc (2:8−0:10) to CH_2Cl_2/EtOAc (2:8−0:10). A total of 5 L of eluent was collected in 200 mL test tubes. These fractions were pooled on the basis of TLC analysis to give three alkaloid fractions: A (1.5 g), B (150 mg), and C (3.5 g). Fraction A (1.5 g) was further separated by column chromatography (CC) using *n*-hexane/EtOAc (2:8) as the eluent to give fraction A1 (500 mg) and fraction A2 (320 mg). Fraction A1 (500 mg) was separated by reversed-phase CC (MeOH/H_2O; 9:1) to give fraction A11 (205 mg). Fraction A11 (205 mg) was further purified by silica gel CC using isocratic eluent (*n*-hexane/EtOAc (4:6)) to give 100.5 mg of pure stichoneurine F (**1**). Fraction A2 (320 mg) was subjected to reversed-phase CC (MeOH/H_2O (7.5:2.5), 100 mL of eluent) to give fraction A21 (23.1 mg). Fractions A21 (23.1 mg) was purified by silica gel CC using petroleum ether/acetone (2:8) to give 3.5 mg of pure sessilistemonamine E (**3**). Fraction B (150 mg) was separated by silica gel CC (petroleum ether/acetone (2:8)) to give fraction B2 (70.5 mg), which was purified again by the same procedure to give stichoneurine G (**2**) (49.2 mg). A portion of fraction C (2.0 g) was separated by silica gel CC (petroleum ether/acetone (3:7)) to give fraction C1 (364.9 mg). This fraction was chromatographed on silica gel (*n*-hexane/acetone (3:7)) to give fraction C12 (137.2 mg). This purification procedure was repeated on fraction C12 (137.2 mg) to give pure sessilistemonamine F (**4**) (50.2 mg).

*Stichoneurine F (**1**):* pale yellow gum; $[\alpha]^{23}_D$ −31 (*c* 0.1, $CHCl_3$); IR film ν_{max} 2927, 2857, 1771, 1734, 1457, 1191, 1163, 1014, 922 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 408.2750 $[M + H]^+$, calcd for $C_{23}H_{37}NO_5$ 408.2753

*Stichoneurine G (**2**):* white amorphous solid; $[\alpha]^{23}_D$ −21 (*c* 0.1, $CHCl_3$); IR film ν_{max} 3520, 2934, 2871, 1761, 1730, 1457, 1377, 1193, 1011, 923 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 424.2699 $[M + H]^+$, calcd for $C_{23}H_{37}NO_6$ 424.2682.

*Sessilistemoamine E (**3**):* pale yellow gum; $[\alpha]^{23}_D$ +11 (*c* 0.03, $CHCl_3$); IR film ν_{max} 3348, 2925, 2884, 1761, 1649, 1461, 1375, 1196, 1024, 978 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 391.2436 $[M + H]^+$, calcd for $C_{22}H_{33}NO_5$ 391. 2376

*Sessilistemoamine F (**4**):* white amorphous solid; $[\alpha]^{23}_D$ +46 (*c* 0.06,$CHCl_3$); IR film ν_{max} 3454, 2930, 2873, 1764, 1458, 1366, 1193, 1150, 1015, 916 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 391.2437 $[M + H]^+$, calcd for $C_{22}H_{33}NO_5$ 391.2376.

hAChE Inhibitory Assays. hAChE inhibitory assays were performed according to Ramli.[14] The multiwell plate was placed directly into the microplate reader, which was thermostated at 25 °C. The absorbances were read using a POLARstar Omega microplate thermostated spectrophotometer (Offenburg, Germany) at 412 nm. Enzyme activity was calculated as a percentage compared to an assay using a buffer without any inhibitor. The hAChE inhibitory data were analyzed with the software package GraphPad Prism (Graph Pad Inc., San Diego, CA). IC_{50} values are means ± SD of three individual determinations each performed in triplicate.

Statistical Analysis. The results were presented as means ± standard deviation from triplicate samples of three independent experiments. Differences between the means were analyzed by *t* test: two samples assuming unequal variances. Results are expressed according to significance: $P < 0.05$.

ASSOCIATED CONTENT

Supporting Information

Copies of the ^{1}H and ^{13}C NMR spectra of alkaloids **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: spyne@uow.edu.au.

Notes

The authors declare no competing financial interest.

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