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Sessilifoliamides K and L: new alkaloids from *Stemona sessilifolia*

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ABSTRACT

Two new alkaloids, sessilifoliamides K and L, having a pyrido[1,2-*a*]azonine skeleton were isolated from the roots of *Stemona sessilifolia* (Miq.) Miq. (Stemonaceae). Their structures were determined by interpretation of their spectroscopic data and computational methods.

Plants of the genus *Stemona* (family Stemonaceae) are known to be a rich source of alkaloids.[1–4] These alkaloids are generally of unique structures often characterized by having a pyrrolo[1,2-*a*]azepine skeleton. Recently, from *Stemona sessilifolia* (Miq.) Miq., we isolated an alkaloid, sessilifoliamide J, incorporating an indolizidine core in lieu of a pyrrolo[1,2-*a*]azepine core.[5] In continuation of our phytochemical investigation on this plant, we isolated two new alkaloids, sessilifoliamides K (**1**) and L (**2**), both having a novel alkaloid skeleton incorporating an unusual pyrido[1,2-*a*]azonine nucleus (Fig. 1). This paper describes their isolation and structure determination.

From 15 kg of the roots of *S. sessilifolia*, 9 kg of a MeOH extract was obtained, from which 360 g of a basic fraction and 160 g of a neutral/acidic fraction were prepared. Chromatographic separation of the latter fraction gave sessilifoliamides K (**1**, 1.9 mg, 0.000013%) and L (**2**, 0.7 mg, 0.000005%).

Sessilifoliamide K (**1**), $[\alpha]_D^{25}$ − 31 (*c* 0.075, MeOH), was obtained as an amorphous solid. Its molecular formula was determined to be $C_{23}H_{35}NO_7$ from the $[M+H]^+$ peak at *m/z* 438.2451 (calcd for $C_{23}H_{36}NO_7$, 438.2492) in the HRESIMS. The IR spectrum indicated the presence of hydroxyl (3391 cm^{-1}) and carbonyl (1760 and 1616 cm^{-1}) groups. Its ^1H NMR spectrum showed characteristic signals corresponding to one terminal methyl (δ_H 1.09), two secondary methyls (δ_H 1.28 and 1.32), one methoxy group (δ_H 3.74), and three heteroatom-substituted methine protons (δ_H 3.25, 4.07, and 4.53) (Table 1). Its ^{13}C NMR spectrum showed 23 signals caused by four methyls, seven methylenes, seven methines, and five quaternary carbons including three carbonyl carbons.

Analysis of the ^1H–^1H COSY and HMQC spectra revealed the presence of three molecular fragments, that is, fragments A, B, and C. Fragment A was of a seven-carbon chain (C-15–C-13–C-12–C-11–C-10–C-16–C-17) in which C-15 was a secondary methyl, C-17 a terminal methyl, and C-11 a heteroatom-substituted methine. Fragment B was of a six-carbon chain (C-2–C-3–C-18–C-19–C-20–C-22) in which C-22 was a secondary methyl, C-3 a heteroatom-substituted methine, and C-18 a hydroxymethine, and fragment C was of a four-carbon chain (C-5–C-6–C-7–C-8) in which C-5 was a heteroatom-substituted methylene (Fig. 2). The HMBC data revealed the relations between those three carbon-chain fragments and also the nature of the other skeleton atoms involved. The HMBC correlations from H-11, H-12, H-13, and H_3-15 to the C-14 (δ_C 181.1) carbonyl carbon indicated the presence of a γ-lactone linkage between C-14 and C-11. Correlations from H-3, H-11, and H-13 to C-1 (δ_C 84.4), H-2b to C-12 (δ_C 49.9), and H-12 to C-2 (δ_C 38.8) indicated that C-1, an oxygen-bearing quaternary carbon, was connected to fragments A and B at C-12 and C-2, respectively. Correlations from H-7b,

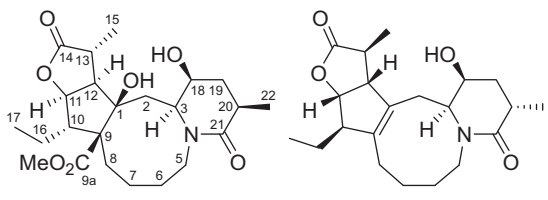

Figure 1. Structures of sessilifoliamides K (**1**) and L (**2**) from *Stemona sessilifolia*.

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Table 1
NMR data for sessilifoliamides K (**1**) and L (**2**) in $CDCl_3$

Position	1		2	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1		84.4		132.6
2	a 2.49 (d, 16.2)	38.8	a 2.53 (d, 14.3)	24.4
	b 2.41 (dd, 16.2, 6.4)		b 2.42 (dd, 14.3, 10.6)	
3	3.25 (t-like, 5.0)	63.2	3.04 (dd, 10.6, 4.6)	63.1
5	a 4.23 (dd, 13.8, 5.9)	51.4	a 4.48 (td, 13.4, 4.0)	46.6
	b 2.69 (td, 13.4, 4.4)		b 2.55 (dt, 14.0, 3.0)	
6	a 2.18 (m)	25.5	a 1.43 (m)	22.4
	b 1.22 (m)		b 1.38 (m)	
7	a 1.52 (m)	20.3	a 1.63 (m)	23.7
	b 1.32 ([c])		b 1.57 (m)	
8	a 1.91 (m)	22.9	a 2.27 (td, 13.2, 5.4)	24.6
	b 1.01 (dd, 14.6, 9.7)		b 1.99 (m)	
9		62.3		141.2
9a		176.2		
10	2.53 (m)	53.6	2.67 (m)	52.6
11	4.53 (dd, 7.9, 4.3)	86.5	4.82 (d, 5.5)	85.4
12	2.64 (d, 7.9)	49.9	3.13 (br d, 4.2)	57.6
13	2.94 (q, 7.7)	35.6	2.73 (qd, 7.6, 1.2)	38.6
14		181.1		180.0
15	1.32 (d, 7.7, 3H)	17.1	1.38 (d, 7.6, 3H)	17.4
16	a 1.93 (m)	22.9	a 1.61 (m)	22.5
	b 1.30 ([c])		b 1.19 (m)	
17	1.09 (t, 7.4, 3H)	12.8	0.93 (t, 7.4, 3H)	11.4
18	4.07 (m)	68.6	3.97 (ddd, 9.8, 8.7, 4.6)	67.2
19	a 2.03 (m)	33.4	a 2.04 (m)	33.5
	b 1.67 (td, 12.7, 9.5)		b 1.76 (m)	
20	2.50 (m)	34.9	2.67 (m)	33.0
21		173.3		174.0
22	1.28 (d, 7.4, 3H)	19.9	1.23 (d, 7.0, 3H)	18.2
OMe	3.74 (s, 3H)	51.9		
OH-1	4.46 (d, 2.1)			
OH-18	2.19 (d, 2.5)		[d]	

[a] Recorded at 600 MHz. Chemical shifts referenced to residual $CHCl_3$ (7.26 ppm); *J*-values given in Hz in parentheses.
[b] Recorded at 150 MHz. Chemical shifts referenced to $CDCl_3$ (77.03 ppm).
[c] Multiplicity patterns were unclear due to signal overlapping.
[d] Not observed in the present experiment.

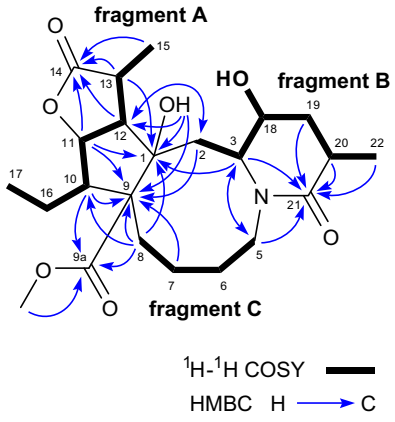

Figure 2. ^{1}H–^{1}H COSY and selected HMBC correlations of **1**.

H-8a, H-8b, H-10, and H-11 to C-9 (δ_C 62.3) and from H-8a to C-10 (δ_C 53.6) indicated that the C-9 quaternary carbon was connected to fragments A and C at C-10 and C-8, respectively. The correlations from H-2a and H-2b to C-9, and from H-8a and H-8b to C-1 revealed that C-1 and C-9 were linked to each other to form a cyclopentane ring along with C-10, C-11, and C-12. The hydroxyl proton at δ_H 4.46 correlating to C-1, C-9, and C-12 showed the presence of a hydroxyl group at C-1. The carbonyl carbon (δ_C 176.2), C-9a, was correlated with H-10, H-8b, and the methoxy protons, indicating that the methoxy group and C-9a constituted a carbomethoxy group connected to C-9. The third carbonyl carbon, C-21 (δ_C 173.3), showed correlations with H-19a, H-20, and H_3-22, indicating that C-21 was connected to C-20 of fragment B. On the basis of the chemical shift values of the signals of C-3 (δ_C 63.2) and C-5 (δ_C 51.4), and the HMBC correlations from H-3 to C-5, from H-5a and H-5b to C-3, and from H-3, H-5a, and H-5b to C-21, the nitrogen atom was shown to be connected to fragment B at C-3, to fragment C at C-5, and to the C-21 carbonyl group. From these observations, alkaloid **1** was concluded to possess the planar structure as shown in Figure 2.

The NOESY experiments reasonably established the relative stereochemistry of **1** (Fig. 3). The correlations between OH-1/H-10, OH-1/H-13, H-8b/H-12, and H-11/H-12 indicated that H-11, H-12, and Me-15 were on the same side of the molecule, whereas OH-1, the C-9a carbomethoxy group, and H-10 were on the opposite side, and that the γ-lactone/cyclopentane rings and the cyclopentane/azonane rings were both *cis*-fused. The correlations between H-3/H-5b, H-3/H-12, H-3/H-18, H-5b/H-8b, and H-18/H-20 revealed that H-3 and H-12 were on the same side of the molecule, whereas OH-18 and Me-22 were on the opposite side. Then, to further verify the NOESY results, the molecule of **1** having the stereochemistry derived from NOE studies was subjected to the Monte Carlo conformational search.[6] In the energetically most stable conformation, the distances between the relevant protons, involved in the above-mentioned diagnostic NOE correlations in **1**, that is, OH-1/H-10 and OH-1/H-13 were 3.0 Å and 3.4 Å,

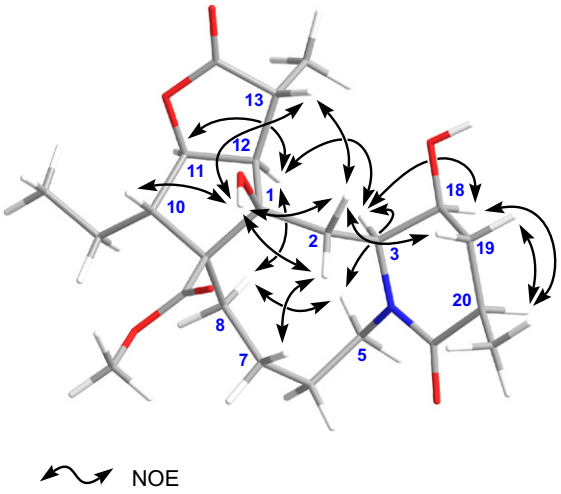

Figure 3. Most stable conformation of **1** found in the Monte Carlo conformational search with the observed key NOE correlations.

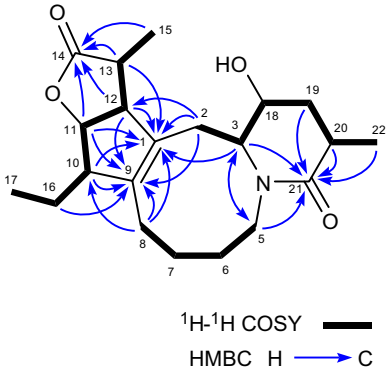

Figure 4. ^{1}H–^{1}H COSY and selected HMBC correlations of **2**.

respectively, and the other NOE cross-peak-producing protons including OH-1/H-2a, OH-1/H-2b, H-2b/H-7b, H-2a/H-13, H-2a/H-19b, and H-19a/H-20 were less than 2.7 Å (Fig. 3), all reasonable for producing NOE cross-peaks. The results also show that in solution, **1** takes the energetically most stable conformation as suggested by the Monte Carlo calculation. Accordingly, alkaloid **1** was determined to have the (1*R**,3*S**,9*S**,10*S**,11*R**,12*R**,13*R**,18*S**,20*R**) relative stereochemistry as shown in Figure 1.[7]

Sessilifoliamide L (**2**), $[\alpha]_D^{25} + 35$ (*c* 0.033, MeOH), was obtained as an amorphous solid. Its molecular formula was determined to be $C_{21}H_{31}NO_4$ from the $[M+H]^+$ peak at *m/z* 362.2346 (calcd for $C_{21}H_{32}NO_4$, 362.2331) in the HRESIMS. The IR spectrum indicated the presence of hydroxyl (3388 cm^{-1}), γ-lactone (1767 cm^{-1}), and amide carbonyl (1623 cm^{-1}) groups. Its ^1H NMR spectrum showed the presence of one terminal methyl (δ_H 0.93) and two secondary methyls (δ_H 1.23 and 1.38) and three heteroatom-substituted methine protons (δ_H 3.04, 3.97, and 4.82) (Table 1). Its ^{13}C NMR spectrum showed 21 signals caused by three methyls, seven methylenes, seven methines, and four quaternary carbons comprising two quaternary sp^2 carbons and two carbonyl carbons. Analysis of the ^1H–^1H COSY, HMQC, and HMBC spectra disclosed that **2** possessed the same skeletal features as **1**, but that it had no carbomethoxy group (Fig. 4). Another marked difference noted between **1** and **2** was that the ^{13}C NMR signals of C-1 (δ_C 132.6) and C-9 (δ_C 141.2) were of sp^2 quaternary carbons in **2**, whereas they were of sp^3 quaternary carbons in **1**. From these observations, the planar structure of **2** was determined to be as shown in Figure 4.

Its relative stereochemistry was deduced from its NOESY data (Fig. 5). The NOE correlations observed between H-3/H-5b, H-3/H-6b, H-3/H-13, H-3/H-18, H-5b/H-18, and H-6b/H-10 indicated that H-3, H-5b, H-6b, and H-10 were on the same side of the molecule, whereas Me-15 and OH-18 were on the opposite side. Other NOE correlations noted between H-2b/H-20, H-11/H-12, H-11/H_3-15, and H-12/H_3-15 revealed that the γ-lactone and cyclopentene rings were *cis*-fused, and that Me-22 and H-3 were on the same side, whereas H-11 and H-12 were on the opposite side.

The stereochemistry of **2** thus established was also verified by the distances between the NOE correlation-causing protons in the energetically most stable structure obtained by the Monte Carlo conformational search.[6] Thus, in the energetically most stable Monte Carlo conformation model of **2**, the distances between the protons showing the above-mentioned and other NOE correlations, that is, between H-2a/H-13, H-2a/H-19a, H-2b/H-8a, H-5a/H-7a, H-11/H-16b, and H-12/H-16b, were all less than 3.1 Å, which are short enough for causing cross-peaks in the NOESY spectrum (Fig. 5). From these observations, alkaloid **2** was concluded to have the (3*R**,10*S**,11*R**,12*S**,13*R**,18*R**,20*R**) relative stereochemistry as shown in Figure 1.

Sessilifoliamides K (**1**) and L (**2**) have a novel alkaloid skeleton incorporating an unusual pyrido[1,2-*a*]azonine core. The pyrido[1,2-*a*]azonine skeleton may be derived from the tuberostemoninol-type alkaloids **A**, previously isolated from this[8] and other *Stemona* plants,[2,9–11] as shown in Figure 6. Thus, an attack by a hydroxide anion on C-9a, a lactam carbonyl carbon of **A**, followed by O → N acyl migration gives acids **B** having a pyrido[1,2-*a*]azonine unit. **B** may be intermediary acids involved in the biosynthesis of present **1** and **2**. *O*-Methylation of **B** may give sessilifoliamide K (**1**), and decarboxylation and concomitant dehydration, sessilifoliamide L (**2**).[12]

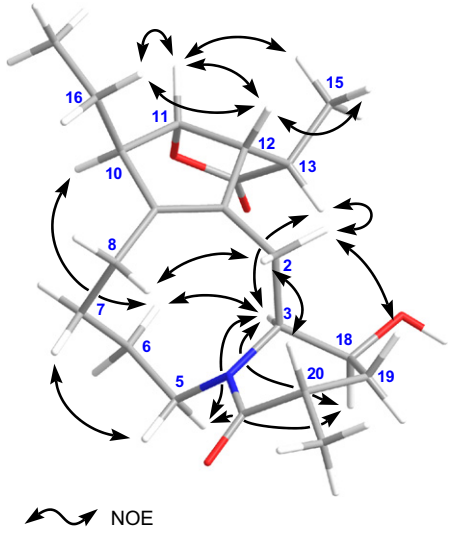

Figure 5. Most stable conformation of **2** found in the Monte Carlo conformational search with the observed key NOE correlations.

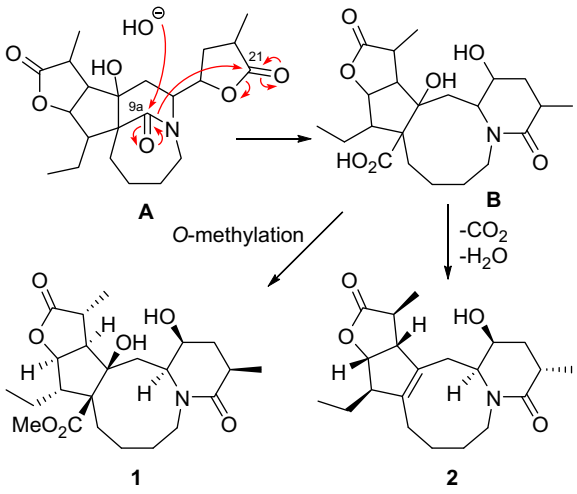

Figure 6. A possible biogenesis of sessilifoliamides K (**1**) and L (**2**) from tuberostemoninol-type alkaloids **A**.

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6. Monte Caro conformation searches were performed using the MacroModel 8.1 implementation of the MMFF force field and the Maestro 5.1 graphical interface. For each molecule, 50,000 search steps were performed.
7. To determine the absolute configuration of **1** through modified Mosher's ester analysis, we tried to prepare the MTPA esters of **1**. However, our attempt failed due to the low reactivity of the hydroxyl group at 18.
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12. Compounds **1** and **2** were shown to have no cytotoxic effect on cultured P-388 leukemia cells (IC_{50} >100 μg/mL).