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SESSILIFOLIAMIDE I, A NEW ALKALOID FROM *STEMONA SESSILIFOLIA*

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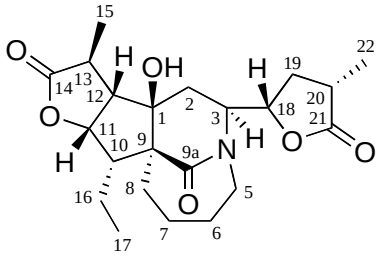
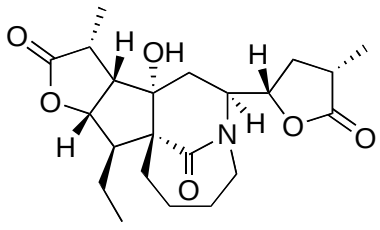
Abstract – A new alkaloid, sessilifoliamide I, was isolated from the roots of *Stemona sessilifolia* (Miq.) Miq. Its structure was established by interpretation of spectral data and X-ray crystallography.

INTRODUCTION

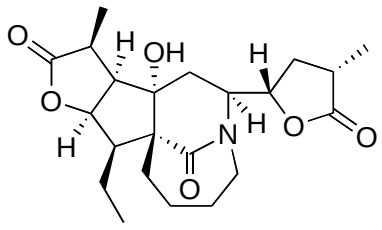
The genus *Stemona* plants (family Stemonaceae) have been used in China and Japan as an insecticide and a cough remedy. A number of alkaloids have been isolated from *Stemona japonica* (Blume) Miq., *Stemona tuberosa* Lour., and *Stemona sessilifolia* (Miq.) Miq.,[1] including tuberostemoninol (**2**)[2] and neotuberostemoninol (**3**)[3] from *S. tuberosa*, and sessilifoliamides A–H from *S. sessilifolia* reported by us.[4] The present paper describes further isolation of a new alkaloid, sessilifoliamide I (**1**), from the roots of *S. sessilifolia* and the structure elucidation of this new alkaloid on the basis of the spectral data and X-ray crystallographic analysis (Figure 1).



sessilifoliamide I (**1**)



tuberostemoninol (**2**)



neotuberostemoninol (**3**)

Figure 1

RESULTS AND DISCUSSION

From 15 kg of the roots of *Stemona sessilifolia*, 8 kg of a crude MeOH extract was obtained, from which 250 g of a basic fraction and 300 g of a neutral and acidic fraction were prepared. The neutral and acidic fraction was subjected to silica gel column chromatography, and the fraction obtained by eluting the column with EtOAc–MeOH (10/1) was then subjected to aminopropyl-bonded silica gel column chromatography, and then to reversed-phase HPLC to give 13.8 mg (0.000092%) of alkaloid **1**.

Sessilifoliamide I (**1**) was obtained as colorless prisms. Its molecular formula was determined to be $C_{22}H_{31}NO_6$ from the $[M + H]^+$ peak at *m/z* 406.2234 (calcd for $C_{22}H_{32}NO_6$ 406.2230) in the HRESIMS. The IR spectrum showed the presence of hydroxyl (3423 cm^{-1}), γ-lactone (1768 cm^{-1}), and lactam carbonyl (1656 cm^{-1}) groups. Its ^1H NMR spectrum showed the presence of one terminal (δ 1.10) and two secondary (δ 1.29 and 1.36) methyl groups, and two oxymethine protons (δ 4.44 and 5.15). Its ^{13}C NMR spectrum showed 22 signals caused by three methyls, seven methylenes, seven methines, and five quaternary carbons, of which three were carbonyl carbons. Analysis of the ^1H–^1H COSY and HMQC spectra revealed the presence of three molecular fragments, a four-carbon chain fragment composed of four methylenes (fragment A, C-5–C-6–C-7–C-8), a six-carbon chain fragment in which C-22 was a terminal methyl and C-18 was an oxymethine (fragment B, C-2–C-3–C-18–C-19–C-20–C-22), and a seven-carbon chain fragment in which C-15 and C-17 were two terminal methyls and C-11 was an oxymethine (fragment C, C-15–C-13–C-12–C-11–C-10–C-16–C-17) (Figure 2). The connectivity of those carbon chain fragments and the five quaternary carbons were determined on the basis of the HMBC data. Correlations from H_2-2, H-3, H-11, H-12, and H-13 to C-1 indicated that C-1 (δ_C 83.7), an oxygen-bearing quaternary carbon, was connected to fragments B and C at C-2 and C-12, respectively. The HMBC correlations from H-2b, H-10, H-11, and H-12 to C-9 indicated that C-9 was connected to both C-1 and C-10 of fragment C to form a cyclopentane ring, and those from H-8b to C-9 and C-10, and

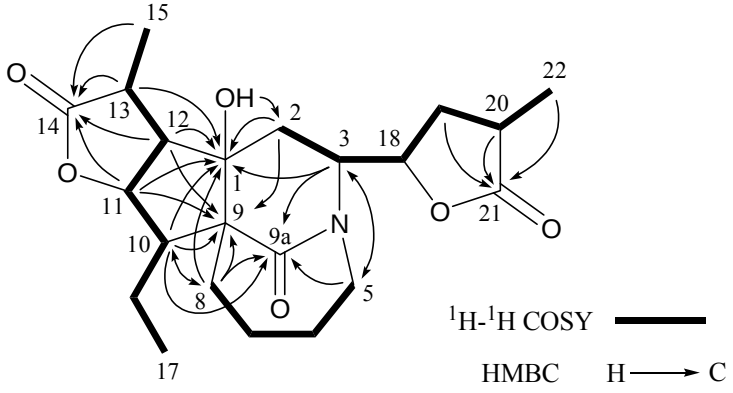


Figure 2. Selected ^1H-^1H COSY and HMBC correlations for sessilifoliamide I (**1**).

from H-10 to C-8 indicated that C-8 of fragment A was connected to C-9. On the basis of the chemical shift values of the signals of C-3 (δ_C 57.1) and C-5 (δ_C 54.1), and the HMBC correlations from H-3 to C-5 and from H-5a to C-3, both C-5 of fragment A, and C-3 of fragment B were connected to the nitrogen. The HMBC correlations from H-11, H-12, H-13, and H_3-15 to C-14 and from H_2-19, H-20, and H_3-22 to C-21 placed each of the two γ-lactones between C-14 (δ_C 179.5) and C-11, and between C-21 (δ_C 178.0) and C-18, respectively. The HMBC correlations from H-3, H-5a, H-8b, and H-10 to C-9a placed the remaining carbonyl carbon (C-9a, δ 182.4) between the nitrogen atom and C-9, and that from the hydroxyl proton (δ 2.92) to C-2 placed the hydroxyl group at C-1. From these observations, alkaloid **1** was concluded to possess the same gross structure as tuberostemoninol (**2**)[2] and neotuberostemoninol (**3**)[3] isolated from *S. tuberosa*. Differences between the chemical shifts of the ^{1}H and ^{13}C NMR signals of those three compounds indicated that **1** was a stereoisomer of **2** and **3**. The NOESY spectrum gave some information about its stereochemistry: the correlations between H-3/H-6, H-3/H-8a, H-8a/H-13, H-11/H_3-15, OH-1/H-10, OH-1/H-12, and H-18/H-20 indicated that in **1**, H-3, C-8, and Et-10 were α-oriented, OH-1, H-11, H-12, and Me-15 were β-oriented, and H-18 and H-20 were in a *cis*-relationship (Figure 3). However, the stereochemical relation between the core unit and the γ-lactone moiety remains

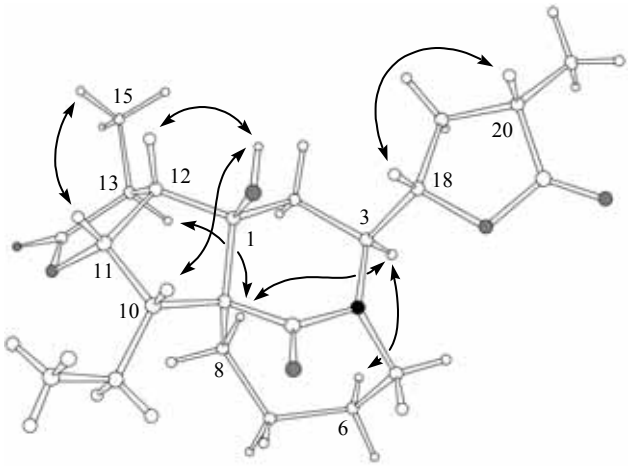


Figure 3. Selected NOE correlations for **1**.

unspecified because of the rotatable C-3–C-18 single bond. The relative stereochemistry of this alkaloid was finally established by X-ray crystallographic analysis as shown in Figure 4.

Sessilifoliamide I (**1**), tuberostemoninol (**2**),[2] and neotuberostemoninol (**3**)[3] are alkaloids of unique structures incorporating a naturally rare 1-azabicyclo[4.3.1]decan-10-one unit, which is seldom seen in natural products. Alkaloids **1**–**3** have the same gross structure, but their relative stereochemistry differs at several chiral centers, which may highlight the interesting biosynthetic route of these alkaloids.

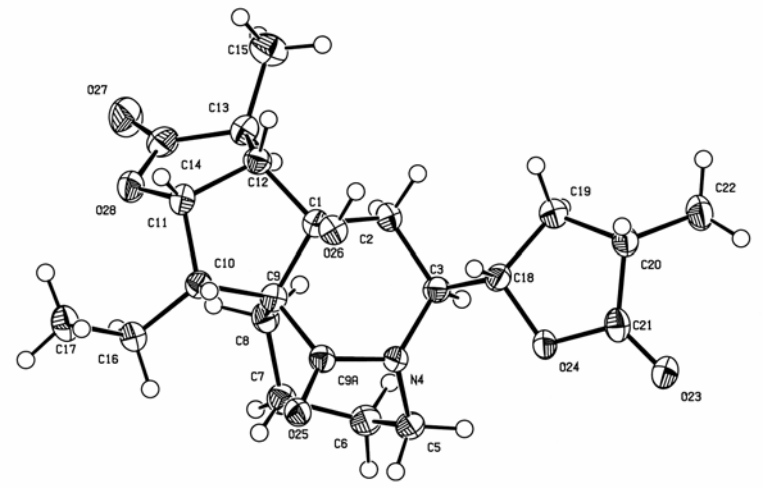


Figure 4. Crystal structure of sessilifoliamide I (**1**).

EXPERIMENTAL

General

The melting point was determined on a Yanagimoto micro-melting point apparatus and is uncorrected. The optical rotation was determined on a JASCO P-1030 digital polarimeter and the CD spectrum on a JASCO J-720 spectropolarimeter. The IR spectrum was recorded on a JASCO FT/IR 620 spectrophotometer. HRESIMS was obtained with a Micromass LCT spectrometer. NMR spectra were obtained on a Bruker DRX-500 spectrometer at 300 K, in the ^{1}H NMR spectrum, the chemical shifts (δ) are given in ppm relative to the resonance of residual $CHCl_3$ at 7.26 ppm, and in the ^{13}C NMR spectrum, the chemical shifts are given in ppm relative to the resonance at 77.03 ppm for $CDCl_3$. Preparative HPLC was carried out on a Shimadzu LC-6AD system equipped with a SPD-10A UV detector (220 nm) and an Inertsil PREP-ODS column (10 μm, 20 × 250 mm), by using a mixed solvent of MeOH/H_2O or MeCN/H_2O at a flow rate of 10 mL/min.

Plant material

Stemona sessilifolia (Miq.) Miq. was harvested in Shandong Province, China in 2000. The botanical origin of the plant was identified by Prof. Z. W. Xie of the China Academy of Traditional Chinese Medicine.

Extraction and isolation

The air-dried roots (15 kg) were extracted with hot MeOH (3 × 35 L). The solvent was removed to give a crude MeOH extract (8 kg), which was, after acidification with 3% aqueous tartaric acid (8 L), treated with EtOAc (3 × 8 L). The combined EtOAc layers were evaporated in vacuo to give a residue (neutral and acidic fraction 300 g). After adjusting the pH to 9 with solid Na_2CO_3, the aqueous layer was extracted

with $CHCl_3$ (3 × 8 L). The combined $CHCl_3$ extracts were evaporated in vacuo to give a residue (basic fraction 250 g).

The neutral and acidic fraction (300 g) was subjected to silica gel (1700 g) column chromatography eluting sequentially with hexane–EtOAc (3/1, 5 L), hexane–EtOAc (1/1, 5 L), EtOAc (5 L), EtOAc–MeOH (10/1, 8 L), and MeOH (8 L) to afford six fractions. The fourth fraction (43.6 g), a part of the EtOAc–MeOH (10/1) eluate, was subjected to aminopropyl-bonded silica gel (570 g) column chromatography eluting sequentially with hexane–EtOAc (1/0, 3/1, 1/1, 1/3, and 0/1, 4 L each), EtOAc–MeOH (10/1, 8 L), and MeOH (8 L) to give seven fractions. After removal of the solvent to dryness, the sixth fraction (5.59 g) was subjected silica gel column chromatography eluting with $CHCl_3$–MeOH (30:1) to afford five fractions. The third fraction (1.26 g) was subjected to HPLC using MeOH–H_2O (40/60, then 100/0) to afford seven fractions. The fifth fraction (32.3 mg) was subsequently purified by HPLC using MeCN–H_2O (22/78) to give sessilifoliamide I (**1**) (13.8 mg).

Sessilifoliamide I (1). Colorless prisms, mp 273–275 °C (MeOH–H_2O), $[\alpha]_D$ −0.2 (MeOH, *c* 0.78); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 226 (+10), 255 (−22) nm; ^{1}H NMR ($CDCl_3$) δ 5.15 (1H, t, *J* = 6.9 Hz, H-11), 4.44 (1H, m, H-18), 3.98 (1H, m, H-5a), 3.60 (1H, dd, *J* = 10.9, 6.3 Hz, H-3), 3.07 (1H, br d, *J* = 14 Hz, H-5b), 2.92 (1H, br s, OH-1), 2.76 (1H, dd, *J* = 14.7, 10.9 Hz, H-2a), 2.70 (1H, m, H-20), 2.68 (1H, m, H-10), 2.63 (1H, dd, *J* = 6.9, 6.0 Hz, H-12), 2.57 (1H, m, H-13), 2.44 (1H, m, H-19a), 1.91 (1H, d, *J* = 14.7 Hz, H-2b), 1.86 (1H, m, H-8a), 1.85 (1H, m, H-16a), 1.78 (1H, m, H-7a), 1.75 (1H, m, H-19b), 1.64 (2H, m, H_2-6), 1.57 (1H, m, H-16b), 1.36 (3H, d, *J* = 7.2 Hz, H_3-15), 1.34 (1H, m, H-7b), 1.29 (3H, d, *J* = 7.0 Hz, H_3-22), 1.24 (1H, m, H-8b), 1.10 (3H, t, *J* = 7.2 Hz, H_3-17); ^{13}C NMR ($CDCl_3$) δ 182.4 (C-9a), 179.5 (C-14), 178.0 (C-21), 83.7 (C-1), 83.2 (C-11), 82.4 (C-18), 60.4 (C-9), 57.9 (C-12), 57.1 (C-3), 54.1 (C-5), 46.4 (C-10), 37.3 (C-13), 35.8 (C-20), 33.7 (C-19), 29.2 (C-6), 28.5 (C-2), 27.8 (C-8), 26.0 (C-7), 18.6 (C-15), 17.9 (C-16), 14.8 (C-22), 12.5 (C-17); IR ν_{max} (KBr) 3423, 2958, 2929, 2874, 1768, 1656, 1192 cm^{-1}; HRESIMS *m/z* 406.2234 ($[M + H]^+$, calcd for $C_{22}H_{32}NO_6$, 406.2230).

X-Ray crystallographic study of 1

$C_{22}H_{31}NO_6$, *M* = 405.48, 0.48 × 0.13 × 0.13 mm, orthorhombic, $P2_12_12_1$, *a* = 10.2660(5) Å, *b* = 10.9560(11) Å, *c* = 19.0250(19) Å, *V* = 2139.8(3) Å^3, *Z* = 4, D_x = 1.259 Mg m^{-3}, μ(Mo Kα) = 0.091 mm^{-1}, 2570 measured reflections, 2570 unique reflections, 1788 observed reflections [$I > 2\sigma(I)$], R1 = 0.0443, wR2 = 0.0973 (observed data), GOF = 0.881; R1 = 0.0626, wR2 = 0.1022 (all data).

The structure was solved by direct methods using the maXus crystallographic software package,[5] and refined by full-matrix least-squares on F^2 using the program SHELXL-97.[6] The absolute structure could not be determined crystallographically.

CCDC 638870 contains the supplementary crystallographic data for compound **1** from this paper. These

data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

REFERENCES AND NOTES

1. R. A. Pilli and M. C. F. Oliveira, *Nat. Prod. Rep.,* 2000, **17**, 117.
2. W. H. Lin, L. Ma, M. S. Cai, and R. A. Barnes, *Phytochemistry*, 1994, **36**, 1333.
3. R.-W. Jiang, P.-M. Hon, P. P.-H. But, H.-S. Chung, G. Lin, W.-C. Ye, and T. C. W. Mak, *Tetrahedron,* 2002, **58**, 6705. Figure 1 of this reference gives a structure having H-13β for neotuberostemoninol. According to their crystallographic data, however, its H-13 should be α-oriented. Therefore, we took the compound as having H-13α in the present paper.
4. D. Kakuta, Y. Hitotsuyanagi, N. Matsuura, H. Fukaya, and K. Takeya, *Tetrahedron,* 2003, **59**, 7779; Y. Hitotsuyanagi, M. Hikita, T. Oda, D. Kakuta, H. Fukaya, and K. Takeya, *Tetrahedron,* 2007, **63**, 1008.
5. S. Mackay, C. J. Gilmore, C. Edwards, N. Stewart, and K. Shankland, *maXus: Computer Program for the Solution and Refinement of Crystal Structures*; Nonius, The Netherlands, MacScience, Japan, and The University of Glasgow, U.K., 1999.
6. G. M. Sheldrick, *SHELXL-97: Program for the Refinement of Crystal Structures*; University of Göttingen, Göttingen, Germany, 1997.