Novel Alkaloids from *Stemona sessilifolia*

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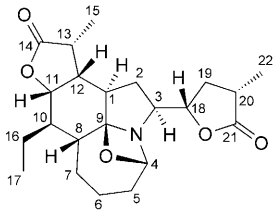
Two new alkaloids, sessilifolines A (**1**) and B (**2a**), were isolated from the stems of *Stemona sessilifolia*, together with three known alkaloids, tuberstemonine (**3**), sessilifoliamide A (**4**), and stemoninoamide (**5**). Their structures were established by mass-spectrometric and spectroscopic methods, especially 2D-NMR techniques.

Introduction. – The roots and leaves of various Stemonaceae species are being used in traditional Chinese, Japanese, and Thai medicine to treat respiratory disease, parasitic infestation, and as insecticides [1]. *Stemona* comprises *ca*. 25 species and represents the largest genus of the small monocotyledonous family Stemonaceae. Phytochemical studies of this genus have resulted in the isolation of more than 70 alkaloids, which have been structurally classified into five different groups [2].

Stemona sessilifolia MIQ. (Stemonacea) is native to South China, and used in traditional Chinese medicine (TCM) as an insecticide and cough remedy [3]. Previous investigations on this species resulted in the isolation of several *Stemona* alkaloids [4–7]. In the present study, two novel alkaloids, sessilifolines A (**1**) and B (**2a**), were isolated from the stems of *S. sessilifolia*, together with three known alkaloids, tuberstemonine (**3**) [8], sessilifoliamide A (**4**) [7], and stemoninoamide (**5**) [9]. Their structures were established by mass-spectrometric and spectroscopic analyses, especially 2D-NMR techniques (^{1}H,^{1}H-COSY, HMQC, HMBC, NOESY).

Results and Discussion. – Sessilifoline A (**1**) was obtained as an optically active, colorless, amorphous powder ($[\alpha]_D^{20} = +31.4$). Its molecular formula was established as $C_{22}H_{31}NO_5$ by HR-ESI-MS (*m/z* 412.2103 ($[M+Na]^+$; calc. 412.2100)), inferring eight degrees of unsaturation. Strong IR absorptions at 1775 and 1763 cm^{-1} were attributed to ester C=O groups, as confirmed by the ^{13}C-NMR signals at δ(C) 178.8 and 178.4 (*Table 1*). The ^{1}H-NMR spectrum of 1 displayed signals for three Me groups at δ(H) 0.99 (*t*, *J* = 7.4), 1.20 (*d*, *J* = 7.3), and 1.27 (*d*, *J* = 7.2 Hz) (*Table 1*). Consistent with the molecular formula of **1**, a total of 22 signals were observed, comprising three quaternary, ten tertiary, and six secondary C-atoms, as well as three Me groups. Among them, two oxygenated methines were observed (δ(C) 78.4, 81.0; δ(H) 4.48, 4.32, resp.), and the two downfield signals at δ(C) 107.7 and 101.6 were due to a hemiacetal function. Two out of the eight degrees of unsaturation were due two COOH groups; the remaining six degrees of unsaturation, thus, had to be accounted for by six rings. These data pointed to a stenine-type alkaloid [1].



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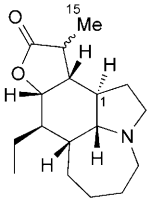
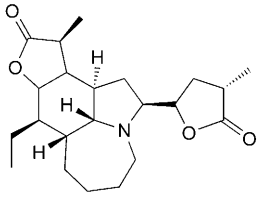


2a 15α
2b 15β (stenine)



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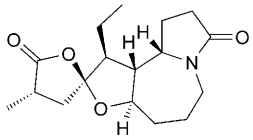

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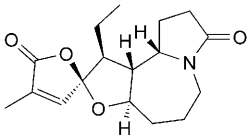

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Table 1. *^{1}H- and ^{13}C-NMR Data for* **1**. At 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	δ(H)	δ(C)
1	1.53–1.57 (*m*)	43.7 (*d*)
2	2.06–2.15, 1.80–1.89 (2*m*)	29.4 (*t*)
3	3.26 (*ddd*, J=8.0, 6.4, 1.4)	71.0 (*d*)
4	5.23 (*d*, J=4.6)	101.6 (*d*)
5	1.90–1.99, 1.64–1.68 (2*m*)	33.1 (*t*)
6	1.68–1.73, 1.45–1.54 (2*m*)	21.4 (*t*)
7	1.22–1.36, 1.90–1.94 (2*m*)	26.9 (*t*)
8	1.78–1.85 (*m*)	42.2 (*d*)
9	–	107.7 (*s*)
10	2.12–2.20 (*m*)	41.7 (*d*)
11	4.48 (*t*, J=3.8)	78.4 (*d*)
12	2.40–2.48 (*m*)	44.6 (*d*)
13	2.88–2.97 (*m*)	41.9 (*d*)
14	–	178.4 (*s*)
15	1.20 (*d*, J=7.3)	11.6 (*q*)
16	1.38–1.64 (*m*)	21.3 (*t*)
17	0.99 (*t*, J=7.4)	11.6 (*q*)
18	4.32 (*dt*, J=10.9, 5.9)	81.0 (*d*)
19	2.37–2.45, 1.50–1.59 (2*m*)	33.7 (*t*)
20	2.60–2.74 (*m*)	35.4 (*d*)
21	–	178.8 (*s*)
22	1.27 (*d*, J=7.2)	15.1 (*q*)

Analysis of the ^{1}H,^{1}H-COSY spectrum of **1**, in combination with HMBC data, permitted us to establish the linkages of all C-atoms, including the quaternary C-atoms and the heteroatoms (*Fig. 1*). The linkage of C(3) and C(4) to an N-atom was established by

the HMBC correlations of H–C(3)/C(4) and H–C(4)/C(3). The HMBC correlation of H–C(4)/C(9) indicated that both C(4) and C(9) were linked by an O-atom. The correlations of H–C(1)/C(9), H–C(8)/C(9), and H–C(1)/C(8) showed the connectivity C(1)–C(9)–C(8). The presence of a 14,11- and a 21,18-olide was confirmed by the HMBC correlations of H–C(11)/C(14) and H–C(18)/C(21). Thus, the planar structure of **1** was figured out.

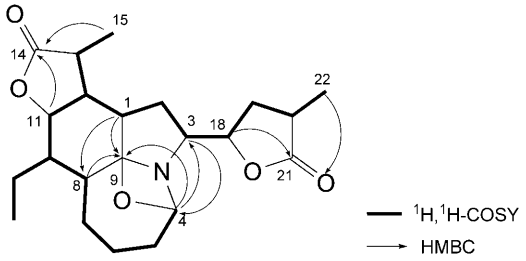

Fig. 1. *Selected 2D-NMR correlations of* **1**

The relative configuration of **1** was deduced by NOESY analysis (*Fig. 2*). NOEs for H–C(11)/H–C(8), H–C(11)/H–C(12), H–C(11)/H–C(13), and H–C(11)/H–C(17) indicated that H–C(8), H–C(11), H–C(12), H–C(13), and H–C(17) were on the same side of the molecular plane, tentatively assumed as β-orientation. As a consequence, H–C(10) and H–C(15) were α-orientated. The NOE correlations of H–C(15)/H–C(1), H–C(1)/H–C(3), and H–C(3)/H–C(4) indicated that H–C(15), H–C(1), H–C(3), and H–C(4) were α-configured. Finally, an NOE between H–C(18) and H–C(20) indicated that these two H-atoms were in β-orientation, thus, H–C(22) being in α-orientation. Complete ^{1}H and ^{13}C assignments (*Table 1*) were achieved through a combination of ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY experiments. Thus, the structure of sessilifoline A (**1**) was established[1])

Sessilifoline B (**2a**) had the molecular formula $C_{17}H_{27}NO_2$, based on HR-EI-MS analysis (*m*/*z* 278.2115 ($[M+H]^+$; calc. 278.2120)). The compound was obtained as a colorless, amorphous powder. Its IR spectrum showed an absorption at 1770 cm^{-1}, which indicated a lactone C=O group, as further confirmed by the ^{13}C-NMR signal at δ(C) 179.6 (*Table 2*). The ^{13}C-NMR spectrum indicated two Me, seven CH_2, and seven CH groups, as well as a quaternary C-atom. Among them, one CH group (δ(C) 70.9, δ(H) 2.42) and two CH_2 groups [δ(C) 55.7, 55.6; δ(H) 3.22/2.45, 2.87/2.34, resp.) were attached to an N-atom. Further, the ^{1}H-NMR spectrum of **2a** showed two Me groups at δ(H) 0.99 and 1.21 (2*t*, J = 7.4 Hz each).

The interpretation of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra showed that **2a** had the same planar structure as stenine (**2b**) [10][11] and isostenine [12], which indicated that they might be isomers of each other. The relative configuration of **2a** was deduced by NOESY analysis (*Fig. 3*). NOE Correlations of H–C(11)/H–C(8), H–C(8)/H–C(9), H–C(11)/H–C(12), H–C(11)/H–C(13), and H–C(11)/H–C(17) indi-

[1]) For systematic names, see *Exper. Part.*

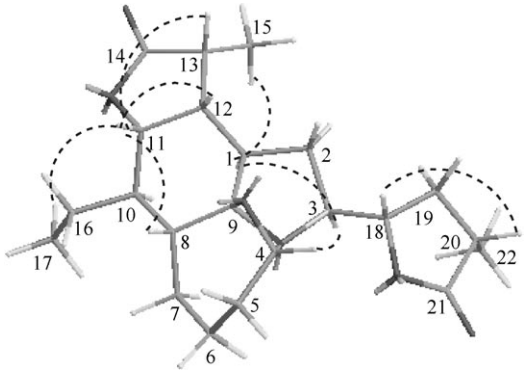

Fig. 2. *Key NOESY correlations and relative configuration of* **1**

Table 2. *^{1}H- and ^{13}C-NMR Data for* **2a**. At 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	δ(H)	δ(C)
1	1.80–1.85 (*m*)	37.5 (*d*)
2	1.95–2.12, 1.38–1.44 (2*m*)	30.2 (*t*)
3	3.22 (*dd*, *J* = 15.3, 7.4), 2.42–2.49 (*m*)	55.7 (*t*)
4	2.85–2.91 (*m*), 2.34 (*t*, *J* = 7.9)	55.6 (*t*)
5	1.64–1.78, 1.70–1.79 (2*m*)	28.4 (*t*)
6	1.62–1.74 (*m*)	21.1 (*t*)
7	1.66–1.73, 1.69–1.80 (2*m*)	28.1 (*t*)
8	1.71–1.82 (*m*)	34.4 (*d*)
9	2.38–2.45 (*m*)	70.9 (*d*)
10	1.73–1.86 (*m*)	37.3 (*d*)
11	4.51 (*d*, *J* = 2.2)	79.4 (*d*)
12	2.21–2.30 (*m*)	43.0 (*d*)
13	2.80–2.87 (*m*)	42.5 (*d*)
14	–	179.6 (*s*)
15	1.21 (*d*, *J* = 7.2)	10.1 (*q*)
16	1.62–1.74, 1.38–1.45 (2*m*)	21.2 (*q*)
17	0.99 (*t*, *J* = 7.4)	11.3 (*q*)

cated that H–C(8), H–C(9), H–C(11), H–C(12), H–C(13), and H–C(17) were all on the same side, assumed as β-orientation. As a consequence, H–C(10) and H–C(15) were α-oriented. Similarly, the correlation of H–C(15)/H–C(1) indicated that H–C(1) was α-configured. From these data, the structure of sessilifoline B (**2a**) was, thus, established as 15-epistenine.

The structures of the three known alkaloids also isolated from *S. sessilifolia*, *i.e.*, tuberstemonine (**3**) [8], sessilifoliamide A (**4**) [7], and stemoninoamide (**5**) [9], were corroborated by comparison of their spectroscopic data with those reported in the literature.

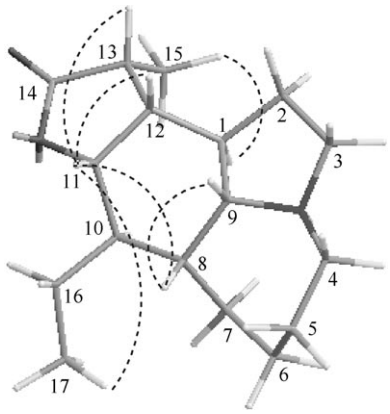

Fig. 3. *Key NOESY correlations and relative configuration of* **2a**

Experimental Part

General. All solvents were of anal. grade (*Shanghai Chemical Plant*). Silica gel (230–400 mesh), and *Lichroprep RP-18* (40–63 μm; *Merck*) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Plant*) were used for thin-layer chromatography (TLC). Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Perkin-Elmer-577* spectrophotometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* apparatus; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Finnigan LCQ^{DECA}* mass spectrometer; in *m*/*z*.

Plant Material. The stems of *S. sessilifolia* were collected from Zhejiang Province, P. R. China, in June, 2005, and authenticated by Prof. *Yong-Hong Zhang*, Fujian Medical University, Fujian, P. R. China. A voucher specimen (No. 20051114X) was deposited at the Zhejiang University of Technology.

Extraction and Isolation. The powdered stems of *S. sessilifolia* (10.0 kg) were percolated with 95% aq. EtOH. After solvent removal, the crude extract was suspended in H_2O (3 l) and acidified with 0.5N aq. H_2SO_4 to pH 5. The acidic suspension was immediately extracted with AcOEt (6×400 ml) to remove non-alkaloidal components. The acidic aq. phase was brought to pH 10 with 2N aq. Na_2CO_3 soln., and then extracted with $CHCl_3$ (6×400 ml) to afford the crude alkaloids (15.2 g). The crude alkaloids were subjected to CC (SiO_2; $CHCl_3$/MeOH 50:1 → 10:1): to afford two major fractions, *Fr. 1* (2.3 g) and *Fr. 2* (530 mg). *Fr. 1* was separated by CC (*RP-18*; MeOH/H_2O 6:4 → 8:2) to yield **2a** (131 mg) and **3** (320 mg) consecutively. *Fr. 2* was also separated by CC (*RP-18*; MeOH/H_2O 6:4 → 8:2) to afford **1** (18.0 mg), **4** (110 mg), and **5** (78 mg).

Sessilifoline A (=*(2*S**,4*S**,7a*R**,8*R**,8a*S**,11*R**,11a*S**,11b*R**,11c*S**)-8-Ethyl-decahydro-11-methyl-2-[(2*S**,4*S**)-4-methyl-5-oxotetrahydrofuran-2-yl]-2*H*-4,11c-epoxyazepino[3,2,1-*hi*]furo[3,2-*e*]indol-10(4*H*)-one*; **1**). Colorless, amorphous powder. $[\alpha]_D^{20} = +31.4$ ($c = 0.8$, $CHCl_3$). IR (KBr): 1775 (C=O), 1763 (C=O), 1562, 1359, 1260, 1064, 690. ^{1}H- and ^{13}C- NMR: see *Table 1*. ESI-MS (pos.): 412 ($[M+Na]^+$). HR-ESI-MS: 412.2103 ($[M+Na]^+$, $C_{22}H_{31}NNaO_5^+$; calc. 412.2100).

Sessilifoline B (=*15-Epistenine* or *(7a*R**,8*R**,8a*S**,11*R**,11a*S**,11b*R**,11c*R**)-8-Ethyl-dodecahydro-11-methylazepino[3,2,1-*hi*]furo[3,2-*e*]indol-10(2*H*)-one*; **2a**). Colorless, amorphous powder. $[\alpha]_D^{20} = +34.0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 1770 (C=O), 1379, 1299, 1137, 937, 667. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 278 ($[M+H]^+$). HR-ESI-MS: 278.2115 ($[M+H]^+$), $C_{17}H_{28}NO_2^+$; calc. 278.2120).

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Received October 24, 2006