New Stephaoxocane Alkaloids from *Stephania longa*

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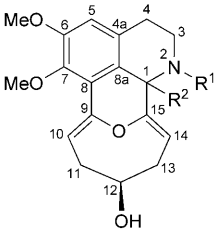
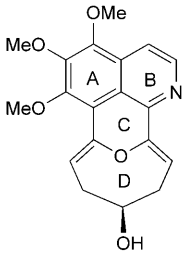
Three new stephaoxocane-type alkaloids, stephalonganines A–C (**1**–**3**), together with the known eletefine (**4**), were isolated from the whole plant of *Stephania longa*. Their structures were fully characterized spectroscopically, and the absolute configurations of the new alkaloids were assigned by comparison of their circular-dichroism (CD) data with those of 1,2-dihydrostephaoxocanine (**5**), in combination with 2D-NMR experiments.

Introduction. – Plants of the genus *Stephania* (Menispermaceae) are rich sources of various bioactive alkaloids [1]. *Stephania longa* LOUR., native to southern China, is used in traditional Chinese medicine (TCM) against fever, inflammation, and dysentery [2]. In chemical studies on *S. longa* performed during the 1980s and 1990s, eight alkaloids and five non-alkaloids were reported [3]. In a more-recent, extensive study on TCM constituents, we have reported 22 hasubanan-type alkaloids [4]. In a further investigation on this plant, we herein report four stephaoxocane-type alkaloids, including the new compounds stephalonganines A–C (**1**–**3**)[1]) and the known eletefine (**4**) [5a]. Their structures were elucidated by spectroscopic methods, and the absolute configurations of the three new alkaloids **1**–**3** were determined by comparison of their CD data with those of 1,2-dihydrostephaoxocanine (**5**) [5b].

The first alkaloid of this type was reported in 1993 by *Miao* and co-workers [5c], and was named stephaoxocane by *Kashiwaba et al.* in 1997 [5d]. Until now, only five ste-



1[1]) R^1 = H R^2 = β-H
2 R^1 = Me R^2 = β-H
3 R^1 = H R^2 = α-H



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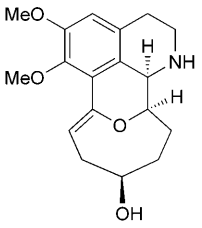

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[1]) Arbitrary numbering.

phaoxocane alkaloids have been described from Menispermaceae [5]. Their interesting structures have given rise to challenging synthetic approaches [6], and some of their simplified analogs show inhibitory activities against acetylcholinesterase [6f].

Results and Discussion. – Stephalonganine A (**1**) was obtained as a colorless, amorphous powder with UV absorptions at 227 (log ε 4.38), 265 (4.03), and 301 (3.46) nm. The molecular formula of **1** was determined as $C_{18}H_{21}NO_4$ by HR-EI-MS (*m/z* 315.1474 (M^+, calc. 315.1471)), with two degrees of unsaturation less than in the case of eletefine (**4**) [5a].

The NMR data of **1** (*Table*) showed the presence of six aromatic C-atoms (a tertiary one at δ(C) 112.2, and five quaternary ones at 124.0, 126.8, 129.6, 142.8, and 151.9, resp.), four olefinic C-atoms (two tertiary ones at δ(C) 104.9 and 113.8, and two quaternary ones at 155.7 and 160.1), two CH (δ(C) 55.3 (N-bonded), 70.5 (O-bonded)), four CH_2 (δ(C) 27.6, 37.2, 37.7, and 43.3), and two MeO groups (δ(C) 56.0 and 60.6) on an aromatic ring. By comparison with the spectroscopic data of **4** and **5** [5b], compound **1**

Table. *^{13}C- and ^{1}H-NMR Data of Compounds* **1**–**3**. At 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm, *J* in Hz. Arbitrary atom numbering.

Position	**1**		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	55.3	4.54 (br. *s*)	64.3	4.00 (br. *s*)	55.2	4.57 (br. *s*)
3	43.3	3.37 (*ddd*, *J* = 12.8, 7.6, 3.2), 3.17–3.25 (*m*)	54.0	3.11 (*ddd*, *J* = 11.7, 6.3, 2.2), 2.58–2.66 (*m*)	43.3	3.38 (*ddd*, *J* = 12.8, 7.6, 3.4), 3.23 (*ddd*, *J* = 12.8, 9.2, 7.0)
4	27.6	2.82–2.91 (*m*), 2.76 (*ddd*, *J* = 16.8, 6.9, 3.2)	27.4	2.94–3.02 (*m*), 2.68–2.75 (*m*)	27.4	2.81–2.89 (*m*), 2.77 (*ddd*, *J* = 16.8, 7.0, 3.4)
4a	129.6	–	129.0	–	129.6	–
5	112.2	6.59 (*s*)	111.4	6.57 (*s*)	112.2	6.60 (*s*)
6	151.9	–	151.8	–	152.0	–
7	142.8	–	143.2	–	143.1	–
8	124.0	–	123.8	–	123.6	–
8a	126.8	–	124.7	–	128.6	–
9	155.7		156.2		155.5	
10	113.8	5.87 (*dd*, *J* = 8.4, 6.3)	113.4	5.94 (*dd*, *J* = 8.5, 6.2)	112.6	5.80 (*dd*, *J* = 8.2, 5.8)
11	37.7	3.21–3.29 (*m*), 2.26–2.33 (*m*)	37.5	3.24 (*ddd*, *J* = 13.6, 9.2, 6.2), 2.29–2.37 (*m*)	34.4	3.10 (*ddd*, *J* = 14.3, 5.8, 0.9), 2.47–2.55 (*m*)
12	70.5	3.46 (br. *t*, *J* = 9.5)	70.3	3.54 (br. *t*, *J* = 9.2)	69.4	4.36 (br. *t*, *J* = 6.7)
13	37.2	2.96–3.04 (*m*), 2.24–2.31 (*m*)	37.4	2.97–3.04 (*m*), 2.29–2.37 (*m*)	34.6	2.83–2.90 (*m*), 2.43–2.51 (*m*)
14	104.9	5.11 (*ddd*, *J* = 8.6, 5.0, 1.9)	106.7	5.17 (*ddd*, *J* = 8.5, 4.8, 1.5)	103.8	5.03 (*ddd*, *J* = 7.9, 4.6, 1.9)
15	160.1	–	158.4	–	160.5	–
6-MeO	56.0	3.83 (*s*)	55.9	3.85 (*s*)	56.0	3.85 (*s*)
7-MeO	60.6	3.80 (*s*)	60.6	3.81 (*s*)	60.6	3.82 (*s*)
N-Me		–	43.6	2.47 (*s*)		–

had to be a stephaoxocane-type alkaloid, the planar structures of rings *A* and *B* being identical with those in **5**, and that of ring *D* in **1** being the same as in **4**. HMQC Analysis allowed us to unambiguously assign all the H- and C-atoms, and an ^{1}H,^{1}H-COSY experiment enabled us to establish two spin systems (*Fig. 1*, bold lines). The planar structure of **1** was finally confirmed by an HMBC experiment (*Fig. 1*).

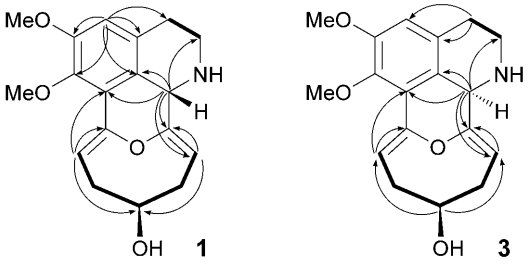

Fig. 1. *^{1}H,^{1}H-COSY* (bold) *and selected HMBC* (→) *correlations for* **1** *and* **3**

The 12-OH group of **1**[1]) was tentatively assigned β-configuration by comparing the ^{13}C-NMR resonances for C(11), C(12), and C(13) at δ(C) 37.7, 70.5, and 37.2, respectively, with those of **4** (38.2, 71.8, and 38.0, resp.). This assignment was confirmed by the NOESY spectrum (*Fig. 2*) and the NOESY correlation pattern of **1**, which, in terms of H–C(12), was similar to that of **4**. In the NOESY spectrum, H–C(12) (δ(H) 3.46) correlated with both CH_2(11) at δ(H) 3.21–3.29 (weak) and 2.26–2.33 (strong), and with both CH_2(13) at 2.96–3.04 (weak) and 2.24–2.31 (strong). Furthermore, compound **5**, with (1*R*)-configuration, showed negative *Cotton* effects at 263 ($\Delta\varepsilon = -6.1$) and 304 (-0.2) nm, while alkaloid **1** exhibited *positive* effects at 265 ($+28.0$) and 303 ($+0.6$) nm, suggesting that the absolute configuration of **1** was (1*S*). The absolute configuration at the second stereogenic center was then determined as (12*S*).

From these data, stephalonganine A (**1**) was identified as (7*Z*,10*S*,12*Z*,13a*S*)-7,13-epoxy-2,3,9,10,11,13a-hexahydro-5,6-dimethoxy-1*H*-cyclodec[*ij*]isoquinolin-10-ol.

Stephalonganine B (**2**) has the molecular formula $C_{19}H_{23}NO_4$, as deduced by HR-EI-MS (*m*/*z* 329.1618 (M^+, calc. 329.1627)), corresponding to 14 mass units more com-

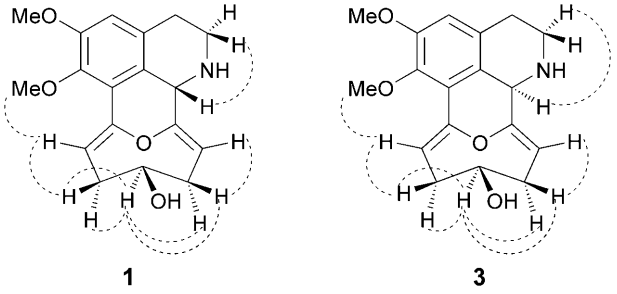

Fig. 2. *Key NOESY correlations for* **1** *and* **3**

pared to **1**. The spectroscopic data of **1** and **2** were very similar, suggesting that the two compounds had the same basic skeleton. The ^{1}H-NMR spectrum of **2** (*Table*) displayed an additional Me group at δ(H) 2.47 (*s*), and the upfield-shifted signal for H–C(1) at δ(H) 4.00 (br. *s*), as compared to **1**, indicated an *N*-Me group. This was supported by the ^{13}C-NMR data of **1** (*Table*), which showed an *N*-Me signal at δ(C) 43.6 and a downfield-shifted signal for C(1) at δ(C) 64.3.

The CD spectrum of **2** resembled that of **1**, with positive *Cotton* effects at 266 ($\Delta\varepsilon = +18.3$) and 303 ($+0.1$) nm, indicating the same absolute configuration as in **1**. From these data, the structure of stephalonganine B (**2**) was identified as (7*Z*,10*S*,12*Z*,13a*S*)-7,13-epoxy-2,3,9,10,11,13a-hexahydro-5,6-dimethoxy-1-methyl-1*H*-cyclodec[*ij*]isoquinolin-10-ol.

Stephalonganine C (**3**) was assigned the same molecular formula ($C_{18}H_{21}NO_4$) as compound **1** by HR-EI-MS (*m*/*z* 315.1477 (M^+, calc. 315.1471)); and the NMR data of **3** (*Table*) indicated that the structures of **1** and **3** were closely related. The main NMR differences were related to the two stereogenic centers. Analysis of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra of **3** (*Fig. 1*) showed that this alkaloid had the same planar structure as **1**, with differences only in terms of configuration at C(1) or C(12). Compared with **1**, the CD spectrum of **3** exhibited completely reversed *Cotton* effects at 260 ($\Delta\varepsilon = -9.0$) and 300 (-0.2) nm, implying that the configuration at C(1) was inverted. As a consequence, the absolute configuration at C(12) was the same as in **1**, otherwise, both compounds would be enantiomers and would have displayed identical NMR data. The epimeric nature of **3** was further confirmed by its NOESY spectrum (*Fig. 2*). Thus, stephalonganine C (**3**) was identified as the (1*R*)-epimer of **1**.

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Experimental Part

General. All solvents were of anal. grade (*Shanghai Chemical Reagent Co., Ltd.*, China). Column chromatography (CC): neutral alumina (200–300 mesh; *Shanghai Chemical Reagent*), silica gel *H* (60 μm; *Qingdao Marine Chemical Inc., Ltd.*, China), Sephadex LH-20 (*Amersham Biosciences*, Japan), and amino silica gel (20–45 μm; *Fuji Silysia Chemical Ltd.*, Japan). Thin layer chromatography (TLC): precoated SiO_2 GF_{254} plates (*Yantai Huiyou Silica Gel Exploitation Co., Ltd.*, China); detection by spraying with *Dragendorff* reagent. UV Spectra: *Varian Cary-300-BIO* spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Perkin-Elmer 341* polarimeter. CD Spectra: *JASCO J-810* spectrophotometer; λ ($\Delta\varepsilon$) in nm. IR Spectra: *Perkin-Elmer 577* spectrometer; with KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* instrument; chemical shifts δ in ppm rel. to Me_4Si, coupling constants *J* in Hz. EI-MS: *Finnigan MAT-95* mass spectrometer, at 70 eV; in *m*/*z* (rel. %). ESI-MS: *Bruker Esquire-3000* mass spectrometer; in *m*/*z*.

Plant Material. The whole plant of *S. longa* was collected from Guangxi Province, P. R. China, in summer 2002, and identified by Prof. *Su-Hua Shi*, Institute of Botany, School of Life Sciences, Zhongshan University. A voucher specimen (SL-2002-1Y) was deposited at the Shanghai Institute of Materia Medica.

Extraction and Isolation. The plant material was extracted as reported previously [4] to give the crude alkaloids. The crude alkaloids were fractionated by CC (Al_2O_3; Et_2O/MeOH 100:1 → 1:1): *Fractions* (*Fr.*) *1–6*. *Fr. 3* was subjected to CC (SiO_2 *H*; petroleum ether/AcOEt/Et_2NH 12:1:0.3 → 3:1:0.3): *Fr. 3.1–3.7*. *Fr. 3.6* was further purified by CC (SiO_2 *H*; $CHCl_3$/MeOH 70:1), followed by prep. TLC

(SiO_2; $CHCl_3$/MeOH 40:1) to afford **4** (4.6 mg). *Fr. 3.7* was purified by CC (*Sephadex LH-20*; MeOH) and prep. TLC (SiO_2; $CHCl_3$/MeOH 20:1) to provide **2** (1.8 mg). *Fr. 4* was subjected to CC (SiO_2 *H*; petroleum ether/AcOEt/Et_2NH 12:1:0.3 → 2:1:0.3): *Fr. 4.1– 4.6*. *Fr. 4.6* was rechromatographed (*Sephadex LH-20*; MeOH) and subjected to prep. TLC (SiO_2; $CHCl_3$/MeOH 10:1) to afford **1** (6.7 mg). *Fr. 5* was purified by CC (SiO_2 *H*; petroleum ether/AcOEt/Et_2NH 5:1:0.3 → 1:1:0.3): *Fr. 5.1–5.9*. *Fr. 5.9* was further purified by CC (1. *Sephadex LH-20*, MeOH; 2. amino silica gel, $CHCl_3$/MeOH 100:1) to afford **3** (12.3 mg).

Stephalonganine A (=*(7Z,10S,12Z,13aS)-7,13-Epoxy-2,3,9,10,11,13a-hexahydro-5,6-dimethoxy-1*H-*cyclodec[*ij*]isoquinolin-10-ol*; **1**). Colorless, amorphous powder. UV (MeOH): 227 (4.38), 265 (4.03), 301 (3.46). $[\alpha]_D^{20} = +157.5$ ($c = 0.12$, $CHCl_3$). CD (MeOH): 236 (+11.2), 265 (+28.0), 303 (+0.6). IR (KBr): 3442, 2920, 2850, 1701, 1653, 1591, 1485, 1429, 1358, 1256, 1103, 1040, 1003, 844, 667. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 315 (90, M^+), 314 (58), 300 (15), 286 (100), 272 (62), 258 (38), 244 (29), 230 (24), 216 (19). ESI-MS: 316.2 ($[M+H]^+$). HR-EI-MS: 315.1474 (M^+, $C_{18}H_{21}NO_4^+$; calc. 315.1471).

Stephalonganine B (=*(7Z,10S,12Z,13aS)-7,13-Epoxy-2,3,9,10,11,13a-hexahydro-5,6-dimethoxy-1-methyl-1*H-*cyclodec[*ij*]isoquinolin-10-ol*; **2**). Colorless, amorphous powder. $[\alpha]_D^{20} = +160.0$ ($c = 0.40$, $CHCl_3$). CD (MeOH): 235 (+10.4), 266 (+18.3), 303 (+0.1). IR (KBr): 3423, 2924, 2852, 1689, 1647, 1591, 1483, 1327, 1254, 1128, 1036, 1003, 883, 814, 752. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 329 (94, M^+), 328 (100), 314 (13), 300 (34), 286 (98), 271 (65), 257 (15), 243 (24), 230 (13). ESI-MS: 330.2 ($[M+H]^+$). HR-EI-MS: 329.1618 (M^+, $C_{19}H_{23}NO_4^+$; calc. 329.1627).

Stephalonganine C (=*(7Z,10S,12Z,13aR)-7,13-Epoxy-2,3,9,10,11,13a-hexahydro-5,6-dimethoxy-1*H-*cyclodec[*ij*]isoquinolin-10-ol*; **3**). Colorless, amorphous powder. UV (MeOH): 227 (4.30), 259 (4.03). $[\alpha]_D^{20} = -155.0$ ($c = 0.08$, $CHCl_3$). CD (MeOH): 232 (−7.9), 260 (−9.0), 300 (−0.2). IR (KBr): 3396, 2926, 2852, 1699, 1657, 1591, 1485, 1429, 1360, 1325, 1257, 1099, 1038, 843, 810. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 315 (47, M^+), 314 (25), 300 (10), 286 (100), 272 (98), 258 (68), 244 (70), 230 (57), 216 (37). ESI-MS: 316.2 ($[M+H]^+$). HR-EI-MS: 315.1477 (M^+, $C_{18}H_{21}NO_4^+$; calc. 315.1471).

REFERENCES

[1] X.-K. Wang, T.-F. Zhao, *Zhongguo Yaoxue Zazhi* **1990**, *25*, 3; J.-M. Huang, J.-X. Guo, *Huaxi Yaoxue Zazhi* **1998**, *13*, 97.

[2] State Administration of Traditional Chinese Medicine, 'Zhonghua Bencao', Shanghai Science & Technology Press, Shanghai, 1999, Fasc. 3, Vol. 8, p. 382–383.

[3] A.-N. Lao, Y.-L. Gao, Z.-J. Tang, R.-S. Xu, *Yaoxue Xuebao* **1980**, *15*, 696; A.-N. Lao, Z.-J. Tang, R.-S. Xu, *Yaoxue Xuebao* **1981**, *16*, 940; A.-N. Lao, Y.-L. Gao, Z.-J. Tang, Y.-H. Wang, X.-X. Zhang, C.-G. Wang, R.-S. Xu, *Huaxue Xuebao* **1982**, *40*, 1038; Z.-Y. Zhu, Y.-X. Feng, L.-Y. He, Y.-C. Wang, *Yaoxue Xuebao* **1983**, *18*, 460; J.-Z. Deng, S.-X. Zhao, *Phytochemistry* **1993**, *33*, 941; J.-Z. Deng, S.-X. Zhao, *Zhongguo Yaoke Daxue Xuebao* **1993**, *24*, 73.

[4] H. Zhang, J.-M. Yue, *J. Nat. Prod.* **2005**, *68*, 1201.

[5] a) E. V. L. da-Cunha, M. L. Cornélio, J. M. Barbosa-Filho, R. Braz-Filho, A. I. Gray, *J. Nat. Prod.* **1998**, *61*, 1140; b) N. Kashiwaba, S. Morooka, M. Kimura, M. Ono, *J. Nat. Prod.* **1996**, *59*, 803; c) J.-Z. Deng, S.-X. Zhao, Z.-C. Miao, *Nat. Prod. Lett.* **1993**, *2*, 283; d) N, Kashiwaba, S. Morooka, M. Kimura, M. Ono, *Nat. Prod. Lett.* **1997**, *9*, 177; e) J.-Z. Deng, S.-X. Zhao, *J. Nat. Prod.* **1997**, *60*, 294.

[6] a) T. S. Kaufman, *Molecules* **2000**, *5*, 491; b) T. S. Kaufman, C. R. Bernardi, M. Cipulli, C. C. Silveira, *Molecules* **2000**, *5*, 493; c) T. S. Kaufman, *Heterocycles* **2001**, *55*, 323; d) D. A. Bianchi, T. S. Kaufman, *ARKIVOC* **2003**, (*x*), 178; e) D. A. Bianchi, M. A. Cipulli, T. S. Kaufman, *Eur. J. Org. Chem.* **2003**, 4731; f) D. A. Bianchi, G. S. Hirschmann, C. Theoduloz, A. B. J. Bracca, T. S. Kaufman, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2711.

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