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Cepharatines A−D, Hasubanan-Type Alkaloids from *Stephania cepharantha*

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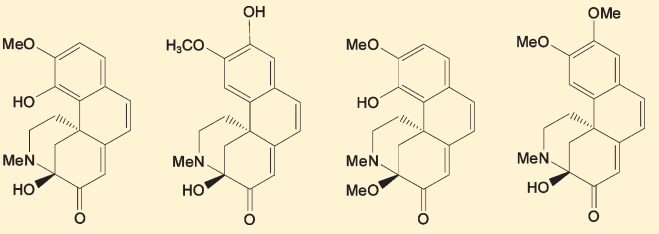
Supporting Information

ABSTRACT: Four new hasubanan-type alkaloids, cepharatines A−D (**1**−**4**), were isolated from the leaves and stems of *Stephania cepharantha*, and their structures were elucidated by spectroscopic analysis. The structure of **1** was further confirmed by X-ray crystallographic diffraction.



The species *Stephania cepharantha* Hayata of the Menispermaceae family is a perennial herbaceous liana that is distributed widely in the northwestern and southwestern areas of China. The tuberous root of this plant has been used as a folk medicine for the treatment of edema, gout, rheumatism, and arthralgia by the Miao minority in the Guizhou Province of China.[1] Previous phytochemical studies have led to the isolation of a number of alkaloids, including the hasubanan cepharamine.[2−9] In the course of our investigation on the hasubanan-type alkaloids in the *Stephania* genus, four new alkaloids, cepharatine A−D (**1**−**4**), were isolated from the leaves and stems of *S. cepharantha*. In this paper, we describe the isolation, structural elucidation, and *in vitro* antibacterial activity of these alkaloids.

RESULTS AND DISCUSSION

Cepharatine A (**1**), obtained as yellow crystals, had the molecular formula $C_{18}H_{19}NO_4$ according to its HREIMS at *m*/*z* 313.1307 $[M]^+$ (calcd 313.1314), implying that the compound has 10 degrees of unsaturation. The UV absorptions of **1** at λ_{max} 255 (4.25) and 388 (4.29) implied the presence of a highly conjugated system. The IR spectrum showed absorption bands that corresponded to hydroxy (3442 cm^{-1}), conjugated carbonyl (1642 cm^{-1}), and aryl (1609 cm^{-1}) groups. The ^{13}C NMR spectrum showed 18 carbon signals corresponding to two methyl (an *O*-methyl and an *N*-methyl group), three methylene, five methine (two aromatic and three olefinic), and eight quaternary (one carbonyl, four aromatic, two saturated, and one olefinic) carbon atoms.

The $^1H-^1H$ COSY spectrum revealed the presence of an isolated $-CH_2CH_2-$ fragment, an isolated $-CH_2-$ fragment, and two pairs of vicinal protons (Figure 1a). The HMQC spectrum established all one-bond $^1H-^{13}C$ connectivities for **1**. Further examination of the ^1H, ^{13}C, and 2D NMR data, together with consideration of its degree of molecular unsaturation, suggested that compound **1** possesses a 6/6/6/6 tetracyclic

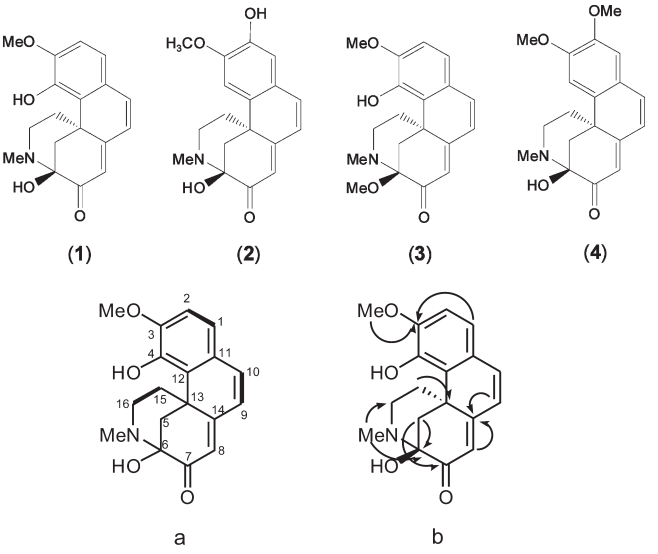


Figure 1. $^1H-^1H$ COSY (a) and key HMBC (b) of **1**.

skeleton bearing *N*-methyl, *O*-methyl, hydroxy, and cyclohexenone moieties, which differed significantly from the ring system of cepharamine, the hasubanan alkaloid that was previously isolated as a major constituent from this plant. In the HMBC spectrum, correlation of *N*Me with C-16 revealed the presence of the $-CH_2CH_2NMe$ group (Figure 1b), which connected to C-6 and C-13 on the basis of the correlation of *N*Me with C-6 and H-15 with C-13. Correlation of H-5/C-6 and H-5/C-7 indicated that the carbonyl carbon of the cyclohexenone moiety was located at C-7, and the double bond of cyclohexenone was thus located at C-8, which was further supported by correlation of

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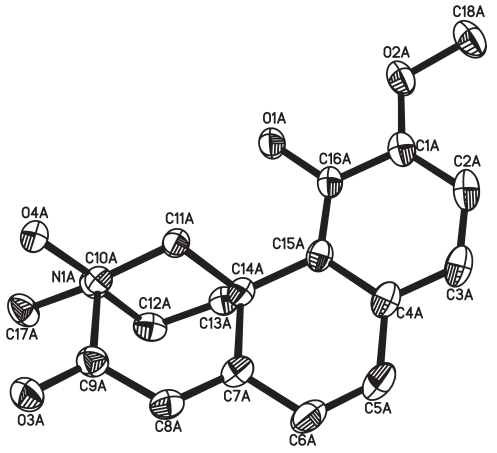

Figure 2. Perspective view of **1**.

H-8, H-9/C-14. The remaining *O*Me was placed at C-3 by the correlation of *O*Me/C-3 and H-1/C-3. Thus, the gross structure of **1** was established.

On the basis of the known biosynthetic pathway, where the configuration of the benzylic quaternary carbon C-13 is kept as *R* and C-13 was involved in the formation of this six-membered pyridine ring together with C-6, the pyridine ring should fuse to the cyclohexenone component at C-6−C-13 from the back side of the molecule, and the configuration of the quaternary carbon C-6 should be deduced as *S*. This deduction was confirmed by its CD spectra[10] (CD (CH_2Cl_2) λ_{max} ([$\Delta\varepsilon$]) 378 (−5.86) nm; CD (CH_2Cl_2+Rh_2 ($OCOCF_3$)$_4$) λ_{max} ([$\Delta\varepsilon$]) 350 (+4.55) nm).

The structure and relative configuration of **1** were unambiguously confirmed by X-ray crystallographic analysis (Figure 2). A structural skeleton similar to **1** has not been reported.

Cepharantine B (**2**) was obtained as orange-yellow crystals, and its molecular formula, $C_{18}H_{19}NO_4$, was determined by HREIMS (measured 313.1309, calcd 313.1314), which was the same molecular formula as compound **1**. The only difference between structures **1** and **2** was the positions of the oxygen substitutions of the A ring. In **2**, the *O*-methyl substituent was located at C-3, as can be seen from the HMBC and ROESY correlations of H_3CO/C-3, H-1, H-4/C-3, and H_3CO/H-4. The hydroxy group at C-2 was apparent by the HMBC correlation of H-1 and H-4/C-2. The presence of two isolated aromatic protons in **2** supported that the hydroxy and *O*-methyl groups were located at C-2 and C-3, respectively. Thus, the structure of **2** was established as above.

Cepharatine C (**3**) was obtained as yellow crystals, and it had the molecular formula $C_{19}H_{21}NO_4$ as determined by HREIMS (measured 327.1476, calcd 327.1471). This compound appeared to be a derivative of the hasubanan-type alkaloids when its spectroscopic date were compared to those of **1** or **2**. The only difference between alkaloids **1** and **3** was the substituent group at C-6. The C-6 substituent group of **3** was determined to be an *O*-methyl instead of a hydroxy group, as seen in the ^{1}H and ^{13}C NMR data (Tables 1 and 2). The HMBC spectrum of **3** showed both H_3CO and *N*Me correlated with C-6. Therefore, the structure of **3** was established as shown.

Cepharatine D (**4**) was also obtained as yellow crystals, and it had the molecular formula $C_{19}H_{21}NO_4$, as determined by HREIMS (measured 327.1471, calcd 327.1471). The ^{1}H and ^{13}C NMR data of **4** were close to those of **2**, with the exception of the presence of one more methyl group in the former. Because its HMBC spectrum showed correlations between H_3CO/C-2 and C-3, respectively, and because of the presence of a pair of isolated aromatic protons in the A ring of **4**, the structure of **4** was established.

Compound **1** possessed weak bacteriostatic activities against *Bacillus subtilis*, *Eberth bacillus*, *Micrococcus luteus*, and *Escherichia coli*. The MIC for *B. subtilis* was found to be 0.21−0.24 mg/mL, as compared to gentamicin, which had an MIC of 0.01−0.02 μg/mL. Compounds **2**−**4** were not active against the four microbes.

Table 1. ^{1}H NMR Data of Cepharatines A−D (1−4) (δ in ppm, J in Hz)

no.	1[a]	2[a]	3[b]	4[b]
1	6.77 d (8.4)	6.80 s	6.77 d (8.4)	6.89 s
2	6.79 d (8.4)		6.88 d (8.4)	
4		6.98 s		7.06 s
5	2.21 m	2.33 m	2.02 m	2.19 m
	3.95 d (12.8)	2.77 d (12.4)	4.14 d (12.4)	2.67 d (12.0)
8	6.13 s	6.16 s	6.03 s	6.10 s
9	6.29 d (9.6)	6.32 d (9.2)	6.30 d (9.2)	6.36 d (9.2)
10	6.71 d (9.6)	6.72 d (9.2)	6.80 d (9.2)	6.84 d (9.2)
15	1.40 m	1.59 m	1.29 m	1.59 m
	2.66 m	2.02 m	2.69 m	2.02 m
16	2.74 m	2.66 m	2.55 m	2.66 m
	2.90 m	2.90 m	2.85 m	2.90 m
2-OCH_3				3.92 s
3-OCH_3	3.94 s	3.92 s	3.90 s	3.83 s
6-OCH_3			3.31 s	
NCH_3	2.25 s	2.18 s	2.10 s	2.18 s

[a] In $CDCl_3$. [b] In methanol-d_4.

Table 2. ^{13}C NMR Data of Cepharatines A−D (1−4) (δ in ppm)

no.	1[a]	2[a]	3[b]	4[b]
1	121.2 CH	114.7 CH	122.6 CH	113.4 CH
2	108.7 CH	144.4 C	110.4 CH	151.7 C
3	148.1 C	147.7 C	150.8 C	149.5 C
4	144.4 C	106.9 CH	146.5 C	109.3 CH
5	43.4 CH_2	44.8 CH_2	40.1 CH_2	46.4 CH_2
6	83.2 C	83.1 C	88.8 C	84.7 C
7	194.0 C	193.3 C	193.6 C	194.7 C
8	124.0 CH	124.1 CH	126.4 CH	126.0 CH
9	123.3 CH	123.2 CH	123.7 CH	124.1 CH
10	136.1 CH	135.7 CH	137.9 CH	136.9 CH
11	125.5 C	135.1 C	126.8 C	137.0 C
12	125.4 C	124.0 C	126.7 C	125.3 C
13	44.3 C	43.6 C	45.4 C	44.8 C
14	161.6 C	161.6 C	162.7 C	162.7 C
15	31.1 CH_2	38.1 CH_2	32.0 CH_2	39.0 CH_2
16	46.6 CH_2	46.8 CH_2	47.7 CH_2	47.8 CH_2
2-OCH_3				56.6 CH_3
3-OCH_3	56.1 CH_3	56.0 CH_3	56.6 CH_3	56.5 CH_3
6-OCH_3			49.0 CH_3	
NCH_3	36.2 CH_3	35.9 CH_3	36.9 CH_3	36.7 CH_3

[a] In $CDCl_3$. [b] In methanol-d_4.

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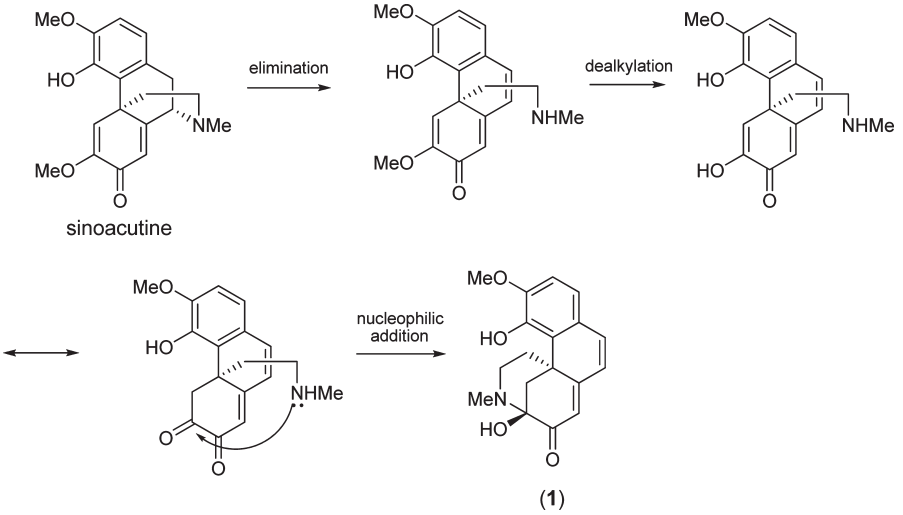
Scheme 1. Plausible Biosynthetic Pathway to the Formation of 1



Compound **1** is structurally related to the known morphinan alkaloid sinoacutine, but differs in the piperidine ring, which has been fused at C-13/C-6 rather than C-13/C-9. We propose a possible biosynthetic pathway for **1** as shown in Scheme 1. Sinoacutine as the precursor of **1** is proposed to undergo elimination, dealkylation, and nucleophilic addition to construct **1**.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained using an X-4 melting point apparatus. Optical rotations were measured on a Rudolph Autopol 1 digital polarimeter (2.5 cm cell). UV spectra were acquired using a Shimadzu UV-2401 PC UV/vis spectrophotometer. CD spectra were recorded on a JASCO-815 spectropolarimeter. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer using KBr disks. NMR spectra were recorded on a Varian INOVA-400 spectrometer using TMS as an internal standard. Chemical shifts were expressed in ppm with reference to the solvent signals. HR-MS were measured on a VG Auto Spec-3000 mass spectrometer. Column chromatography was performed over silica gel (200−300 mesh and 10−40 μm, Qindao Marine Chemical Ltd., Qindao, P. R. China), MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Corporation, Japan), RP-18 gel (50 μm, YMC, Japan), and Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was performed on glass plates precoated with silica gel GF254, and spots were visualized using Dragendorff"s reagent.

Plant Material. The stems and leaves of *S. cepharantha* were collected in Guizhou Province, People's Republic of China, in June 2008, and identified by Prof. De-Yuan Chen of the GuiYang College of Traditional Chinese Medicine. A voucher specimen (No. Zhang20080616) has been deposited at the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation. Dried and powered stems and leaves (27 kg) of *S. cepharantha* were percolated with 95% aqueous EtOH at room temperature (3 × 5 days). After the solvent was removed under reduced pressure, the residue was suspended in H_2O and extracted successively with petroleum ether and $CHCl_3$. The $CHCl_3$ extract (400 g) was chromatographed on a silica gel column, eluting with $CHCl_3$/MeOH in a gradient from 100:0 to 0:100, to give eight major fractions (F1−F8). F3 (11 g) was purified by column chromatography over MCI gel using MeOH/H_2O as the eluent. The fraction that eluted with 50% aqueous MeOH was further separated and purified on a silica gel column, eluting with petroleum ether/acetone (8:2) and Sephadex LH-20 (eluted with MeOH) to yield compounds **1** (375 mg) and **2** (50 mg). F4 (4 g) was also subjected to silica gel column chromatography, eluting with petroleum ether/acetone (8:2), and also to an RP-18 column (eluted with MeOH/H_2O at 30, 50, and 70%). The fraction eluted by 50% MeOH was further purified, using Sephadex LH-20 (eluted with MeOH) and silica gel (eluted with $CHCl_3$/MeOH, 100:0.5) to yield compound **3** (16 mg). The fraction eluted by 30% MeOH was further purified by Sephadex LH-20 (eluted with MeOH) and silica gel (eluted with $CHCl_3$/MeOH, 100:0.5) to yield compound **4** (16 mg).

*Cepharatine A (**1**)*:. yellow needles (MeOH); mp 178−180 °C; $[\alpha]^{15}_D$ −716 (*c* 0.98, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 388 (4.29), 255 (4.25) nm; CD (MeOH) λ_{max} ($[\Delta\varepsilon]$) 202 (−7.09), 228 (+29.98), 384 (−11.19) nm. IR (KBr) ν_{max} 3442, 2930, 2852, 1642, 1610, 1561, 1292 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data ($CDCl_3$), see Tables 1 and 2; EIMS *m*/*z* 313 $[M]^+$, 240, 228, 213; HREIMS *m*/*z* 313.1307 (calcd for $C_{18}H_{19}NO_4$ $[M]^+$, 313.1314).

*Cepharatine B (**2**)*:. orange-yellow needles (MeOH); mp 194−196 °C; $[\alpha]^{16}_D$ −517 (*c* 0.91, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 259 (4.60), 276 (4.47), 406 (4.27) nm; IR (KBr) ν_{max} 3454, 2923, 2851, 1643, 1614, 1557, 1289 cm^{-1}; EIMS *m*/*z* 313 $[M]^+$, 242, 228, 86; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data ($CDCl_3$), see Tables 1 and 2; HREIMS *m*/*z* 313.1309 (calcd for $C_{18}H_{19}NO_4$ $[M]^+$, 313.1314); CD (MeOH) λ_{max} ($[\Delta\varepsilon]$) 220 (−6.72), 258 (+11.44), 389 (−11.26) nm.

*Cepharatine C (**3**)*:. yellow needles (MeOH); mp 174−176 °C; $[\alpha]^{16}_D$ −332 (*c* 1.01, CH_3OH); UV ($CHCl_3$) λ_{max} (log ε) 225 (4.73), 393 (4.55), 255 (4.53) nm ; IR (KBr) ν_{max} 3418, 2925, 2850, 1652, 1611, 1565, 1274 cm^{-1}; EIMS *m*/*z* 327 $[M]^+$, 240, 212, 100; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data (methanol-d_4), see Tables 1 and 2; HREIMS *m*/*z* 327.1476 (calcd for $C_{19}H_{21}NO_4$ $[M]^+$, 327.1471); CD (MeOH) λ_{max} ($[\Delta\varepsilon]$) 207 (−6.39), 231 (+41.48), 382 nm (−20.50) nm.

*Cepharatine D (**4**)*:. yellow needles (MeOH); mp 98−100 °C; $[\alpha]^{17}_D$ −321 (*c* 1.01, CH_3OH); UV ($CHCl_3$) λ_{max} (log ε) 263 (4.87), 212 (4.81), 406 (4.58) nm; IR (KBr) ν_{max} 2925, 2851, 1654, 1608, 1552, 1341, 1275 cm^{-1}; EIMS *m*/*z* 327$[M]^+$, 256, 242, 86; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data (methanol-d_4), see Tables 1 and 2; HREIMS *m*/*z* 327.1471 (calcd for $C_{19}H_{21}NO_4$ $[M]^+$, 327.1471); CD (MeOH) λ_{max} ($[\Delta\varepsilon]$) 218 (−7.93), 263 (+13.47), 389 (−10.19) nm.

Single-Crystal X-ray Structure Determination of Cepharatine A (1). Crystal analysis was performed with a yellow crystal (dimensions 0.10 × 0.22 × 0.42 mm) obtained from $CHCl_3$/MeOH (1:1) and

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measured on a Rigaku MicroMax 002+ diffractometer with a graphite monochromator ($\omega-\kappa$ scan, $2\theta_{max}$ = 144.9°) using Cu Kα radiation. Crystal data: $C_{18}H_{19}NO_4 \cdot CH_3OH$; MW = 313.35(no solvent of crystallization); monoclinic system, space group $P2_1$; crystal cell parameters a = 11.916(2) Å, b = 6.673(1) Å, c = 21.319(4) Å, β = 90.46(3)°, V = 1695.1(6) Å^3, Z = 4, d = 1.353 g/cm^3. The total number of independent reflections measured was 5940, of which 4928 were observed ($|F|^2 \geq 2\sigma|F|^2$). Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC-779947 (available free of charge at http://www.ccdc.cam.ac.uk).

Bacteriostatic Effects. The bacteriostatic activities of compounds **1**−**4** were measured and evaluated semiquantitatively using the KB method.[11] Gentamicin was used as positive control. Each sample was tested twice.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR, EI/MS, CD, UV, and IR spectra for compound **1** and ^{1}H, ^{13}C NMR, EI/MS, and CD spectra for compounds **2**, **3**, and **4** are available free of charge via the Internet at http://pubs.acs.org.

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