Alkaloidal Constituents of the Leaves of *Stephania cepharantha* Cultivated in Japan: Structure of Cephasugine, a New Morphinane Alkaloid

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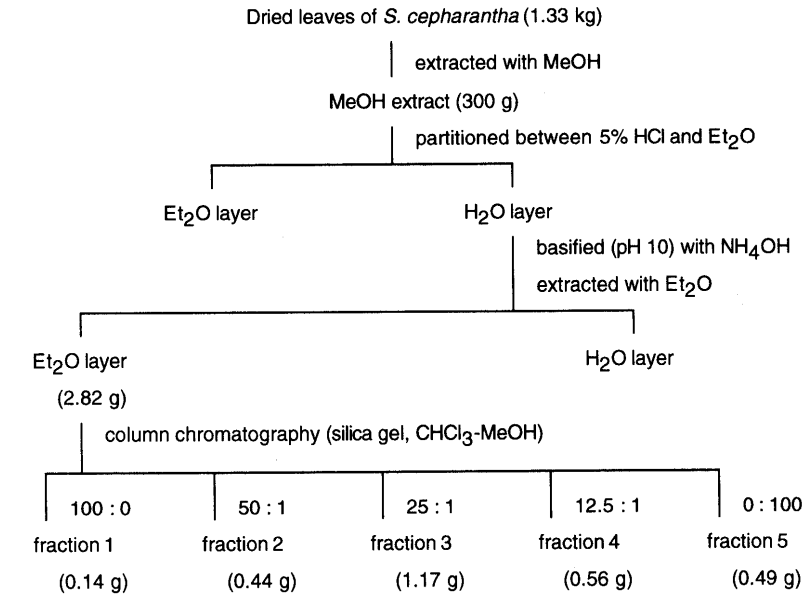
From the leaves of *Stephania cepharantha* HAYATA (Menispermaceae) cultivated in Japan, a new morphinane alkaloid, cephasugine (1), was isolated together with 14 known alkaloids. The structure of 1 was elucidated as the *N*-methyl derivative of sinococuline (17) by comparison of spectroscopic data with those of cephakicine (16). This is the first report of the alkaloidal constituents of the leaves of *S. cepharantha*.

Key words cephasugine; *Stephania cepharantha*; leaf; morphinane alkaloid; sinococuline; cephakicine

The alkaloidal constituents of the seeds,[1] roots,[2] and tubers,[3] of *Stephania cepharantha* HAYATA (Menispermaceae), a folk medicine in China, have been studied, but those of the leaves have not been reported. We have investigated the alkaloidal constituents of the leaves of this plant cultivated in Japan and obtained cephasugine (**1**) as a new morphinane alkaloid, together with 14 known alkaloids. Here, we describe the isolation and structural determination of **1** and the isolation and characterization of several known alkaloids. This is the first report of the alkaloidal constituents of the leaves of *S. cepharantha*.

The alkaloid-containing fraction from the methanol



fraction 1	cepharamine (**2**, 45 mg, 0.00338%)	
fraction 2	aknadinine (**3**, 140 mg, 0.01053%)	aknadilactam (**4**, 19 mg, 0.00143%)
	cephatonine (**5**, 40 mg, 0.00301%)	stephodeline (**9**, 4 mg, 0.00030%)
fraction 3	aknadinine (**3**, 39 mg, 0.00293%)	sinomenine (**6**, 67 mg, 0.00504%)
	cephamonine (**7**, 410 mg, 0.03083%)	cephamuline (**8**, 5 mg, 0.0003 %)
	litseferine (**1 4**, 25 mg, 0.00188%)	stepharine (**1 5**, 20 mg, 0.00150%)
fraction 4	sinomenine (**6**, 78 mg, 0.00586%)	cepharanoline (**1 0**, 67 mg, 0.00504%)
	juziphine (**1 1**, 32 mg, 0.00241%)	norjuziphine (**1 2**, 13 mg, 0.00098%)
	(+)-reticuline (**1 3**, 47 mg, 0.00353%)	litseferine (**1 4**, 15 mg, 0.00113%)
fraction 5	cephasugine* (**1**, 20 mg, 0.00150%)	

* new alkaloid

Chart 1. Fractionation of *Stephania cepharantha* HAYATA

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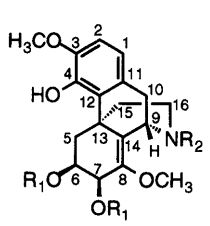
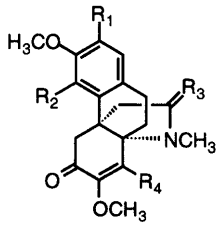
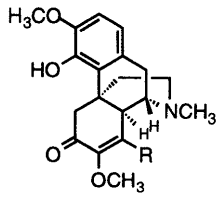


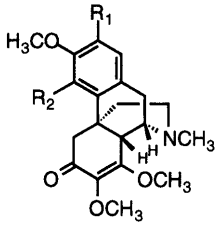
	R_1	R_2
1	H	CH_3
16	Ac	CH_3
17	H	H



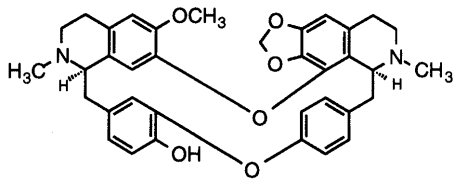
	R_1	R_2	R_3	R_4
2	H	OH	H_2	H
3	H	OH	H_2	OCH_3
4	H	OH	O	OCH_3
5	OH	H	H_2	OCH_3



	R
6	H
7	OCH_3



	R_1	R_2
8	H	OH
9	OCH_3	H



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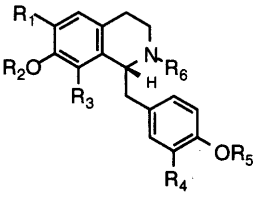
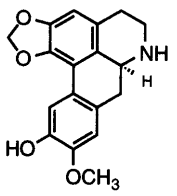


	R_1	R_2	R_3	R_4	R_5	R_6
11	H	CH_3	OH	H	H	CH_3
12	H	CH_3	OH	H	H	H
13	OCH_3	H	H	OH	CH_3	CH_3



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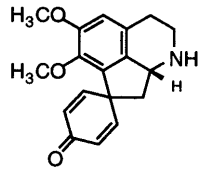

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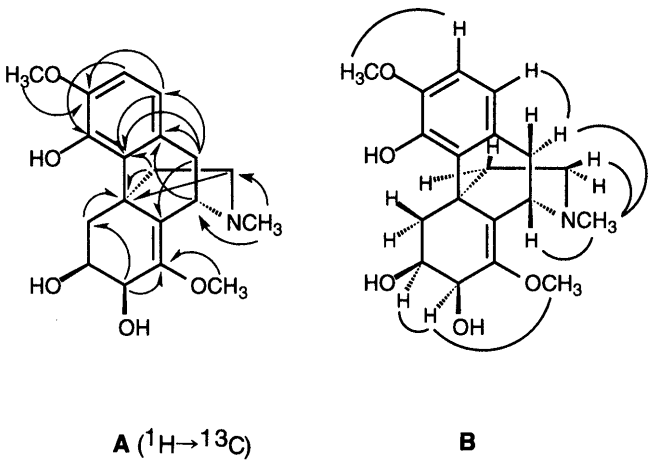
Chart 2

extract of the leaves of *S. cepharantha* was separated by a combination of crystallization, column chromatography, and preparative TLC, to give a new morphinane alkaloid, cephasugine (**1**), together with 14 known alkaloids: four hasubananes, cepharamine (**2**),[3b] aknadinine (**3**),[3b] aknadilactam (**4**),[3b] and cephatonine (**5**)[3b]; four morphinanes, sinomenine (**6**),[3c] cephamonine (**7**),[3c] cephamuline (**8**),[3c] and stephodeline (**9**)[4]; one bisbenzylisoquinoline, cepharanoline (**10**)[3d]; three benzylisoquinolines, juziphine (**11**),[5] norjuziphine (**12**),[6] and (+)-reticuline (**13**)[7]; one aporphine, litseferine (**14**),[8] and one proaporphine, stepharine (**15**).[9]

Cephasugine (**1**) was obtained as an amorphous powder, and its molecular formula was established as $C_{19}H_{25}NO_5$ by the high-resolution mass spectrum (HR-MS). The IR spectrum indicated the presence of a hydroxy group (broad absorption at 3400 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed the signals of one *N*-methyl group (δ_H 2.38), two methoxy groups (δ_H 3.69, 3.85), and a set of coupled aromatic protons (δ_H 6.56, 6.67) and was similar to that of cephakicine (**16**),[3b] which is a morphinane alkaloid possessing two acetoxy groups on C-6 and C-7, isolated by us from the tubers of this plant, except for the absence of the signals due to two acetyl groups and the upfield shifts of H-6 (δ_H 3.96) and H-7 (δ_H 4.34) compared with those (δ_H 5.24, 5.92) of **16**. The ^{13}C-NMR spectrum (Table 1) was also similar to that of **16**, except for the absence of the signals due to two acetyl groups. These results suggested that the structure of **1**, including the relative stereochemistry should be 6,7-dediacetylcephakicine, namely, the *N*-methyl derivative of sinococuline (**17**).[10] This structure was also supported by the results of correlation *via* long-range coupling (COLOC) and nuclear Overhauser effect spectroscopy (NOESY) experiments

Table 1. ^{1}H- and ^{13}C-NMR Data for **1** and **16**[3b]

Position	^{1}H		^{13}C	
	1	**16**	**1**	**16**
1	6.56 d (8.2)	6.62 d (8.2)	118.33	118.38
2	6.67 d (8.2)	6.72 d (8.2)	108.75	108.81
3			144.99	145.03
4			143.41	143.40
5	2.17 dd (13.1, 12.5)	2.35 dd (13.3, 12.8)	35.45	32.75
	2.85 dd (13.1, 3.4)	2.86 dd (12.8, 3.8)		
6	3.96 ddd (12.5, 3.4, 3.4)	5.24 ddd (13.3, 3.8, 3.8)	67.33	68.40
7	4.34 d (3.4)	5.92 dd (3.8, 0.9)	65.65	64.37
8			144.76	141.18
9	4.16 d (5.2)	4.15 d (5.2)	51.82	52.06
10	2.80 ddd (17.7, 5.8, 0.9)	2.87 ddd (17.7, 5.8, 1.0)	29.32	29.92
	3.11 d (17.7)	3.14 d (17.7)		
11			130.64	130.63
12			128.41	128.01
13			38.07	38.12
14			122.68	125.78
15	1.88 ddd (12.2, 3.7, 1.8)	1.87 ddd (12.5, 3.1, 1.8)	35.54	35.06
	1.96 ddd (12.2, 12.2, 4.6)	2.10 ddd (12.5, 12.2, 4.6)		
16	2.30 ddd (12.2, 12.2, 3.7)	2.38 ddd (12.5, 12.2, 3.1)	48.19	48.05
	2.52 ddd (12.2, 4.6, 1.8)	2.55 ddd (12.5, 4.6, 1.8)		
N-CH_3	2.38 s	2.40 s	42.06	42.15
3-OCH_3	3.85 s	3.87 s	56.22	56.26
8-OCH_3	3.69 s	3.55 s	56.77	56.98
6-$\underline{C}OCH_3$				170.29
6-CO$\underline{CH_3}$		2.01 s		21.03
7-$\underline{C}OCH_3$				170.64
7-CO$\underline{CH_3}$		2.04 s		21.04



A ($^{1}H \rightarrow ^{13}C$) B

Fig. 1. COLOC (A) and NOE (B) Correlations of **1**

(Fig. 1).

The absolute configuration was deduced as 6*S* (α-H), 7*S* (α-H), and 9*S* (β-H), since the optical activity showed the same sign as that of cephakicine (**16**) and sinococuline (**17**). Thus, the structure of **1** was determined to be the *N*-methyl derivative of **17**.

The identification of known alkaloids was confirmed by direct comparison with authentic samples or by comparison of the spectroscopic data with the literature values. Among 15 alkaloids from the leaves, cephasugine (**1**), stephodeline (**9**), norjuziphine (**12**), and litseferine (**14**) were not isolated from the tubers of the same plant.[11] Interestingly, bisbenzylisoquinoline alkaloids, the major alkaloidal group of the tubers, formed a minor alkaloidal group in the leaves, since only cepharanoline (**10**) was isolated, and morphinane and hasubanane alkaloids, which were not obtained from the seeds,[1] were the major alkaloidal groups.

Experimental

Melting points were measured on a Yanagimoto hot-stage melting point apparatus without correction. IR spectra were recorded on an FT/IR-5000 (JASCO) spectrometer as KBr pellets. UV spectra were measured on a Ubest-35 (JASCO) spectrometer. NMR spectra were taken on a JNM-α500 (JEOL) (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. Optical rotations were determined on a DIP-140 (JASCO) spectrometer. MS were taken on a JMS-D300 (JEOL) spectrometer at 30 eV. Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd.). Preparative TLC was done on precoated Silica gel 60 F_{254} (0.25 mm thick) plates (Merck).

Plant Material *Stephania cepharantha* HAYATA was cultivated at Yasato-machi, Ibaraki prefecture, Japan and collected in August 1995.

Extraction and Isolation Dried leaves of *S. cepharantha* (1.33 kg) were extracted twice with hot MeOH. The extract was evaporated *in vacuo*, and the residue (300 g) was treated with 5% HCl. The mixture was filtered, and the filtrate was extracted with Et_2O. The aqueous layer was basified with NH_4OH to pH 10 and extracted with Et_2O to yield the alkaloid-containing fraction (2.82 g). This fraction was subjected to silica gel column chromatography using $CHCl_3$, 2%, 4%, and 8% MeOH–$CHCl_3$, and MeOH as eluents to afford fractions 1 (0.14 g), 2 (0.44 g), 3 (1.17 g), 4 (0.56 g), and 5 (0.49 g), respectively. Fractions 1—5 were further subjected to a combination of crystallization, column chromatography, and preparative TLC to afford the following alkaloids. From fraction 1; cepharamine (**2**, 45 mg). From fraction 2; aknadinine (**3**, 140 mg), aknadilactam (**4**, 19 mg), cephatonine (**5**, 40 mg), stephodeline (**9**, 4 mg). From fraction 3; aknadinine (**3**, 39 mg), sinomenine (**6**, 67 mg), cephamonine (**7**, 410 mg), cephamuline (**8**, 5 mg), litseferine (**14**, 25 mg), stepharine (**15**, 20 mg). From fraction 4; sinomenine (**6**, 78 mg), cepharanoline (**10**, 67 mg), juziphine (**11**, 32 mg), norjuziphine (**12**, 13 mg), (+)-reticuline (**13**, 47 mg), litseferine (**14**, 15 mg). From fraction 5; cephasugine (**1**, 20 mg).

Cephasugine (1) Amorphous powder. $[\alpha]_D^{25}$ −98° (c=0.30, $CHCl_3$). IR: 3400, 1678, 1605, 1487, 1439, 1282, 1220, 1143 cm^{-1}. UV (MeOH) λ_{max} nm (log ε): 283 (3.22). EI-MS *m/z* (%): 347 (M^+, 15), 333 (20), 332 (93), 330 (16), 273 (27), 272 (100), 258 (40), 256 (13), 242 (10), 230 (17), 214 (11). HR-MS *m/z*: 347.1720 ($C_{19}H_{25}NO_5$ requires 347.1730).

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