Two New Morphinane Alkaloids from *Stephania cepharantha* HAYATA (Menispermaceae)

Noriaki KASHIWABA,*,[a] Shigeo MOROOKA,[a] Michiko KIMURA,[a] Yoshie MURAKOSHI,[a] Jun TODA,[b] and Takehiro SANO[b]

Department of Research, Kaken Shoyaku Co., Ltd.,[a] 3–37–10, Shimorenjaku Mitaka-shi, Tokyo 181, Japan and Showa College of Pharmaceutical Sciences,[b] 3–3165, Higashi-tamagawagakuen, Machida-shi, Tokyo 194, Japan.
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Two new morphinane alkaloids named cephamonine (1) and cephamuline (2) were isolated from the tuber of *Stephania cepharantha* HAYATA (Menispermaceae), cultivated in Japan, along with eleven known alkaloids. By comparison of spectroscopic data with those of sinomenine (3), the structures of 1 and 2 were elucidated to be the 8-methoxy derivative of 3 and its C-14 stereoisomer, respectively.

Keywords *Stephania cepharantha*; cephamonine; cephamuline; morphinane alkaloid; sinomenine; Menispermaceae

Stephania cepharantha HAYATA (Menispermaceae), a perennial plant, has been used as a folk medicine in Taiwan. This plant, native to Taiwan and cultivated in Japan, has been shown to contain six bisbenzylisoquinoline alkaloids and one hasubanane alkaloid from the tuber[1] and two bisbenzylisoquinoline, two dehydroaporphine and four aporphine alkaloids from the seeds.[2] Recently, it was reported that eight alkaloids, including three bisbenzylisoquinolines, four morphinanes and one aporphine, were obtained from the roots of *S. cepharantha*, native to China.[3]

In this paper, we describe the isolation and structural elucidation of two new morphinane alkaloids, cephamonine (**1**) and cephamuline (**2**), from the tuber of *S. cepharantha* cultivated in Japan.

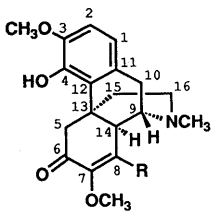
Results and Discussion

The alkaloidal fraction (see Experimental) was repeatedly subjected to silica gel column chromatography to give cephamonine (**1**) and cephamuline (**2**) along with eleven known alkaloids; aromoline,[4] berbamine,[1b] cepharanthine,[1b] cepharanoline,[1b] (−)-cycleanine,[1b] homoaromoline,[1b] isotetrandrine,[1b] (−)-norcycleanine,[5] obamegine,[6] sinomenine[7] (**3**) and stepharine.[8]

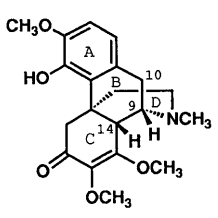
Compound **1** was obtained as an amorphous powder. The molecular formula $C_{20}H_{25}NO_5$ was established by high-resolution (HR) MS. The IR spectrum showed the presence of an α,β-unsaturated carbonyl moiety (1669 and 1611 cm^{-1}). The ^{1}H-NMR spectrum indicated the presence of one *N*-methyl group (δ 2.42), three methoxy groups (δ 3.36, 3.80, 4.00), a set of coupled aromatic protons (δ 6.54, 6.61, each d, J = 8.3 Hz) and characteristic active methylene protons (δ 2.31, 4.22, each d, J = 15.4 Hz). The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of **3**, except that a methoxy signal (δ 4.00) and a quaternary carbon signal (δ 161.92) appeared instead of the H-8 proton signal (δ 5.47) and C-8 tertiary carbon signal (δ 115.15) in sinomenine (**3**). In nuclear Overhauser and exchange spectroscopy (NOESY) experiments, the methoxy signal correlated to the C-7 methoxy signal (δ 3.36), suggesting that the methoxy group is located at C-8. In the ^{13}C-NMR spectrum of **1**, this structural assignment was supported by the fact that the C-7 carbon signal (δ 137.76) was shifted upfield due to the electron-donating effect of the introduced C-8 methoxy group, compared to that (δ 152.35) of **3**.

The relative stereochemistry of **1** was confirmed by means of differential nuclear Overhauser effect (NOE) experiments. The irradiation of the H-14 (δ 3.00) signal enhanced the H-5 (δ 2.31) and H-15 (δ 1.81) signals. These enhancements were observed mutually among the three protons, indicating that H-14, H-5 and H-15 have the same orientation and, therefore, the junction of rings C and D is *trans*. This stereochemical relationship is the same as that of **3**. In the circular dichroism (CD) spectrum (Fig. 3), **1** showed negative ($\Delta\varepsilon$, −12.5) and positive ($\Delta\varepsilon$, +7.0) Cotton effects at 270 and 231 nm, respectively. This CD curve was similar to that of **3**. Therefore, the absolute configuration of **1** was concluded to be 9*S* and 14*S*. On the basis of these data, the structure of **1** was established to be the 8-methoxy derivative of **3**.

Compound **2**, amorphous powder, had the same molecular formula as that of **1** and the fragmentation patterns in the low-resolution (LR) MS were similar to those of **1**. Furthermore, the IR, ^{1}H- and ^{13}C-NMR spectra indicated the presence of the same functional groups as those of **1**. However, the CD curve of **2** was entirely different from that of **1** (Fig. 3). These data suggested that **2** is the C-14 stereoisomer of **1**. This suggestion was confirmed by NOESY experiments, in which the H-14 (δ 2.86) signal was correlated to the H-5 (δ 2.61) and H-10 (δ 2.75) signals. The results indicated that the junction of rings B and C of **2** is *trans*, namely,



1: R=OCH_3
3: R=H



2

Fig. 1

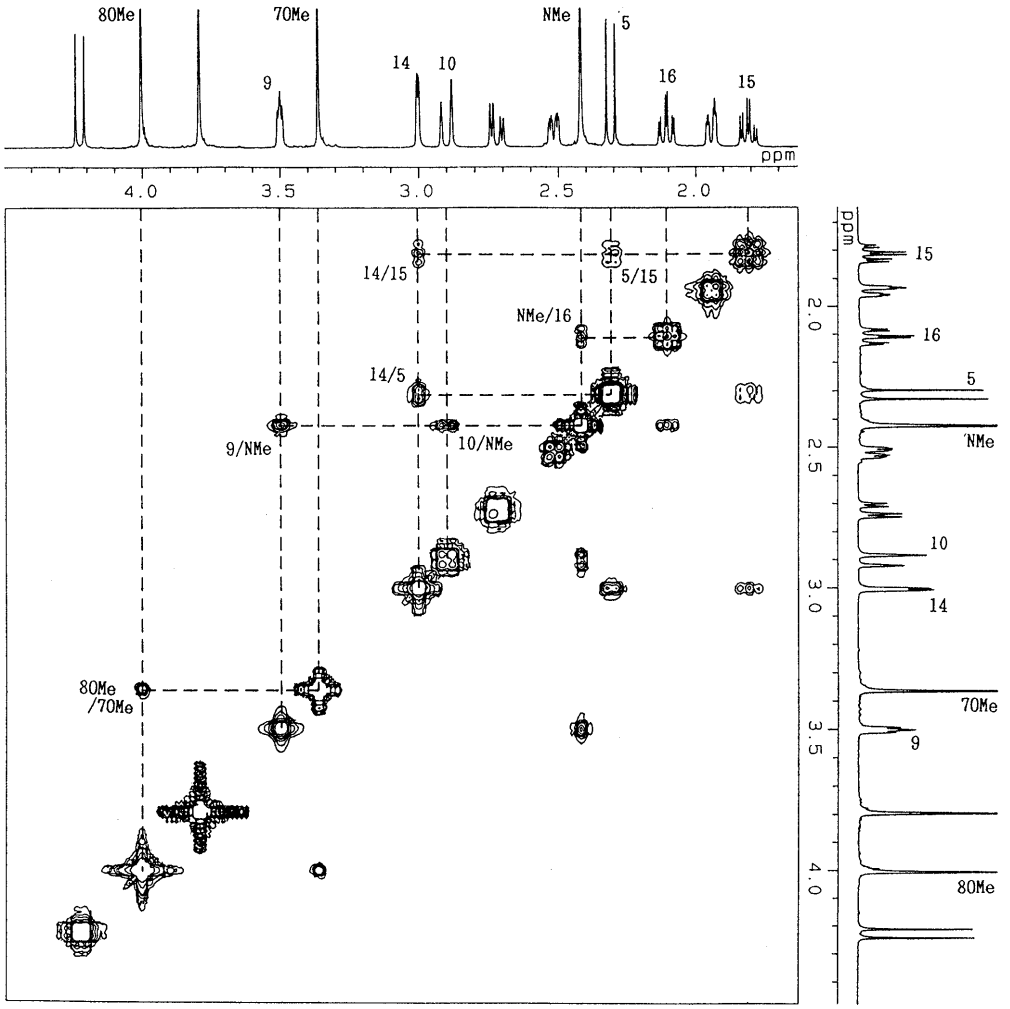


Fig. 2. NOESY Spectrum of **1**

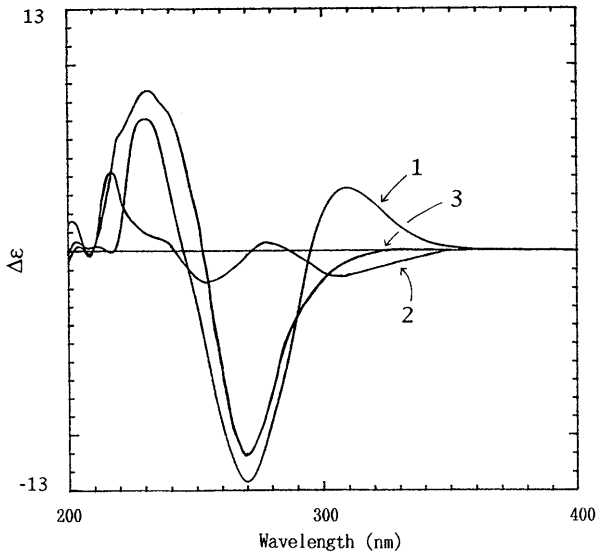
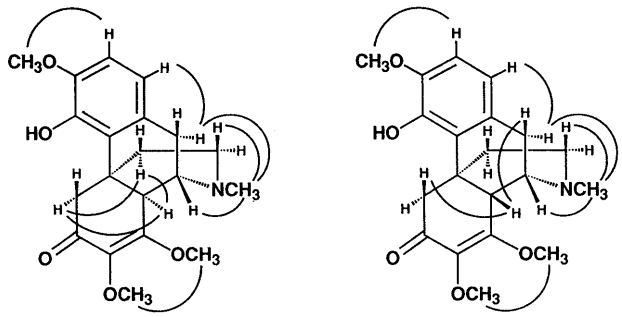


Fig. 3. CD Spectra of **1**, **2** and **3**



1 **2**

Fig. 4. Main NOE Correlations of **1** and **2**

rings C and D are in a *cis*-relationship. The structure of **2** was concluded to be the C-14 stereoisomer of **1**.

This conclusion was also supported by chemical evidence. When **1** was treated with sodium ethoxide in ethanol at room temprature for 22 h, **2** was formed in 4% yield and the starting material was recovered in 84% yield. Under a similar basic condition, sinomenine (**3**) also epimerized into 14-episinomenine in a yield of 2.5%.[9] Although the experiment implies that the minor constituent **2** might be an artifact derived from **1**, we believe that **2** is a natural product since **1** did not epimerize into **2** under the weakly basic or acidic conditions used in the isolation procedures.

The identification of known alkaloids was accomplish-

ed by direct comparison with authentic samples or by comparison of the spectral data with published values. Among the known alkaloids, (−)-norcycleanine was first isolated from *Stephania* genus.

Experimental

Melting points were measured on a Yanagimoto hot-stage melting point apparatus without correction. 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 as an internal standard and the chemical shifts are given in δ values. IR spectra were recorded on an FT/IR-5000 (JASCO) spectrometer as KBr pellets and data are given in cm^{-1}. UV spectra were measured on a Ubest-35 (JASCO) spectrometer in MeOH and data are given as λ_{max} nm ($\log\varepsilon$). MS were taken on a D-300 (JEOL) spectrometer. Optical rotations were determined on a DIP-140 (JASCO) spectrometer in $CHCl_3$. CD spectra were measured on a J-600 (JASCO) spectrometer in MeOH and data are given as $\Delta\varepsilon$ (nm). 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 winter, 1987.

Extraction and Isolation Dried and cut tubers of *S. cepharantha* (37.4 kg) were extracted twice with hot MeOH. The extract was evaporated *in vacuo* and the residue was treated with 5% HCl. The mixture was filtered and the filtrate was extracted with ether. The aqueous layer was adjusted to pH 7 with NH_4OH and extracted with ether to yield fraction A (270.2 g). Then, the aqueous layer was basified with NH_4OH to pH 10 and extracted with ether and $CHCl_3$, successively, to yield fractions B (289.4 g) and C (60.4 g), respectively. Fraction B was repeatedly subjected to column chromatography on silica gel, using 2%, 4%, 6%, 8% and 50% MeOH–$CHCl_3$ as eluents. The elution with 2—4% MeOH–$CHCl_3$ afforded cepharanthine (3.9 g), isotetrandrine (45.6 g), (−)-cycleanine (16.6 g), stepharine (0.9 g), cephamonine (**1**) (1.8 g) and cephamuline (**2**) (0.03 g). The elution with 6% MeOH–$CHCl_3$ afforded sinomenine (**3**) (1.4 g), homoaromoline (9.7 g) and cepharanoline (1.1 g). The elution with 8—50% MeOH–$CHCl_3$ afforded (−)-norcycleanine (2.5 g), berbamine (54.3 g), obamegine (2.5 g) and aromoline (8.3 g).

Cephamonine (1) Amorphous powder (HCl salt, mp 183—186 °C, prisms from MeOH–ether), $[\alpha]_D^{22}$ −36° (c=0.11). IR: 3376, 1669, 1611, 1487, 1439, 1379, 1346. UV: 225 sh (4.03), 275 (3.94). LR-MS *m/z*: 359 (M^+ 48), 344 (78), 328 (100), 316 (9), 222 (29), 178 (8), 146 (2). HR-MS *m/z*: 359.1694 (Calcd for $C_{20}H_{25}NO_5$: 359.1730). CD: −12.5 (270), +7.0 (231). ^{1}H- and ^{13}C-NMR: Tables I and II.

Cephamuline (2) Amorphous powder, $[\alpha]_D^{22}$ −63° (c=0.18). IR: 3235, 1657, 1609, 1484, 1439, 1383, 1340. UV: 232 sh (3.88), 272 (4.05). LR-MS *m/z*: 359 (M^+, 17), 344 (35), 328 (100), 316 (21), 222 (26), 178 (2). HR-MS *m/z*: 359.1753 (Calcd for $C_{20}H_{25}NO_5$: 359.1730). CD: −1.7 (254), +4.2 (218). ^{1}H- and ^{13}C-NMR: Tables I and II.

Sinomenine (3) mp 164—166 °C (lit.[5a] mp 161 °C), needles from ethyl acetate, $[\alpha]_D^{22}$ −54° (c=0.22). IR: 3510, 1690, 1630, 1605, 1582, 1487, 1439, 1379, 1346. UV: 231 sh (3.82), 261 (3.69). LR-MS *m/z*: 329 (M^+, 77), 314 (100), 301 (20), 286 (9), 192 (19), 178 (13), 130 (10). HR-MS *m/z*: 329.1625 (Calcd for $C_{19}H_{23}NO_4$: 329.1625). CD: −11.2 (271), +8.6 (232). ^{1}H- and ^{13}C-NMR: Tables I and II.

Isomerization of 1 into 2 A solution of **1** (620 mg) and NaOEt (3.42 g) in EtOH (40 ml) was stirred for 22 h at room temperature under an N_2 atmosphere. The solution was concentrated *in vacuo*, diluted with H_2O and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to preparative TLC [with MeOH–EtOAc (1 : 1)] to afford **1** (523 mg, 84%) and **2** (26 mg, 4%), which were found to be identical with authentic samples by comparison of TLC and HPLC behaviors, and IR and ^{1}H-NMR spectra.

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TABLE I. ^{1}H-NMR Assignments of **1**, **2** and **3**

H	1	2	3
1	6.54, d (8.3)	6.65, d (8.3)	6.53, d (8.3)
2	6.61, d (8.3)	6.72, d (8.3)	6.62, d (8.3)
5	2.31, d (15.4)	2.61, d (17.4)	2.44, d (15.5)
	4.22, d (15.4)	4.14, d (17.4)	4.35, d (15.5)
8			5.47, d (2.1)
9	3.50, dd (5.5, 3.6)	3.68, br d (5.8)	3.17, dd (5.5, 3.8)
10	2.72, dd	2.75, dd	2.69, dd
	(18.3, 5.5)	(18.0, 5.8)	(18.3, 5.5)
	2.90, d (18.3)	3.13, d (18.0)	3.01, d (18.3)
14	3.00, d (3.6)	2.86, br s	2.99, dd (3.8, 2.1)
15	1.81, ddd	1.60, ddd	1.87, ddd
	(12.8, 12.4, 4.6)	(12.8, 3.4, 1.2)	(12.5, 12.2, 4.5)
	1.94, ddd	2.21, ddd	1.92, ddd
	(12.8, 3.4, 1.8)	(12.8, 12.2, 4.9)	(12.5, 3.7, 2.2)
16	2.11, ddd	2.01, ddd	2.07, ddd
	(12.4, 11.9, 3.4)	(12.2, 11.9, 3.4)	(12.2, 11.9, 3.7)
	2.52, ddd	2.38, ddd	2.53, ddd
	(11.9, 4.6, 1.8)	(11.9, 4.9, 1.2)	(11.9, 4.5, 2.2)
NMe	2.42, s	2.33, s	2.42, s
3-OMe	3.80, s	3.86, s	3.80, s
7-OMe	3.36, s	3.73, s	3.49, s
8-OMe	4.00, s	4.17, s	

Values in parentheses are coupling constants (Hz). Abbreviations: s = singlet; d = doublet; dd = double doublet; ddd = double double doublet; br = broad.

TABLE II. ^{13}C-NMR Assignments of **1**, **2** and **3**

C	1	2	3
1	118.25	118.56	118.23
2	108.77	109.02	108.94
3	144.73	144.68	144.95
4	144.25	143.69	144.70
5	48.37	47.49	49.23
6	194.68	195.39	194.03
7	137.76	137.42	152.35
8	161.92	162.61	115.15
9	53.01	51.73	56.70
10	24.02	27.47	24.23
11	131.42	130.93	130.45
12	122.57	127.03	122.63
13	38.27	36.34	40.50
14	49.95	46.20	45.97
15	35.95	29.21	36.05
16	47.21	46.89	47.14
NMe	42.81	42.95	42.81
3-OMe	56.01	56.23	56.05
7-OMe	60.55	60.71	54.77
8-OMe	60.60	61.05	

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