ALKALOIDS OF THE LEAVES OF *STEPHANIA JAPONICA*[1]

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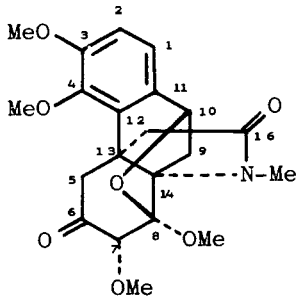
Twenty-one alkaloids have already been isolated from the stems and roots of *Stephania japonica* Miers (Menispermaceae) native to Japan. As a result, it was recognized that the basic constituents of the plant materials are somewhat different in different habitats (table 1). For example, the hasubanan congeners, oxostephamiersine (**1**), 16-oxoprometaphanine (**2**), stephamiersine, and epistephamiersine occurred in the plants grown in the southernmost part of Japan, Kagoshima (1, 2), while hasubanonine and homostephanoline were found in those indigenous to the southwest, Kumamoto and Shikoku (3). In contrast, bisbenzylisoquinoline congeners, stebisimine (**3**), epistephanine, and hypoepistephanine, were common constituents distributed in the plants grown in the various parts in Japan (1, 4). Undoubtedly, the plant materials used for the chemical examinations were conspecific in a morphological feature, but there has been no adequate comment in any literature to explain the reason for the above difference. At the present time, to understand the problems from a chemotaxonomic viewpoint, it may be necessary to presume that *S. japonica* growing in Japan was of two different strains.



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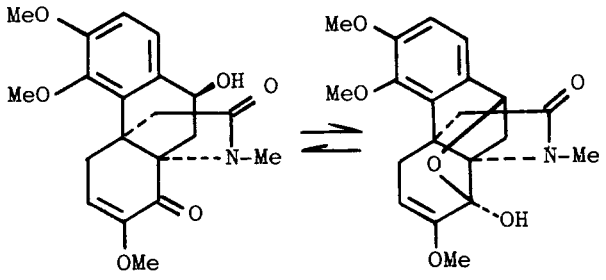

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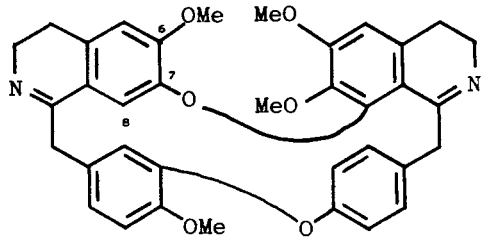

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[1]Part 274 in the series "Studies on the Alkaloids of Menispermaceous Plants". Part 273: M. Matsui, Y. Watanabe, and T. Hinomoto, *J. Nat. Prod.*, in press. This work was presented at the 116th meeting of the Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, October 1980.

TABLE 1. Distribution of the alkaloids from *Stephania japonica* Miers.[a]

alkaloids	stems and roots[b]		leaves[c]
	I[d]	II[e]	I[d]
hasubanan type			
oxostephamiersine (**1**)	+	−	+ (284 mg)
16-oxoprometaphanine (**2**)	+	−	+ (238.5 mg)
stephamiersine	+	−	−
epistephamiersine	+	−	−
stephasunoline	+	−	−
metaphanine	+	+	−
hasubanonine	−	+	−
homostephanoline	−	+	−
miersine	−	+	−
bisbenzylisoquinoline type			
epistephanine	+	+	−
hypoepistephanine	+	+	−
insularine	−	+	−
stebisimine (**3**)	+	+	+ (192.5 mg)
stepinonine	+	−	−
aporphine type			
stephanine	+	+	−
lanuginosine	+	−	−
magnoflorine	+	−	−
oxostephanine	+	−	−
protoberberine type			
steponine	−	+	−
cyclanoline	+	−	−
dibenz [*d,f*] azonine type			
protostephanine	+	+	−

[a]Symbolization: +, detectable; −, not detectable.
[b]See references, 1, 2, 3, 4, 5, 6, and 7.
[c]From 6.0 Kg plant materials.
[d]From the plant source grown in the southernmost part in Japan.
[e]From the plant source grown i the south-west part in Japan.

For the purpose of elaboration of the chemical studies on Menispermaceous plants, we have recently investigated the alkaloids in the leaves from the southernmost plants. The present paper describes the isolation of the alkaloids and the comparison of them with those reported in the stems and roots.

Three known alkaloids, **1**, **2**, and **3** were isolated from a methanolic extract of the leaves (see table 1). Unexpectedly, epistephanine and hypoepistephenine, which have been identified from the stems and roots as major constituents, were not found in the leaves.

From these findings, it was known that the alkaloids occurring in the leaves of this plants are in a more oxidized state than those found in the stems and roots. Therefore, this fact suggested consideration of metabolic modifications of alkaloids in the plant tissues.

EXPERIMENTAL[2]

PLANT MATERIAL.—The leaves of *Stephania japonica* Miers were collected in Bohnotsu-Cho, Kagoshima district, Japan, in June 1979 by Mr. K. Shukuri. A voucher speci-

[2]Melting points are uncorrected. Uv spectra were obtained on a Jasco model UVIDEC-500 spectrophotometer and ir spectra were taken on a Jasco model A-102 spectrophotometer in $CHCl_3$. ^{1}H-nmr spectra were recorded in $CDCl_3$ on a Hitachi Perkin-Elmer model R-20A with TMS as internal standard and chemical shifts reported in δ(ppm) units. Mass spectra were taken with Jeol model JNM-D-100 mass spectrometer by direct inlet probe at 70 eV. Optical rotations were measured on a Jasco model DIP-4 polarimeter. Silicic acid (100 mesh) (Mallinckrodt) and neutral alumina (activity II-III) (E. Merck) were used for column chromatography. Tlc was performed by Aluminiumoxid 150 F_{254} neutral type T (layer thickness 0.2 mm) (E. Merck), and the alkaloids after tlc were detected by treatment with I_2 vapor and by spraying with Dragendorff's reagent.

men is deposited in the Herbarium of the Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan. The plant materials were harvested in the same season and from the same habitat as the stems and roots reported on in a previous study.

EXTRACTION AND FRACTIONATION OF WEAK-BASIC ALKALOIDS.—The fresh leaves (6.0 kg) with the petioles removed were extracted repeatedly with methanol. The solvent was removed at reduced pressure to give 1.24 kg of residue. The residue was triturated with 2% aqueous citric acid, and the combined acid solution extracted with chloroform. The chloroform layer was washed with 2% aqueous sodium hydroxide, dried over anhydrous sodium sulfate and evaporated to dryness to give a tertiary non-phenolic extract (16.0 g) (fraction A). To the sodium hydroxide solution was added ammonium chloride, and the ammoniacal solution was extracted with chloroform. After the solution was dried over anhydrous sodium sulfate, removal of the solvent gave a tertiary phenolic extract (3.2 g) (fraction B).

EXTRACTION AND FRACTIONATION OF STRONG-BASIC ALKALOIDS.—The citric acid solution separated from the foregoing chloroform layer was made alkaline by the addition of ammonium hydroxide, and the solution was extracted with chloroform. The chloroform layer was shaken with 2% aqueous sodium hydroxide, dried over anhydrous sodium sulfate and evaporated to dryness to give a tertiary non-phenolic extract (16.3 g) (fraction C). The sodium hydroxide solution was treated with ammonium chloride, and the ammoniacal solution was extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness to yield a tertiary phenolic extract (3.6 g) (fraction D).

ISOLATION OF TERTIARY NON-PHENOLIC ALKALOIDS FROM WEAK-BASE FRACTION.—Fraction A (16 g) was percolated repeatedly with water at 60° for 8 hrs, and the combined aqueous layer was extracted with benzene. The benzene extract was washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness at reduced pressure to give a brownish extract (3.58 g). The extract dissolved in benzene was chromatographed through an alumina column (2.8 x 30 cm) and eluted with the same solvent to give oxostephamiersine (**1**) (238 mg). The water-insoluble residue was triturated with 10% acetic acid (5 x 40 ml), and the acid solution was made alkaline with ammonium hydroxide. After extraction of the ammoniacal solution with chloroform, the chloroform extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in methanol and allowed to stand at room temperature for a few days to give **1** (20 mg). The mother liquor from which **1** was removed was evaporated to dryness, and the residue was subjected to chromatography through an alumina column (1 x 15 cm) in benzene. Elution with the same solvent gave **1** (26 mg).

ISOLATION OF TERTIARY NON-PHENOLIC ALKALOIDS FROM STRONG-BASE FRACTION.—Fraction C dissolved in chloroform was extracted with MacIlvain buffer solution (double strength) (1, 8) of pH 3.6. The buffer layer was made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in methanol and, on standing, gave stebisimine (**3**) (192.5 mg). The chloroform layer separated from the buffer solution was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness. The residue was dissolved in benzene and chromatographed through a silica gel column (3 x 26 cm) in sequence with the following solvents: benzene, chloroform, chloroform-ethanol (9:1), and chloroform-ethanol (1:1). Each eluate which showed the existence of a few minor alkaloids by tlc behavior was difficult to purify. However, tlc revealed no spot identical with that of epistephanine.

TREATMENT OF PHENOLIC BASE FRACTION.—Fraction B dissolved in chloroform was chromatographed through a silica gel column (2.5 x 32 cm) in sequence with the following solvents: chloroform, chloroform-ethanol (99:1), chloroform-ethanol (95:5), and chloroform-ethanol (9:1). Fraction D was worked up in a similar manner as that described for fraction B. No alkaloid in crystaline form was isolated from either fraction and no spot identical with that of hypoepistephanine could be found on tlc.

OXOSTEPHAMIERSINE (**1**).—Colorless prisms, mp 290°, 256° (bimorphism) (from MeOH); $[\alpha]^{20}D$ +88.05° (*c* 1.0, $CHCl_3$); uv λmax (MeOH) 286 (ϵ 2.1 x 10^3) nm; ir νmax ($CHCl_3$) 1730 (six-membered C=O), 1680 (γ-lactam) cm^{-1}; ^1H nmr (60 MHz, $CDCl_3$) δ 6.77 (2H, s, aromatic H), 4.79 (1H, d, *J*=6.5 Hz, C_{10}-H), 3.63 (1H, s, C_7-H), 3.92, 3.83, 3.33, 3.29 (each 3H, s, 4 x OCH_3), 3.12 (3H, s, NCH_3), 1.63 (1H, d, *J*=10.5 Hz, C_9-H_α); ms *m/e* (rel. int.) 403 (M+, 16%), 258 (39%), 257 (100%), 242 (13%), 227 (23%); *Rf* 0.72 (Al_2O_3, $CHCl_3$); yield 284 mg. *Anal.* Calcd for $C_{21}H_{25}O_7N$: C, 62.52; H, 6.25; N, 3.47. Found: C, 62.34; H, 6.32; N, 3.45. Identical (mixed mp, ir, and co-tlc) with an authentic sample (1). On spraying with Dragendorff's reagent, the spot showed a pale-yellow coloration which faded soon after.

16-OXOPROMETAPHANINE (**2**).—Colorless prisms; mp 195° (from MeOH); $[\alpha]^{20}D$ −52.3° (*c* 0.5, $CHCl_3$); uv λmax (MeOH) 230 (ϵ 8.5 x 10^3), 273 (ϵ 8.0 x 10^3) nm; ir νmax ($CHCl_3$) 3550–3310 (OH), 1680 (conj. C=O and γ-lactam), 1640 (enolic C=C) cm^{-1}; ^1H nmr [60 MHz, $(CD_3)_2CO$]δ 7.35 (1H, d, *J*=8.5 Hz, aromatic H), 7.01 (1H, d, *J*=8.5 Hz, aromatic H), 6.01 (1H, dd, *J*=6.7 and 3.1 Hz, C_6-H), 4.65 (1H, m, C_{10}-H), 4.58 (1H, s, C_{10}-OH), 3.96, 3.87, 3.62 (each 3H, s, 3 x OCH_3), 2.95 (3H, s, NCH_3); ms *m/e* (rel. int.) 373 (M^+, 46%), 358 (M^+−15, 16%), 275 (98%), 258 (99%), 257 (90%), 242 (100%); *Rf* 0.16 (Al_2O_3); yield 239 mg. *Anal.* Calcd for $C_{20}H_{23}O_6N$; C, 64.33; H, 6.21, N, 3.75.

Found: C, 64.29; H, 6.47; N, 3.61. Identical (mixed mp, ir, and co-tlc) with an authentic sample (2).

STEBISIMINE (3).—Colorless prisms; mp 233° (from MeOH); $[\alpha]^{23}D = 0$ (c 0.5, $CHCl_3$); uv λmax (MeOH) 238 (ε 5.1 x 10^4), 280 (ε 2.4 x 10^4) nm; ir νmax ($CHCl_3$) 1606 (C=N) cm^{-1}; ^1H nmr (60 MHz, $CDCl_3$) 7.26–6.51 (8H, aromatic H), 5.88 (1H, aromatic H), 3.96, 3.88, 3.86, 3.52 (each 3H, s, 4 x OCH_3); ms m/s (rel. int.) 590 (M^+, 100%), 295 (M^{2+}, 15%); Rf 0.44 (Al_2O_3, $CHCl_3$); yield 193 mg. *Anal.* Calcd for $C_{36}H_{34}O_6N$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.38; H, 5.90; N, 4.74. Identical (mixed mp, ir, and co-tlc) with an authentic sample (1).

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