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Alkaloids from the Fruits of *Stephania japonica* MIERS. I. Structure of Stephabenine: A New Hasubanan Ester-Ketal Alkaloid[1)]

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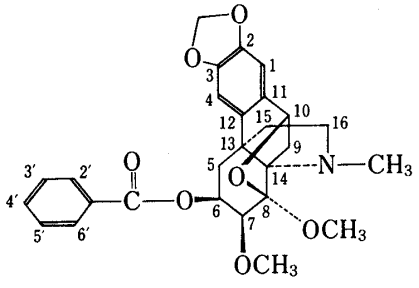
A new hasubanan ester-ketal alkaloid, named stephabenine, was isolated from the fresh fruits of *Stephania japnica* MIERS (Menispermaceae). Alkaline hydrolysis of the new base gave benzoic acid and a basic component, whose proton nuclear magnetic resonance spectrum was identical with that of authentic *N,O*-dimethylstephine.

Keywords——*Stephania japonica*; Menispermaceae; fruit; hasubanan ester-ketal alkaloid; stephabenine; benzoic acid; *N,O*-dimethylstephine

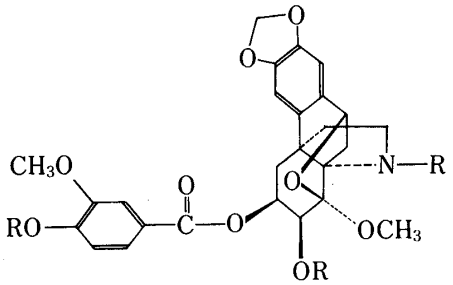
Although eleven hasubanan alkaloids[2)] have been isolated from the stems and roots of *Stephania japonica* MIERS (Menispermaceae) together with many other alkaloids, as far as we know no research has been carried out on alkaloids in the fruits. Recent investigation of the fruits collected in Amakusa, the southwestern region of Japan, resulted in the isolation of a new hasubanan ester-ketal alkaloid, named stephabenine (**1**), together with two unidentified alkaloids. The present paper deals with the structure of the new alkaloid, stephabenine (**1**).

The petroleum ether extract prepared from the fresh fruits (8.22 kg) of *Stephania japonica* was digested with dilute aqueous citric acid, and the solution was shaken with chloroform to separate a chloroform layer ("weak base" fraction) and an acid layer ("strong base" fraction). Each fraction was elaborated by the usual method, as described in the experimental section, to yield a phenolic extract and a non-phenolic extract. The non-phenolic extract (762 mg) from the "weak base" fraction was subjected to extensive column chromatography and preparative thin-layer chromatography (TLC) to give **1** (42 mg) and unidentified base-A (5 mg). A similar treatment of the non-phenolic extract (259 mg) from the "strong base" fraction also gave **1** as a minor component together with unidentified base-B (15 mg).

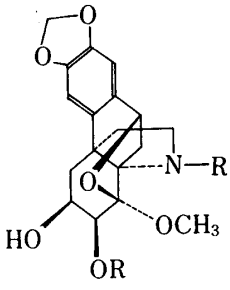
Stephabenine (**1**) was obtained as colorless prisms, $C_{27}H_{29}NO_7$, mp 170 °C, $[\alpha]_D^{16}$ −15.24° ($CHCl_3$). Its infrared (IR) spectrum showed bands at 1702 cm^{-1} and 1600 cm^{-1}, and its ultraviolet (UV) spectrum exhibited absorption maxima at 260.0 nm and 294.0 nm. The mass



1



2 : R=H
3 : R=CH_3



4 : R=CH_3
5 : R=H

Chart 1

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spectrum of **1** revealed a molecular ion peak at *m/e* 479 (11.28%), with the most abundant ion at *m/e* 228, and another significant ion at *m/e* 229 (77.5%).[3]

The proton nuclear magnetic resonance (^{1}H-NMR) spectral data of **1** (Table I) coupled with the above mass spectral findings suggested that **1** is of hasubanan type and probably carries a benzoate moiety. Furthermore, the noticeable high-field shift and splitting of the methylenedioxy protons could be ascribed to the close proximity of the aromatic ring of benzoate ester to the methylenedioxy protons, indicating β-axial orientation for the ester moiety. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** showed 27 carbon resonances in the region of δ 29.37—166.28 ppm, and the spectral pattern exhibited the characteristic resonances of a ketal hasubanan alkaloid[4] carrying the benzoate ester.

In 1970, Kupchan *et al.*[5] reported the structure of a hasubanan ester alkaloid, stephavanine (**2**), isolated from *Stephania abyssinica*. They unequivocally established the

Table I. ^{1}H-NMR Signals of Stephabenine (**1**)[a]

Proton[b]			
C-1	6.44	s	
C-4	6.58	s	
C-6	5.54	m	
C-7	3.82	d	J=4.40 Hz
C-9 α	1.55	d	J=10.77 Hz
C-9 β	2.73	dd	J=10.77, 6.37 Hz
C-10	4.91	d	J=6.37 Hz
C-2,3 $-O-CH_2-O-$	5.15	d	J=1.54 Hz
	5.70	d	J=1.54 Hz
C-7 OCH_3	3.41	s	
C-8 OCH_3	3.55	s	
NCH_3	2.58	s	
Other aromatic C-2′, C-3′, C-4′ C-5′, C-6′	7.09—7.54		

a) Measured in $CDCl_3$, values in δ scale relative to internal TMS.
b) The C-5 methylene protons were not assigned clearly because these signals overlapped with other signals.

Table II. ^{13}C-NMR Signals of Stephabenine (**1**)[a]

Carbon			Carbon		
1	107.01	d	14	77.08	s
2	147.69	d	15	29.37	dd
3	144.71	s	16	53.91	t
4	106.01	d	$-O-CH_2-O-$	100.56	dd
5	36.68	t	C-7 OCH_3	57.65	q
6	68.00	d	C-8 OCH_3	51.58	q
7	81.60	d	NCH_3	38.58	q
8	103.38	s	C=O	166.28	s
9	37.49	dd	Other aromatic		
10	77.10	d	1′	129.64	s
11	137.01	s	2′, 6′	129.87	d
12	133.50	s	3′, 5′	127.49	d
13	49.68	s	4′	132.31	d

a) Measured in $CDCl_3$, values in δ scale relative to internal TMS; abbreviations s, d, t, dd, and q indicate the multiplicity due to direct ^{13}C–^{1}H coupling (^1J).

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structure of **2** on the basis of chemical and X-ray crystallographic evidence. In 1974, moreover, van Wyk *et al.*[6] isolated the hasubanan ester-ketal alkaloid (**3**) from the same species, and the structure of **3** was determined by the following chemical correlation; basic hydrolysis of **3** in methanol gave two components, methyl veratrate and *N,O*-dimethylstephine (**4**), which is derivable from Kupchan's base, stephine (**5**).[5] Thus, from the foregoing spectral data, **1** was assumed to be a new hasubanan congener closely related to **2** and **3**.

Alkaline hydrolysis of **1** gave an acid and a basic component. The former was identical with authentic benzoic acid, and the latter was identical with an authentic sample of **4** with respect to the ^{1}H-NMR spectrum as well as other spectral and physical properties.

Based on the results presented here, the structure of **1** was established to be as drawn in the formula.

Experimental

Melting points were measured with a Yanaco micro melting point apparatus and are uncorrected. UV spectra were taken with a JASCO UVIDEC-500 digital spectrophotometer, and IR spectra were obtained with a JASCO A-120 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL JNM-FX-90 Q spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard, and chemical shifts are given as δ (ppm) (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet). Optical rotations were determined on a JASCO DIP-140 digital polarimeter. Mass spectra (MS) were run on a JEOL JMS-D-100 mass spectrometer at 70 eV using a direct inlet system. Precoated plates (Kieselgel 60 F_{254}, E. Merck) were used for TLC, and preparative TLC was carried out on a 20 × 20 cm plate coated with a 2.0 mm thick layer of silica gel (Kieselgel nach Stahl G 60, E. Merck). The spots on TLC were visualized under UV light, by exposure to I_2 vapor, and by spraying with Dragendorff's reagent. Column chromatography was carried out on silica gel (Kieselgel 60, 70—230 mesh, E. Merck).

Plant Material——The fruits of *Stephania japonica* MIERS used in this study were collected in Hondo-shi, Kumamoto-ken, Japan, during the autumn of 1981.

Extraction and Fractionation of "Weak Base" Fraction——The fresh fruits (8.22 kg) were percolated repeatedly with hot petroleum ether (total of 86 l), and the solvent was removed under reduced pressure to give 40 g of residue. The residue was triturated with 5% aqueous citric acid, and the acid solution was extracted with chloroform. The chloroform phase was washed with 2% aqueous sodium hydroxide, dried over anhydrous sodium sulfate, and evaporated to dryness to leave a tertiary non-phenolic extract (762 mg) (fraction A). Ammonium chloride was added to the sodium hydroxide solution, and the ammoniacal solution was extracted with chloroform. After the solution had been dried over anhydrous sodium sulfate, removal of the solvent gave a tertiary phenolic extract (17 mg). This minor fraction was not evaluated in detail.

Extraction and Fractionation of "Strong Base" Fraction——The citric acid solution separated from the "weak base" fraction was made alkaline by addition of ammonium hydroxide, and the solution was extracted with chloroform. The chloroform phase was extracted with 2% aqueous sodium hydroxide, dried over anhydrous sodium sulfate, and evaporated to dryness to leave a tertiary non-phenolic extract (259 mg) (fraction B). The sodium hydroxide solution was treated with ammonium chloride, and the ammoniacal solution was extracted with chloroform. The chloroform phase was dried over anhydrous sodium sulfate, and the solvent was evaporated off to yield a tertiary phenolic extract (40 mg), though this extract remained untreated.

Isolation of Non-Phenolic Alkaloids from "Weak Base" Fraction——Fraction A (762 mg) was subjected to column chromatography on silica gel (20 g, 1.5 × 18 cm) in benzene, and eluted successively with benzene, benzene–chloroform (1 : 1), chloroform, and then with increasing proportions of methanol in chloroform. The fractions eluted with benzene and benzene–chloroform (1 : 1) were oils, negative to Dragendorff's reagent. The remaining fractions were combined, rechromatographed over a silica gel column (25 g, 2 × 16.5 g) in hexane–diethylamine (95 : 5), and eluted with the same solvent. After the solvent had been evaporated off, the residue was subjected to preparative TLC with hexane–diethylamine (95 : 5), and the major band (about *Rf* 0.5) was collected from the plate and extracted with chloroform. The solvent was evaporated off, and the residue was subjected to preparative TLC again with acetone–methanol–chloroform (7 : 3 : 90). The bands at *Rf* 0.56 and *Rf* 0.48 were scraped off and extracted with chloroform. Removal of the solvent from the band of *Rf* 0.56 afforded a pure base which, on treatment with ethanol, gave stephabenine (**1**) as colorless prisms (40 mg). Similar treatment of the band of *Rf* 0.48 gave unidentified base-A as colorless prisms (5 mg) from methanol.

Isolation of Non-phenolic Alkaloids from "Strong Base" Fraction——Fraction B (259 mg) was dissolved in benzene and subjected to chromatography on silica gel (10 g, 1.5 × 15 cm) with benzene, chloroform, and then with increasing proportions of methanol in chloroform. The eluates from chloroform and methanol–chloroform (1 : 99)

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were combined and rechromatographed on silica gel (5 g, 1.0 × 14 cm) in chloroform followed by methanol–chloroform (1 : 9). The chloroform eluate was evaporated to dryness, and the residue was treated with ethanol to give **1** (2 mg) as colorless prisms. The methanol–chloroform (1 : 9) eluate was subjected to preparative TLC with chloroform. The band of *Rf* 0.39 was collected and extracted with chloroform. Removal of the solvent yielded a pure base which, on treatment with methanol, gave unidentified base-B as light yellow prisms (15 mg).

Stephabenine (1) (New Base)——Colorless prisms, mp 170 °C (from EtOH), $[\alpha]_D^{16}$ −15.24° (c=1.65, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{29}NO_7$: C, 67.63; H, 6.10; N, 2.92. Found: C, 67.77; H, 6.10; N, 2.88. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 294 (4450), 260 (1770). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1702, 1600, 1502, 1483, 1280. MS *m/e*: 479 (M^+, 11.2%), 358 (4.8%), 229 (77.5%), 228 (base peak). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. Yield: 42 mg.

Base-A (Unidentified Alkaloid)——Colorless prisms, mp 152—153 °C (from MeOH). $[\alpha]_D^{15}$ −269.1° (c=0.37, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1665, 1600, 1510, 1490. High resolution MS *m/e*: Calcd for $C_{20}H_{23}NO_5$: 357.1577. Found: 357.1540. Yield: 5 mg.

Base-B (Unidentified Alkaloid)——Light yellow prisms, mp 225—226 °C (dec.) (from MeOH), $[\alpha]_D^{15}$ −219.1° (c=0.25, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 273.5 (7200). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670, 1640, 1505, 1485. ^{1}H-NMR: 7.02 (1H, s), 6.72 (1H, s), 6.62 (1H, d, J=11.65 Hz), 5.90 (2H, d, J=1.54 Hz), 5.68 (1H, m), 4.80 (1H, m), 3.60 (3H, s), 3.49 (1H, d, J=3.51 Hz), 2.57 (3H, s). High resolution MS *m/e*: Calcd for $C_{19}H_{21}NO_5$: 343.1422. Found: 343.1450. Yield: 15 mg.

Alkaline Hydrolysis of 1——A solution of **1** (40 mg) in 5% sodium hydroxide (ethanol–water = 2 : 3) was refluxed on a water bath for 3 h. After cooling, the mixture was extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to yield **4** (30 mg). The aqueous phase was acidified with 2 N hydrochloric acid to pH 2 and extracted with chloroform. Evaporation of the solvent gave benzoic acid (5 mg), which was identical with an authentic sample with respect to the IR spectrum.

***N,O*-Dimethylstephine (4)**——Amorphous, colorless solid. *Rf* 0.54 (silica gel, chloroform–methanol = 9 : 1). IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3555, 3010, 2950, 2850, 1619, 1510, 1480, 1260, 1210, 1040 (lit.,[6] 3555, 3010, 2950, 2850, 1620, 1518, 1498, 1270, 1200, 1040). MS *m/e*: 375 (M^+, 5.6%), 230 (20.1%), 229 (base peak), 228 (81.6%). ^{1}H-NMR: 6.69 (1H, s, C-4H), 6.52 (1H, s, C-1 H), 5.92 (1H, d, J=1.32 Hz, methylenedioxy H), 5.90 (1H, d, J=1.32 Hz, methylenedioxy H), 4.82 (1H, d, J=6.37 Hz, C-10 H), 4.17 (1H, m, C-6 H), 3.65 (1H, d, J=4.18 Hz, C-7 H), 3.52 (3H, s, C-8 methoxyl H), 3.44 (3H, s, C-7 methoxyl H), 2.70 (1H, dd, J=10.5, 6.37 Hz, C-9β H), 2.54 (3H, s, *N*-methyl H), 1.51 (1H, d, J=10.5 Hz, C-9α H). The ^{1}H-NMR spectrum was identical with that of an authentic sample of **4**.

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References and Notes

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