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ALKALOIDS FROM THE FRUITS OF *STEPHANIA JAPONICA* 2.[1] STRUCTURES OF OXOSTEPHABENINE AND *N,O*-DIMETHYLOXOSTEPHINE

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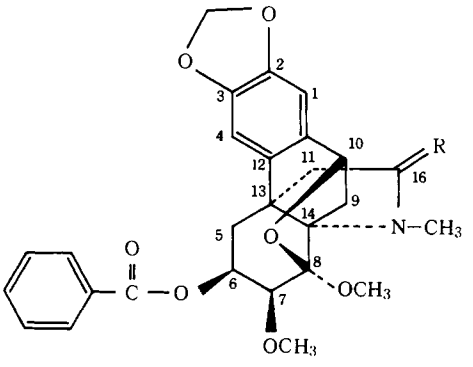
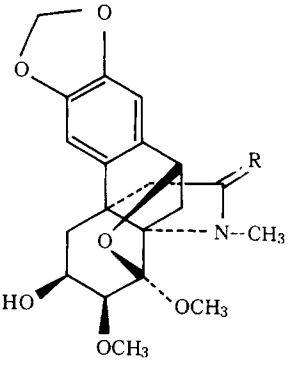
ABSTRACT.—A new ester-ketal hasubanalactam alkaloid, oxostephabenine (**1**), was isolated from a methanolic extract of the fruits of *Stephania japonica* (Menisperamaceae). Alkaline hydrolysis of oxostephabenine (**1**) gave a new hasubanalactam alkaloid, *N,O*-dimethyloxostephine (**3**) and benzoic acid. The structures of the new alkaloids, **1** and **3**, were elucidated by ^{1}H nmr, ^{13}C nmr, and mass spectral arguments. Further, permanganate oxidation of stephabenine (**2**), a known ester-ketal hasubanan alkaloid, gave a γ-lactam identical with the naturally occurring **1**.

In the first paper of this series (1), we reported the isolation and structure elucidation of a new hasubanan ester-ketal alkaloid, stephabenine (**2**), present in a petroleum ether extract from the fruits of *Stephania japonica* Miers (Menispermaceae). In the continuation of our studies on alkaloids of the fruits of this plant, we have recently isolated a new hasubananlactam ester-ketal alkaloid named oxostephabenine (**1**).

The present paper deals with the structures of the new alkaloid, oxostephabenine (**1**), and its derivative, *N,O*-dimethyloxostephine (**3**).



1 R=O
2 R=H_2



3 R=O
4 R=H_2

RESULTS AND DISCUSSION

The fresh fruits (8.24 kg) of *S. japonica* were extracted with petroleum ether and then with MeOH. The MeOH extract was treated by the usual method, as described in the experimental section, to yield a nonphenolic extract (42.4 g). The extract was subjected to column chromatography on alumina and on silica gel to give **1** (123.7 mg).

Oxostephabenine (**1**) was obtained as colorless needles, mp 272-273° (dec.) (from MeOH), $C_{27}H_{27}NO_8$, $[\alpha]_D$ +65° ($CHCl_3$). Its ir spectrum exhibited bands at 1700, 1680, and 1600 cm^{-1}, and the uv spectrum showed absorption maxima at 294.4 and 230.0 nm. The electron impact mass spectrum revealed a molecular ion peak at *m/z* 493 (17%) with the most abundant peak at *m/z* 241 and another significant peak at *m/z* 242.

[1]For Part 1 in this series, see S. Kondo, M. Matsui, and Y. Watanabe, *Chem. Pharm. Bull.*, **31**, 2574 (1983).

^{1}H-nmr and ^{13}C-nmr data are summarized in Tables 1 and 2, indicating the close resemblance of **1** and **2**.

TABLE 1. ^{1}H-nmr Assignments for Oxostephabenine (**1**) and *N,O*-Dimethyloxostephine (**3**)[a]

Proton	**1**[b]	**3**[c]
C-1	6.48 s	6.57 s
C-4	6.62 s	6.70 s
C-5α	2.13 dd (J=3.08, 15.49)	1.94 dd (J=2.91, 14.77)
C-5β	2.54 dd (J=3.52, 15.49)	2.41 dd (J=3.42, 14.77)
C-6	5.45-5.59 m	4.15-4.09 m
C-7	3.60 d (J=4.18)	3.44 d (J=3.91)
C-9α	1.67 d (J=10.77)	1.64 d (J=10.87)
C-9β	2.96 dd (J=6.15, 10.77)	2.96 dd (J=6.34, 10.87)
C-10	4.96 d (J=6.15)	4.88 d (J=6.34)
C-15α		2.45[d] d (J=15.86)
C-15β		2.58[d] d (J=15.86)
C-2,3 -OCH_2O-	5.75 d (J=1.54)	5.96 d (J=1.46)
	5.23 d (J=1.54)	5.95 d (J=1.46)
C-6 OH		2.34 d (J=9.58)
C-7 OCH_3	3.43 s	3.46 s
C-8 OCH_3	3.61 s	3.60 s
NCH_3	3.05 s	3.01 s
Other aromatic		
C-2′, C-3′	7.13-7.49	
C-4′, C-5′, C-6′		

[a]Chemical shifts are expressed in δ ppm from TMS, and coupling constants (J) are expressed in Hz.
[b]Spectrum was recorded in $CDCl_3$ solution at 90 MHz.
[c]Spectrum was measured in $CDCl_3$ solution at 300 MHz.
[d]Assignments may be reversed.

Among the above spectral findings, the carbonyl absorption band at 1680 cm^{-1}, the downfield shift (δ 3.05) of the *N*-methyl protons, and the fragmentation pattern of the mass spectrum suggested that **1** was a member of the hasubanalactam family carrying a benzoate moiety at C-6 (1-3). The highfield shifts (δ 5.75, 5.23) and splitting pattern (d, J=1.54 Hz) of the methylenedioxy protons indicated the close proximity of the aromatic ring of the benzoate ester to the methylenedioxy protons. Additionally, the value of the coupling constants (J=3.08 Hz, 3.52 Hz) between the C-5 methylene protons and the C-6 proton were indicative of axial-equatorial or equatorial-equatorial coupling and not of diaxial coupling. These findings suggested that the benzoate moiety attached to C-6 should be axial (β), as we discussed earlier (1).

Alkaline hydrolysis of **1** gave benzoic acid and a basic component (**3**), which was obtained as colorless needles, mp 229-231° (dec.) (from MeOH), $C_{20}H_{23}NO_7$, [α]D +222° ($CHCl_3$). The ir spectrum of **3** exhibited bands at 3550, 1690, and 1620 cm^{-1} and the absence of an ester carbonyl band near 1700 cm^{-1}, and the uv spectrum showed absorption maxima at 293.6 and 245.0 nm. The mass spectrum revealed a molecular ion peak at *m/z* 389 ($C_{20}H_{23}NO_7^+$) with the most abundant ion at *m/z* 243 and 241, indicating the fragmentation pattern characteristic of hasubanalactam alkaloids (2, 3). The ^{13}C-nmr and ^{1}H-nmr data are summarized in Tables 1 and 2.

Structure assignment of **3** was accomplished by comparison of its mass and ^{1}H-nmr spectra with those of the closely related known compound, *N,O*-dimethylstephine (**4**) (1). The mass spectrum of **4** showed a molecular ion peak at *m/z* 375 ($C_{20}H_{25}NO_6^+$) and the most abundant ion at *m/z* 229 ($C_{14}H_{15}NO_2^+$). Comparison of the mass spectral fragmentations of **3** and **4** suggested that both alkaloids possess the same substituents

TABLE 2. ^{13}C-nmr Data for Oxostephabenine (**1**), Stephabenine (**2**), and *N,O*-Dimethyloxostephine (**3**)[a]

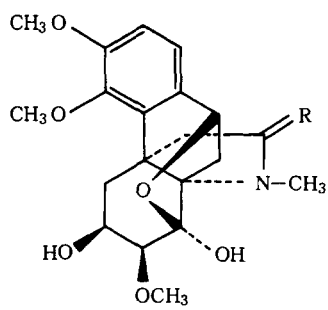
Carbon	1	2[b]	3
1	107.06 d	107.01 d	107.98 d
2	148.13 s	147.69 s	148.61 s
3	145.47 s	144.71 s	145.86 s
4	106.41 d	106.01 d	105.76 d
5	36.86 dd	36.68 t	41.02 dd
6	66.48 d	68.00 d	66.10 d
7	81.97 d	81.60 d	81.79 d
8	101.48 s	103.38 s	102.24 s
9	33.16 dd	37.49 dd	33.32 dd
10	76.10 d	77.10 d	76.23 d
11	133.55 s	137.01 s	134.69 s
12	132.58 s	133.50 s	132.42 s
13	46.62 s	49.68 s	43.67 s
14	73.85 s	77.08 s	73.74 s
15	45.78 dd	29.37 dd	45.84 dd
16	173.32 s	53.91 t	173.27 s
-OCH_2O-	100.94 dd	100.56 dd	101.37 dd
C-7 OCH_3	57.87 q	57.65 q	57.00 q
C-8 OCH_3	52.07 q	51.58 q	52.34 q
NCH_3	28.34 q	38.58 q	28.17 q
ester C=O	166.06 s	166.28 s	—
Other aromatic			
1′	129.38 s[c]	129.64 s	—
2′, 6′	129.55 d[c]	129.87 d	—
3′, 5′	127.54 d	127.49 d	—
4′	129.82 d[c]	132.31 d	—

[a]Chemical shifts are expressed in δ ppm from TMS. Spectra were measured in $CDCl_3$ solution at 22.5 MHz.

[b]See Kondo *et al.* (1).

[c]Assignments may be reversed.

located in the same positions except for C-16, as in the case of oxostephasunoline (**5** and stephasunoline (**6** (3, 4). The ^{1}H-nmr spectrum of **3** revealed the signals of the C-7 proton at δ 3.44 (J=3.91 Hz) as a doublet, the C-5α proton at δ 1.97 (J_1=2.81 Hz, J_2=14.77 Hz), and the C-5β proton at δ 2.41 (J_1=3.42 Hz, J_2=14.77 Hz) as double doublets. Irradiation of the higher field C-5α proton (δ 1.97) gave a nOe enhancement of the C-7 proton signal corresponding to the 1,3-diaxial interaction. Further, a 2D-J-correlated spectrum revealed a long-range coupling of the C-7 proton (δ 3.44) with the C-10 equatorial(α) proton (δ 4.88, doublet, J=6.34 Hz) through five σ bonds and an



5 R=O
6 R=H_2

oxygen atom (Figure 1). These findings indicated that the C-7 proton was axial(α), and therefore the C-7 methoxyl group should be equatorial(β), as in **4**.

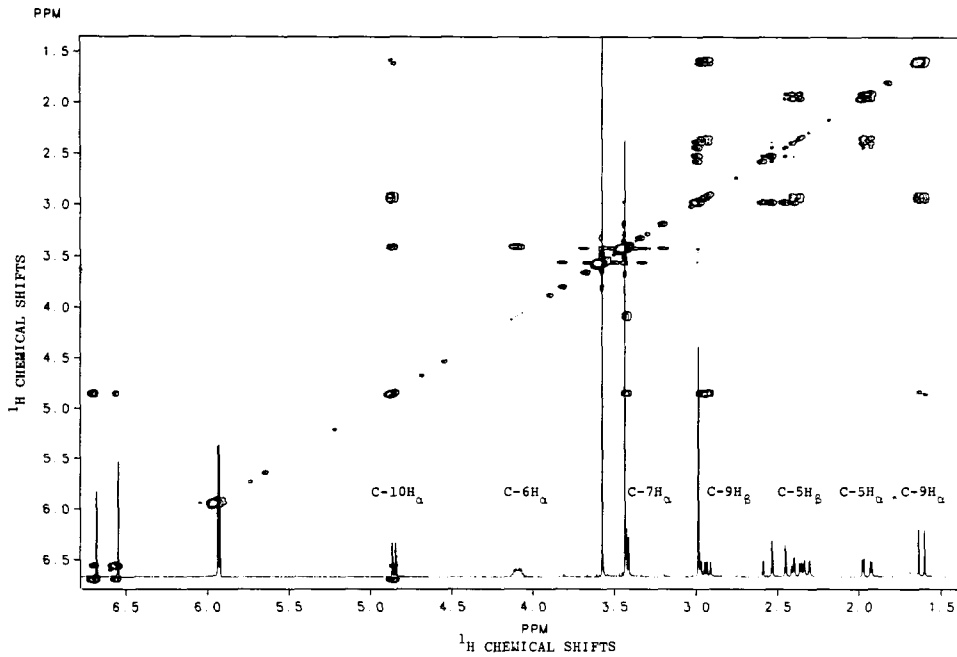


FIGURE 1. Proton homonuclear *J* correlated 2D nmr contour plot of *N,O*-dimethyloxostephine (**3**).

The orientation of the C-6 hydroxy group was shown to be axial(β) by the splitting pattern of the neighboring C-5 methylene protons as discussed for **1**. Thus, the structure of **3** derived from **1** was established, and we named **3**, *N,O*-dimethyloxostephine.

On the other hand, oxidation of **2** with permanganate gave a hasubanalactam alkaloid identical with **1**. Thus, the structures of **1** and **3** were unambiguously established by the spectral and chemical evidence.

EXPERIMENTAL

GENERAL METHOD.—Mps were determined on a YANACO micro melting point hot stage apparatus and are uncorrected. Elemental analyses were in agreement with molecular formulas. Uv spectra were taken on a JASCO UVIDEC-500 spectrophotometer, and ir spectra were obtained on a JASCO A-120 spectrophotometer in $CHCl_3$ solution. ^{1}H-nmr spectra and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a JEOL JNM-FX 90Q spectrometer or a Varian XL-300 spectrometer with TMS as an internal standard, and chemical shifts are quoted in δ (ppm) unit. Mass spectra by electron impact ionization at 70 eV were measured on a JEOL DX-300 mass spectrometer using a direct inlet probe. Optical rotations were taken on a JASCO DIP-140 polarimeter. Silica gel 60 (70-230 mesh) and aluminum oxide (activity II-III) (E. Merck) were used for column chromatography. Tlc was performed on Kieselgel $60F_{254}$ (0.20 mm) plates (E. Merck), and the spots were visualized under uv light and by spraying with Dragendorff's reagent.

PLANT MATERIAL.—The fruits of *S. japonica* were collected in Hondo, Kumamoto-ken, Japan, in September to November, 1982, by pharmacist S. Fukuda. A voucher specimen is deposited in the Herbarium of Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan.

EXTRACTION AND SEPARATION OF OXOSTEPHABENINE (**1**).—The fresh fruits (8.24 kg) were exhaustively extracted with petroleum ether, and the residue was repeatedly extracted with MeOH (25×5 liters). The solvent was evaporated to dryness under reduced pressure to give a MeOH extract (730 g). The extract was digested with 5% aqueous citric acid at 60° (8×2 liters), and the acid solution was shaken with $CHCl_3$. The $CHCl_3$ solution was washed with 2% aqueous NaOH, dried over anhydrous Na_2SO_4, and concentrated to dryness to leave a crude tertiary nonphenolic alkaloid extract (42.39 g). This extract was divided into two equal portions and subjected to column chromatography on Al_2O_3 (425 g), each column 5×25 cm), eluting both columns with hexane, hexane-C_6H_6 (1:1), C_6H_6, $CHCl_3$, $CHCl_3$-MeOH (9:1),

and MeOH. The fractions eluted with hexane and with hexane-C_6H_6 (1:1) were oils and gave a negative test with Dragendorff's reagent. The remaining fractions were combined, and the solvent was evaporated to dryness to give a residue (6.59 g). This residue was chromatographed on a silica gel column (80 g, 3.3×28.5 cm) placed in C_6H_6 and eluted successively with C_6H_6, C_6H_6-$CHCl_3$ (1:1), $CHCl_3$, and then with increasing proportion of MeOH in $CHCl_3$ (1:99, 2:98, 5:95, 10:90, 1:1) followed by elution with only MeOH. The fraction eluted with the mixture of MeOH-$CHCl_3$ (1:99) was rechromatographed on a silica gel column (100 g, 3.3×29 cm) placed in $CHCl_3$ and eluted with $CHCl_3$, and then with increasing proportions of MeOH in $CHCl_3$ (1:99, 3:97, 10:90). Evaporation of the solvent from the eluate (MeOH:$CHCl_3$, 1:99) gave a solid. Recrystallization of the solid from MeOH gave **1** as colorless needles (123.7 mg).

OXOSTEPHABENINE (**1**).—Colorless needles, mp 272-273° (dec.) (from MeOH); $C_{27}H_{27}NO_8$; $[\alpha]^{24}D$ +65° (c=1.04, $CHCl_3$); uv λ max (EtOH) (log ε) 230.0 (4.16), 294.4 (3.64) nm; ir ν max 1700 (ester C=O), 1680 γ-lactam C=O), 1600 (C=C) cm^{-1}; ms *m/z* 493 (M^+, 493.1709, $C_{27}H_{27}NO_8$, 17%), 242 (242.0767, $C_{14}H_{12}NO_3$, 60%), 241 (241.0707, $C_{14}H_{11}NO_3$, 100%); ^{1}H-nmr and ^{13}C-nmr data, see Tables 1,2. Yield, 123.7 mg.

ALKALINE HYDROLYSIS OF **1**.—A solution of **1** (120 mg) in 15 ml of 5% NaOH (EtOH-H_2O, 2:3) was refluxed for 3 h. After cooling, the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4, and the solvent evaporated to yield **3** (56.6 mg). The aqueous layer was acidified with 2 N HCl to pH 2, and the acidic solution was extracted with $CHCl_3$. Evaporation of the solvent gave benzoic acid (89 mg), which was identical wih an authentic sample in mmp, ir, and ms.

N,O-DIMETHYLOXOSTEPHINE (**3**).—Colorless needles, mp 229-231° (dec.) (from MeOH); $C_{20}H_{23}NO_7$; $[\alpha]^{22}D$ +222° (c=1.02, $CHCl_3$); uv λ max (EtOH) (log ε 293.6 (4.77), 245.0 (3.60) nm; ir ν max 3550 (OH), 1690 γ-lactam C=O), 1620 (C=C) cm^{-1}; ms *m/z* 389 (M^+, 389.1434, $C_{20}H_{23}NO_7$, 17%), 243 (243.0871, $C_{14}H_{13}NO_3$, 32%), 242 (242.0802, $C_{14}H_{12}NO_3$, 100%), 241 (241.0726, $C_{14}H_{11}NO_3$, 55%); ^{1}H-nmr and ^{13}C-nmr data, see Tables 1, 2.

OXIDATION OF **2** TO **1**.—A solution of anhydrous $MgSO_4$ (135 mg) in H_2O (20 ml) was added to a solution of **2** (135 mg) in Me_2CO (10 ml). To the mixture was added dropwise a solution of $KMnO_4$ (135 mg) in Me_2CO (15 ml), cooling with ice, and stirred for 6 h at room temperature; then the excess reagent and precipitated MnO_2 were decomposed with a solution of $NaHSO_3$ (300 mg) in 5% H_2SO_4 (10 ml). The solution was concentrated under reduced pressure and the residue was extracted with $CHCl_3$. The $CHCl_3$ solution was washed successively with 2% HCl, 2% NaOH, H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness to leave an amorphous residue. The residue was subjected to a preparative tlc with $CHCl_3$. The band of alkaloid at Rf 0.39 was collected and extracted with $CHCl_3$-MeOH (95:5). Removal of the solvent gave a pure alkaloid on tlc which, on treatment with MeOH, gave colorless needles, mp 272-273° (dec.) (58.6 mg) identical with an authentic sample of **1** in ir, nmr, ms, and co-tlc.

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