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STRUCTURE OF OXOSTEPHASUNOLINE, A NEW HASUBANALACTAM ALKALOID FROM *STEPHANIA JAPONICA*[1]

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ABSTRACT.—In a reinvestigation of the basic components of *Stephania japonica*, a new hasubanalactam alkaloid, named oxostephasunoline (**1**), was isolated from the roots. Its structure was established from consideration of spectral properties and by chemical conversion to 16-oxometaphanine (**5**) *via* 16-oxoprometaphanine (**4**). Oxostephasunoline (**1**), having a carbonyl group at C-16, is the sixth example of this type of compound isolated from natural sources.

Previously, twelve hasubanan alkaloids have been isolated and characterized from the stems, roots (1), leaves (2), and fruits (3) of *Stephania japonica* Miers (Menispermaceae), native to Japan. Recently, in the course of a reinvestigation of this plant collected in the southwest region of Japan, a new hasubanalactam,[2] named oxostephasunoline (**1**), was isolated from the roots.

This paper describes in detail the structure elucidation of the new alkaloid.

RESULTS AND DISCUSSION

The dried and chipped roots (6.5 kg) of *S. japonica* were extracted with MeOH, and after evaporation of the solvent, the residue was digested with dilute aqueous citric acid. The acid solution was extracted by shaking with $CHCl_3$ to obtain a $CHCl_3$-soluble fraction. This fraction was treated, as described in the experimental section, to yield a nonphenolic extract containing the new alkaloid. The extract was chromatographed on an alumina column and then on silica gel to give **1** (34 mg).

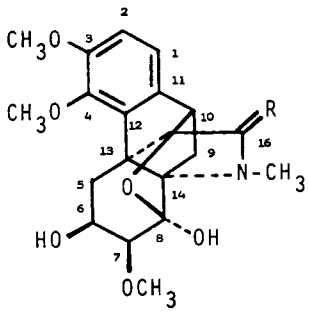
Oxostephasunoline (**1**) was obtained as colorless prisms, mp 217° (from Me_2CO), $C_{20}H_{25}O_7N$, $[\alpha]^{14}D$ +199.36°. Its ir spectrum depicted bands at 3550, 3500, and 1670 cm^{-1} indicating the presence of alcoholic hydroxyl groups and a carbonyl group, and the uv spectrum showed absorption maximum at 286 nm. The high resolution mass spectrum revealed a molecular ion peak at *m/z* 391.1658 (40.2%) with the most abundant peak at *m/z* 258.1109, and the fragmentation pattern was characteristic of the hasubanan group (4).

The pmr spectrum (Table 1) exhibited signals for two aromatic protons at δ 6.80 and 6.70 as doublets, three methoxyls at δ 3.92, 3.84, and 3.48, and one N-methyl group at δ 3.06. The downfield shift of the N-methyl signal suggested that **1** was a γ-lactam. The assignment was further supported by the band at 1670 cm^{-1} in the ir spectrum and by the base ion at *m/z* 258.1019 corresponding to the formula $C_{15}H_{16}O_3N$ in the mass spectrum. The hydroxyl groups observed in the ir spectrum were confirmed by the following pmr experiments. The signal, due to a tertiary alcoholic proton, showed at δ 4.20 as a singlet, and a secondary alcoholic proton was observed at δ 2.57 as a doublet. Each signal disappeared after treatment with D_2O. From these findings, the molecular formula $C_{20}H_{25}O_7N$ may rationally be represented as follows: $C_{15}H_{11}\cdot(OH)_2\cdot(OCH_3)_3\cdot(CONCH_3)\cdot(-O-)$.

Furthermore, the pmr spectrum of **1** exhibited a doublet attributable to C-10 proton at δ 4.94 (J=6.37 Hz), and a doublet at δ 1.66 (J=10.76 Hz) and a double doublet at δ3.19 (J=6.37, 10.76 Hz) were assigned to C-9 methylene protons by double resonance technique. However, no signal assignable to C-8 proton could be recognized.

[1]Part 280 in the series "Studies on Alkaloids of Menispermaceous Plants." Part 279: J. Kunitomo, M. Satoh, and T. Shingu, *Tetrahedron*, **39**, 3261 (1984).

[2]In this paper, the hasubanan skelton with a carbonyl group at C-16 is called "hasubanalactam."



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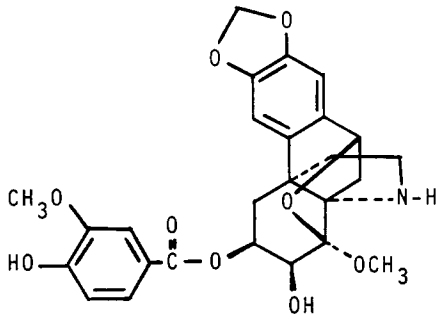

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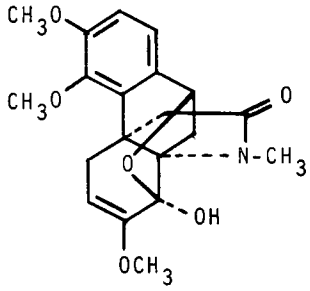

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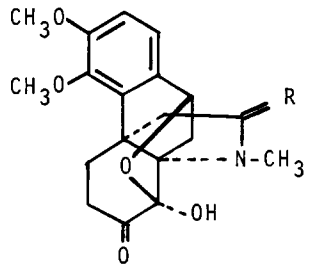

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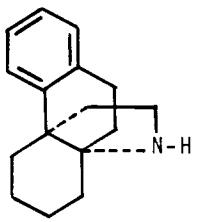

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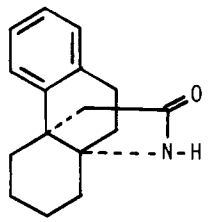

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It is possible, therefore, that an ether bridge links C-8 and C-10, as we reported for some hasubanan alkaloids (1). The cmr spectrum (Table 2) of **1** showed 20 carbon signals in the region of δ 27.8-174.08, and the spectral pattern was similar to that of hasubanalactam alkaloids closely related to stephasunoline (**2**) (1).

In 1970, Kupchan *et al.* reported the structure of stephavanine (**3**) (6) related to the new alkaloid (**1**). They unequivocally established the stereostructure of **3** on the basis of chemical and X-ray crystallographic evidence. The six-membered C-ring was found to be substantially flattened in comparison with an ideal cyclohexane ring. The conformation of the C-ring in **1**, therefore, was assumed to be analogous to that of **3**. In the pmr spectrum of **1**, the C-5 methylene protons exhibited signals at δ 2.83 (J=3.96, 14.73 Hz) and δ 1.79 (J=2.41, 14.73 Hz), each as double doublets. The former was due to an axial (α) proton and the latter to an equatorial (β), as we reported for **2** (1). These splittings, due to the vicinal C-6 proton, correspond to axial-equatorial and equatorial-equatorial interaction. On the other hand, a nOe enhancement (9.8%%) was observed between the downfield C-5 and the C-7 proton. From these findings, it was reasonably

TABLE 1. Pmr Spectral Assignments for Oxostephasunoline (**1**)

Proton	Chemical shift[a]	Multiplicity (*J* in Hz)
C-1	6.80	d (8.13)
C-2	6.70	d (8.13)
C-5α	1.79	dd (2.41, 14.73)
C-5β	2.83	dd (3.96, 14.73)
C-6	4.23-4.10	m
C-7	3.39	d (3.73)
C-9α	1.66	d (10.76)
C-9β	3.19	dd (6.37, 10.76)
C-10	4.94	d (6.37)
C-15α	3.05[b]	d (17.14)
C-15β	2.48[b]	d (17.14)
C-3 OCH_3	3.92[c]	s
C-4 OCH_3	3.84[c]	s
C-7 OCH_3	3.48	s
C-6 OH	2.57	d (10.33)
C-8 OH	4.20	s
NCH_3	3.06	s

[a]Measured in $CDCl_3$, chemical shifts in δ scale relative to internal TMS.
[b]Assignments may be interchangeable.
[c]Assignments may be interchangeable.

TABLE 2. Cmr Chemical Shifts for Oxostephasunoline (**1**)[a]

Carbon		Carbon	
1	120.01	11	132.74
2	110.58	12	132.53
3	153.79	13	49.90
4	148.29	14	72.17
5	33.30	15	44.92
6	64.63	16	174.08
7	75.26	C-3 OCH_3	55.70
8	100.51	C-4 OCH_3	60.52
9	37.90	C-7 OCH_3	56.73
10	79.81	NCH_3	27.81

[a]Measured in $CDCl_3$, values in δ scale relative to internal TMS.

assumed that the C-6 hydroxyl group should be an axial (β) and the C-7 methoxyl group be an equatorial (β).

Heating **1** in 1% ethanolic NaOH under mild conditions gave 16-oxoprometaphanine (**4**). This facile reaction by the basic reagent suggested that *trans*-elimination takes place involving simultaneous removal of the C-6 axial hydroxyl group and the C-7 axial hydrogen of **1** (7). Treatment of **4** with dilute HCl gave 16-oxometaphanine (**5**), whose spectral and physical properties were identical with authentic **5** derived from naturally occurring metaphanine (**6**) by permanganate oxidation. Thus, these reaction sequences chemically substantiated the structure of **1** and the relationship of **1, 2, 4, 5,** and **6**.

Based on the spectral and chemical results presented here, the structure of oxostephasunoline was established as drawn in the formula **1**.

The term hasubanan skelton was first proposed in 1964 for 2,3,4,5-tetrahydro-3a,9b-butano-1H-benz[*e*]indole (**7**) by Tomita *et al.* (8). Since that time, 32 congeners with the hasubanan skelton have been found in *Stephania* species (4). Among them were six alkaloids with a C-16 carbonyl group. Because these congeners exhibited a charac-

teristic property due to the γ-lactam system, we propose the designation "hasubanalactam" for the skelton (**8**).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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 $CHCl_3$. The pmr and cmr spectra were recorded in $CDCl_3$ on a JEOL JNM-FX 90Q with TMS as internal standard, and chemical shifts were reported in δ (ppm) units. Mass spectra were taken with JEOL JNM-D-100 mass spectrometer by direct inlet probe at 70 eV. Optical rotations were measured on a JASCO model DIP-14 polarimeter. Silica gel 60 (70-230 mesh) (E. Merck) and neutral alumina (activity I-II) (E. Merck) were used for a column chromatography. Tlc was performed by Aluminiumoxid 150 F_{254} neutral type T (layer thickness 0.2 mm) (E. Merck), and the spots on tlc were visualized under uv light and by spraying with Dragendorff's reagent.

PLANT MATERIAL.—The roots of *S. japonica* were collected in Bohnotsu-cho, Kagoshima-ken, Japan, in June, 1979, by Mr. K. Shukuri. A voucher specimen is deposited in the Herbarium of the Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan. The plant material was collected in the same season and from the same habitat as we previously reported for the roots and leaves (1,2).

EXTRACTION AND SEPARATION.—The dried and chipped roots (6.5 kg) were successively extracted with MeOH at 60°, and the solvent was removed under reduced pressure to yield 580 g of residue. The crude extract was digested with 5% aqueous citric acid, and the acid solution was extracted by shaking with $CHCl_3$. The $CHCl_3$ phase was washed with 2% aqueous NaOH to remove phenolic bases. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness to give a tertiary nonphenolic extract (13 g). The extract was chromatographed on an alumina column (350 g, 3.5×32 cm) in C_6H_6 and eluted successively with C_6H_6-$CHCl_3$ (1:1), and then $CHCl_3$. Fractions of 50 ml each were collected and examined by tlc. After removal of the solvent, the fraction eluted with C_6H_6-$CHCl_3$ (1:1) (450 ml) containing the new alkaloid, was subjected to rechromatography on silica gel (50 g, 2.0×22 cm). Elution with $CHCl_3$-MeOH (9:1) (200 ml) furnished a light-brown solid. The solid was further purified by preparative tlc on silica gel in CH_2Cl_2-MeOH (9:1), and recrystallization of the crude alkaloid from Me_2CO gave **1** (34 mg).

OXOSTEPHASUNOLINE (**1**).—Colorless prisms, mp 217° (from Me_2CO), $[\alpha]^{14}D$ +199.36° (*c* 0.84, MeOH), analysis: found, C, 61.29; H, 6.36; N, 3.58; cald for $C_{20}H_{25}O_7N$: C, 61.37; H, 6.44; N, 3.58. Uv λ max (ε) (MeOH) 286 (2100) nm; ir ν max ($CHCl_3$) 3550, 3500, 1670 cm^{-1}. Ms *m/z* (rel. int.) 391.1658 (40.2, M^+), 259.1173 (54.3), 258.1109 (100), 257.1019 (44.0), 243.0852 (13.4), 242.0792 (33.2), 227.0933 (36.4); pmr: Table 1; cmr: Table 2. Yield 34 mg.

CONVERSION OF OXOSTEPHASUNOLINE (**1**) TO 16-OXOPROMETAPHANINE (**4**).—A solution of **1** (28 mg) in 1% ethanolic NaOH (5 ml) was refluxed for 10 min and the solvent evaporated to dryness. After addition of H_2O (5 ml) to the residue, the aqueous mixture was extracted with $CHCl_3$, and the $CHCl_3$ solution was washed with H_2O and dried over anhydrous Na_2SO_4. Removal of the solvent gave a colorless residue. The residue was chromatographed on an alumina column (20 g, 1×6 cm) in $CHCl_3$. Elution with the same solvent furnished an amorphous solid which on crystallization from MeOH gave colorless prisms (17 mg), mp 195°. The product was identical (mmp, tlc, pmr) with an authentic sample of naturally occurring **4**.

CONVERSION OF 16-OXOPROMETAPHANINE (**4**) TO 16-OXOMETAPHANINE (**5**).—To a solution of **4** (15 mg) in MeOH (3 ml) was added 1 N HCl (0.5 ml), and the mixture was warmed for 5 min at 60°. After removal of the solvent, the residue was dissolved in $CHCl_3$, and the solution was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was submitted to preparative tlc on alumina with $CHCl_3$, and a band of Rf 0.22 was treated by the usual work-up. Crystallization from EtOH gave colorless prisms (8 mg), mp 194°, which were identical (mmp, tlc, ir) with authentic 16-oxometaphanine (**5**) prepared from metaphanine (**6**) (7).

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