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ALKALOIDS FROM THE FRUITS OF *STEPHANIA JAPONICA*, 3.[1] STRUCTURES OF PROSTEPHANABERRINE AND STEPHANABERRINE, TWO NEW HASUBANAN ALKALOIDS

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ABSTRACT.—Prostephanaberrine (**1**), a new hasubanan alkaloid, was isolated from the fresh fruits of *Stephania japonica*. The new alkaloid and its acetate **2** were converted into the metaphanine-type base stephanaberrine (**3**). The reaction sequence and spectral data showed that the structures of **1** and **3** are related to prometaphanine (**7**) and metaphanine (**10**).

In the previous papers of this series (1,2), we reported the structures of three new hasubanan alkaloids, stephabenine (**4**), oxostephabenine (**5**), and *N,O*-dimethyloxostephine (**6**), together with two unidentified alkaloids tentatively named Base-A and Base-B. The unidentified alkaloids were isolated from a petroleum ether extract and we briefly reported their physical and spectral data (2). In a continuing search for this plant material, Base-B, isolated also from a methanolic extract, now named prostephanaberrine (**1**), was shown to be a new hasubanan alkaloid.

In the present work, we report the isolation and characterization of the new alkaloid prostephanaberrine (**1**) and its derivative, stephanaberrine (**3**).

RESULTS AND DISCUSSION

The methanolic extract prepared from the defatted fresh fruits of *Stephania japonica* Miers (Menispermaceae) was fractionated into a nonphenolic tertiary alkaloid fraction, which, after chromatography on two silica gel columns, yielded the new alkaloid **1**.

Prostephanaberrine (**1**) crystallized from MeOH as light yellow prisms, mp 225° (dec.), $C_{19}H_{21}NO_5$, $[\alpha]_D$ −219.1°. Its uv spectrum showed an absorption maximum at 273.5 nm, and ir bands at 3580, 1670, and 1640 cm^{-1}, indicating the presence of a hydroxyl and α,β-unsaturated ketone. The ^{1}H-nmr and ^{13}C-nmr data are summarized in Tables 1 and 2. The ^{1}H-nmr spectrum indicated the presence of a methylenedioxy, a methoxyl, an *N*-methyl, an olefinic proton, and two aromatic protons. The hrms revealed a molecular ion at *m/z* 343, and the most abundant ion characteristic of a C-ring enone hasubanan alkaloid appeared at *m/z* 257 ($C_{15}H_{13}O_4$) (3). This ion is presumably derived by rupture of the ethanamine linkage followed by loss of a CO unit from the methylenedioxy group. Further, the presence of a nitrogen-containing ion at *m/z* 245 ($C_{14}H_{15}NO_3$) (**a**) suggested the substitution pattern in A- and B-ring. It was, therefore, assumed that the C- ring of **1** possesses a conjugated ketone system bearing an enolic methyl ether and an olefinic proton adjacent to methylene protons. The splittings of the aromatic protons suggested that the methylenedioxy group should be located in C-2 and C-3. Upon irradiation of the aromatic C-1 proton (δ 6.73), the double of doublet signal (δ 4.70) exhibited an nOe enhancement (12.5%) indicating the presence of a proton on the hydroxyl bearing C-10. The structure of **1** is, therefore, of the hasubanan type and is related to prometaphanine (**7**) (4) and prostephabyssine (**8**) (5) except for the methylenedioxy group. The structures of **7**, **8**, and 16-oxoprometaphanine (**9**) (6) have been reported as existing in a solvent-dependent equilibrium mixture of an α,β-unsaturated ketone and a hemiketal, but the new alkaloid **1** was found to be in the ketone form alone.

[1]For part 2 in this series, see Y. Yamamura and M. Matsui (1).

TABLE 1. ^{1}H-nmr Data of Prostephanaberrine (**1**)[a]

Proton No.	Chemical shift[b] δ ppm, *J*(Hz)
1	6.731 (1H, s)
4	7.027 (1H, s)
5α	2.612 (1H, dd, 3.31, 14.84)
5β	2.804 (1H, dd, 6.42, 14.84)
6	5.682 (1H, dd, 3.31, 6.42)
9α	1.812 (1H, dd, 7.82, 13.37)
9β	2.386 (1H, dd, 5.42, 13.37)
10	4.703 (1H, dd, 5.42, 7.82)[c]
15	2.144, 2.253 (2H, m)
16	2.531, 2.738 (2H, m)
$-OCH_2O-$	5.914 (1H, d, 1.47)
	5.926 (1H, d, 1.47)
OCH_3	3.609 (3H, s)
NCH_3	2.372 (3H, s)

[a]Spectrum was measured in $CDCl_3$ at 400 MHz with TMS as an internal standard.

[b]Chemical shifts were sustantiated by homonuclear 2-D measurements (i.e., COSY and 2-D*J*).

[c]After exchange with D_2O.

Further confirmation of the structure of **1** could be achieved by the following chemical experiments. Treatment of **1** with Ac_2O and pyridine gave 10-*O*-acetylprostephanaberrine (**2**), mp 169.5°, $C_{21}H_{23}NO_6$, crystallized from MeOH as light yellow prisms. Its ir spectrum showed bands at 1730, 1675, and 1638 cm^{-1}; no hydroxyl band was present. The ^{1}H-nmr spectrum revealed the presence of an acetyl methyl signal at δ 2.07. The hrms exhibited a molecular ion peak at *m/z* 385 with the most abundant ion (**b**) at *m/z* 227 ($C_{14}H_{13}NO_2$).

TABLE 2. ^{13}C-nmr Chemical Shifts of Prostephanaberrine (**1**)[a]

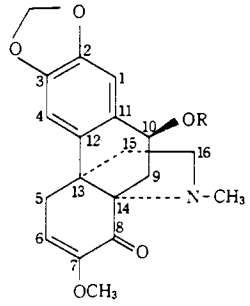
Carbon No.	Chemical shift[b] (δ ppm)	Carbon No.	Chemical shift[b] (δ ppm)
1	106.21	11	137.26[d]
2	147.38[c]	12	131.25[d]
3	146.10[c]	13	49.37
4	106.36	14	69.67
5	34.70	15	36.35
6	112.23	16	51.19
7	151.49	$-OCH_3O-$	100.95
8	195.77	C-7 OCH_3	55.11
9	32.04	NCH_4	35.75
10	66.11		

[a]Spectrum was measured in $CDCl_3$ at 100.4 MHz with TMS as an internal standard.

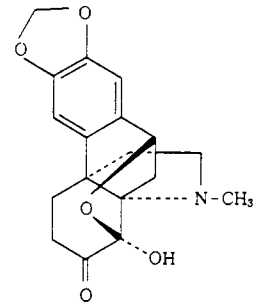
[b]Chemical shifts were substantiated by ^{1}H-^{13}C heteronuclear 2-D measurement in combination with INEPT experiment.

[c,d]Assignment may be interchangeable.

On treatment with aqueous HCl under mild conditions, **2** was transformed into stephanaberrine (**3**), which could also be prepared from **1** by the same manner. Stephanaberrine (**3**) was obtained as colorless prisms from MeOH, mp 205° (dec.),



1 R=H
2 R=Ac



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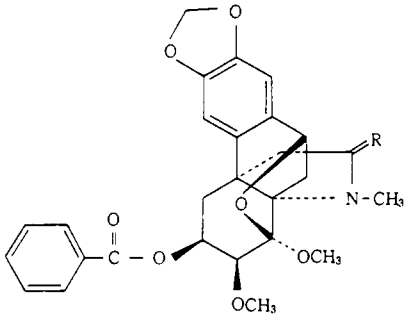
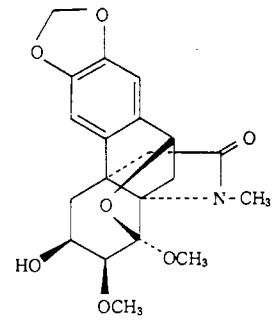


4 R=H_2
5 R=O



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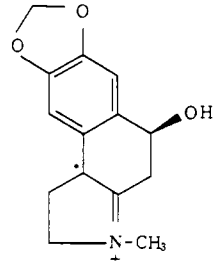
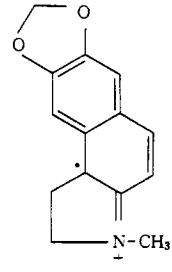
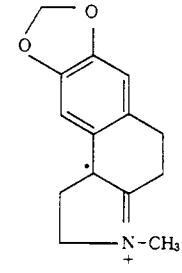


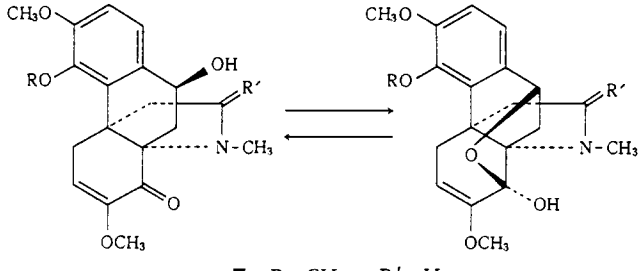
a



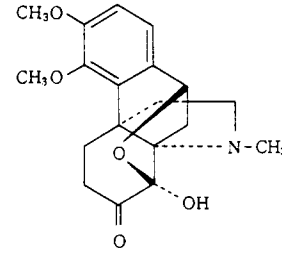
b



c



7 R=CH_3, R′=H_2
8 R=H, R′=H_2
9 R=CH_3, R′=O



10

$C_{18}H_{19}NO_5$, $[\alpha]D$ −47.5°. The uv spectrum showed absorption maxima at 293 and 240.5 nm, and the ir spectrum displayed bands at 3460 and 1725 cm^{-1} corresponding to a hydroxyl and a six-membered ketone. The ^{1}H-nmr spectrum indicated the presence of a methylenedioxy, an *N*-methyl, and a C-10 proton as a doublet (J=6.16 Hz).

No signals due to methoxyl and olefinic protons were observable. The hrms revealed the most abundant ion characteristic of the metaphanine-type hasubanan alkaloid (8) at *m/z* 229 (**c**) ($C_{14}H_{15}NO_2$), and no fragment ion characteristic of the C-ring enone hasubanan alkaloid was observable. The reaction sequence and spectral data demonstrated that the C-7 enolic methyl ether was hydrolyzed to a ketonic function, and the C-10 hydroxyl and C-8 ketonic carbonyl group were converted into a hemiketal linkage between C-8 and C-10 to yield the metaphanine-type alkaloid **3**.

Upon direct comparison of **3** and metaphanine (**10**) (7), the close resemblance of them was indicated by the similarity of ^{1}H-nmr, hrms, and optical property. Thus, the structure of **3** was assigned to stephanaberrine.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Mps were determined on a YANACO micro melting point apparatus and are uncorrected. Uv spectra were obtained on a JASCO UVIDEC-500 spectrophotometer and ir spectra were taken on a JASCO A-120 spectrophotometer in $CHCl_3$ solution. ^{1}H-nmr and ^{13}C-nmr spectra were measured in $CDCl_3$ on JEOL JNM-FX 90Q and on JEOL JNM-GX 400 spectrometers with TMS as an internal standard, and chemical shifts are given in δ unit. The abbreviations s, d, dd, and t in the spectra signify singlet, doublet, double doublet, and triplet. Hrms were recorded on a JEOL JMS-300 mass spectrometer at 70 eV using a direct inlet probe. Optical rotations were measured on a JASCO DIP-400 polarimeter. Silica gel 60 (70-230 mesh, E. Merck) was used for column chromatography. Tlc was performed on pre-coated silica gel plates (E. Merck, type 5717), and the spots were visualized under uv light and by spraying with Dragendorff's reagent.

PLANT MATERIAL.—The fruits of *S. japonica* were collected by Mrs. A. Fukuda in Hondo city, Kumamoto, Japan, during the autumns of 1981-1983. A voucher specimen is deposited in the Herbarium of the Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan.

EXTRACTION AND ISOLATION OF PROSTEPHANABERRINE (**1**).—The fresh fruits (19 kg) defatted with petroleum ether[2] were successively extracted with MeOH (total of 72 liter) at 60°, and the solvent was removed under reduced pressure to yield a MeOH extract (1.5 kg). The extract was digested with 5% aqueous citric acid at 60°, and the acid solution was made alkaline with NH_4OH. The ammoniacal solution was extracted by shaking with $CHCl_3$ to separate a tertiary alkaloid fraction. The $CHCl_3$ solution, after extraction with 2% aqueous NaOH, was evaporated to dryness to leave a nonphenolic alkaloid extract (17.1 g). The extract was dissolved in $CHCl_3$ and subjected to chromatography on silica gel column (220 g, 4.6×29 cm) with $CHCl_3$, $CHCl_3$-MeOH (95:5, 9:1, 8:2, 1:1), and MeOH collecting each of 100-ml fractions. The fractions eluted from $CHCl_3$-MeOH (95:5), after evaporation of the solvent, were chromatographed on silica gel column (70 g, 3×26.5 cm) with C_6H_6/$CHCl_3$ followed by $CHCl_3$. The eluates (400 ml) from $CHCl_3$ were combined, and the solvent was evaporated to dryness to give an amorphous solid, which was crystallized from MeOH to give **1** (105.2 mg).

PROSTEPHANABERRINE (**1**).—Light yellow prisms, mp 225° (dec.), $[\alpha]^{15}D$ −219.1° (*c* 0.25, $CHCl_3$); uv λmax EtOH (log ε) 273.5 (3.86) nm, ir νmax 3580, 1670, 1640, 1505, 1485, cm^{-1}; ms *m/z* 343 (M^+, 343.1430, $C_{19}H_{21}NO_5$, 16.3%), 257 (257.0794, $C_{15}H_{13}O_4$, 100%), 245 (245.1033, $C_{14}H_{15}NO_3$, 51.4%), 227 (227.0949, $C_{14}H_{13}NO_2$, 18.5%). ^{1}H-nmr and ^{13}C-nmr data, see Tables 1 and 2. Yield 105.2 mg.

ACETYLATION OF **1** TO 10-*O*-ACETYLPROSTEPHANABERRINE (**2**)..—The mixture of **1** (78 mg), Ac_2O (1.5 ml), and pyridine (1.5 ml) was allowed to stand for 40 h at room temperature, and the reaction mixture was evaporated to dryness to yield a brownish oil. The oil dissolved in $CHCl_3$ (20 ml) was washed twice with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness to leave a residue, which was crystallized from MeOH to give **2** (63.6 mg).

10-*O*-ACETYLPROSTEPHANABERRINE (**2**).— Light yellow prisms, mp 169.5° from MeOH; ir νmax 1730, 1675, 1638, 1505, cm^{-1}; ms *m/z* 385 (M^+, 385.1540, $C_{21}H_{23}NO_6$, 35.2%), 257 (257.0803, $C_{15}H_{13}O_4$, 36.1%), 228 (228.1004, $C_{14}H_{14}NO_2$, 21.8%), 227 (227.0938, $C_{14}H_{13}NO_2$, 100%); ^{1}H-nmr 6.79 (1H, s, C-4H, 6.66 (1H, s, C-1H), 5.94 (2H, s, -OCH_2O-), 3.59 (3H, s, C-7 OCH_3), 5.64 (1H, t, C-10H), 2.58 (3H, s, NCH_3), 2.07 (3H, s, OAc); Rf 0.76 ($CHCl_3$). Yield 63.6 mg.

CONVERSION OF **2** TO STEPHANABERRINE (**3**).—To a MeOH solution (3 ml) of **2** (61.8 mg) was

[2]The extract prepared from petroleum ether solution gave 15 mg of **1**. Kondo *et al.* (2).

added 1 N HCl (0.5 ml), and the mixture was allowed to stand for 18 h at room temperature. The mixture was evaporated to dryness to give an amorphous solid that was suspended in 2% NH_4OH, and the suspension was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness to give a light yellow amorphous solid. The solid was crystallized from MeOH to give **3** (51.9 mg).

CONVERSION OF **1** TO **3**.—A MeOH solution of **1** (23 mg) was treated by the same manner as described above. The resulting product was identical with **3** derived from **2** by direct comparison of mmp, ir, hrms, and co-tlc.

STEPHANABERRINE (**3**).—Colorless prisms, mp 205° (dec.) from MeOH, $[\alpha]^{23}D$ −47.5° (*c* 0.78, $CHCl_3$); uv λmax EtOH (log ε) 293 (3.71, 240.5 (3.52) nm; ir νmax 3460, 1725 cm^{-1}; ms *m/z* 329 (M^+, 329.1309, $C_{18}H_{19}NO_5$, 17.1%), 229 (229.1103, $C_{14}H_{15}NO_2$, 100%), 228 (228.1027, $C_{14}H_{14}NO_2$, 91.5%), 213 (213.0785, $C_{13}H_{11}NO_2$, 2.5%); ^1H nmr 6.72 (1H, s, C-4H), 6.64 (1H, s, C-1H), 5.95 (2H, s, $-OCH_2O-$), 5.10 (1H, s, C-8H), 4.98 (1H, d, J=6.16 Hz, C-10H), 2.75 (1H, dd, J=6.16, 10.88, C-9H β), 2.53 (3H, s, NCH_3), 1.68 (1H, d, J=10.88, C-9H α).

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