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Constitution of Four New Hasubanan Alkaloids from *Stephania japonica* Miers[1]

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Four new hasubanan alkaloids, stephamiersine (**1**), epistephamiersine (**2**), oxostephamiersine (**3**) and stephasunoline (**4**) were isolated together with seven known and three unidentified alkaloids from *Stephania japonica* Miers (Menispermaceae). Among these new alkaloids, **1** and **2** were found to be epimeric isomers with respect to the C-7 methoxyl group. Permanganate oxidation of **1** gave **3**, and borohydride reduction of **1** and **2** gave the highly stereoselective products, dihydrostephamiersine (**6**) and dihydroepistephamiersine (**5**). On mild treatment of **5** with hydrochloric acid gave **4**. Acetolyses of **1**, **2**, **5** and **6** gave the phenanthrene derivatives, **7**, **8** and **9**, respectively. Further, both **1** and **2** were converted to the conjugated carbonyl compound (**14**) which was obtained from dihydro-16-oxohasubanonine (**17a**) (**17b**). On the other hand, the stereochemistry of **1**, **2**, **3** and **4** was elucidated by nuclear magnetic resonance (NMR) spectroscopic studies as follows: the C-7 methoxyl group of **1** has the α-axial and that of **2** and **4** has the β-equatorial configuration, and the hydroxyl group of **4** has the β-axial one. On the basis of the above chemical correlation coupled with the spectral arguments, the constitution of the new alkaloids was represented as drawn in the formulas, **1**, **2**, **3** and **4**.

In the course of an investigation on the non-quaternary bases of *Stephania japonica* Miers (Menispermaceae) collected in Kagoshima Prefecture, we have recently isolated four new hasubanan alkaloids, named stephamiersine (**1**), epistephamiersine (**2**), oxostephamiersine (**3**) and stephasunoline (**4**) together with seven known and three unidentified bases. The present paper deals with the elucidation of structure and absolute stereochemistry of the new alkaloids.

The methanolic extract prepared from the stems and rhizomes of *Stephania japonica* Miers, as described in the experimental section, was digested with dilute aqueous citric acid, then the acid solution was shaken with chloroform to separate the chloroform layer ("weak base fraction") and the acid layer ("strong base fraction"). Each fraction was worked up by the usual method and the "weak base fraction" gave stephamiersine (**1**), epistephamiersine (**2**), oxostephamiersine (**3**), metaphanine,[3] stephanine[4] and unidentified bases, tentatively named base-X, base-S and base-P (non-phenolic), and hypoepistephanine[5] (phenolic). The

1) Preliminary communication of this work appeared in *Tetrahedron Letters*, **1973**, 4263. This paper forms Part CCLXIII of the series "Studies on the Alkaloids of Menispermaceous Plants." Part CCLXII: J. Kunitomo, Y. Hasegawa, Y. Imori and E. Yuge, *Yakugaku Zasshi*, **92**, 1496 (1972).

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3) *a*) H. Kondo and T. Sanada, *Yakugaku Zasshi*, **45**, 5, 1039 (1924); *idem*, *ibid.*, **48**, 177, 930 (1927); H. Kondo and T. Watanabe, *ibid.*, **58**, 268 (1938); *b*) M. Tomita, T. Ibuka, Y. Inubushi and K. Takeda, *Tetrahedron Letters*, **1964**, 3605; *idem*, *Chem. Pharm. Bull.* (Tokyo), **13**, 695, 704 (1965).

4) M. Tomita and H. Shirai, *Yakugaku Zasshi*, **62**, 381 (1942); H. Shirai, *ibid.*, **64B**, 208 (1944); H. Shirai and N. Oda, *ibid.*, **76**, 1287 (1956).

5) H. Kondo and T. Nozoe, *Yakugaku Zasshi*, **63**, 333 (1943); M. Tomita and E. Fujita, *Chem. Pharm. Bull.* (Tokyo), **2**, 378 (1954).

6) H. Kondo and T. Sanada, *Yakugaku Zasshi*, **514**, 1039 (1924); *idem*, *ibid.*, **541**, 177 (1927); *idem*, *ibid.*, **549**, 930 (1927); H. Kondo and K. Tanaka, *ibid.*, **63**, 267, 273 (1943); K. Tanaka, *ibid.*, **64**, 28 (1944).

"strong base fraction" gave stephasunoline (**4**), epistephanine,[6] protostephanine[7] and stebisimine[8] (non-phenolic), hypoepistephanine and stepinonine[9] (phenolic). The phenolic base, hypoepistephanine was the only base obtainable from the both fractions. Physical constants and ultraviolet (UV), infrared (IR) and mass spectral data of the new bases, **1**, **2**, **3** and **4** thus obtained are shown in Table I, and nuclear magnetic resonance (NMR) spectral data are given in Table II.

Table I. Physical Constants and UV, IR and Mass Spectral Data of Stephamiersine (**1**), Epistephamiersine (**2**), Oxostephamiersine (**3**) and Stephasunoline (**4**)

Comp. No.	mp (°C) (uncorr.)	$[\alpha]_D$ ($CHCl_3$)	UV λ_{max}^{EtOH} nm (ε)	IR $\nu_{max}^{CHCl_3}$ cm^{-1}	Mass Spectra (*m/e*) M^+ and base peak
1	165	+33.0	286(2200)	1725	389, 243
2	98	+64.1	286(2300)	1735	389, 243
3	290, 256[a]	+88.3	286(2000)	1730, 1680	403, 257
4	233	+121.4	286(2000)	3550	377, 245

a) bimorphism

Table II. NMR Spectral Data[a] of Stephamiersine (**1**), Epistephamiersine (**2**), Oxostephamiersine (**3**), and Stephasunoline (**4**)

Compd. No.	Aromatic H	C(7)-H	C(10)-H	OCH_3	NCH_3	Other signals
1	6.67(2H, s)	3.52(1H, d) (J =1.5 Hz)	4.72(1H, d) (J =6.5 Hz)	3.92(3H, s) 3.82(3H, s) 3.34(3H, s) 3.31(3H, s)	2.64(3H, s)	
2	6.66(2H, s)	4.27(1H, s)	4.82(1H, d) (J =6.5 Hz)	3.89(3H, s) 3.76(3H, s) 3.52(3H, s) 3.45(3H, s)	2.63(3H, s)	
3	6.77(2H, s)	3.63(1H, s)	4.79(1H, d) (J =6.5 Hz)	3.92(3H, s) 3.83(3H, s) 3.33(3H, s) 3.29(3H, s)	3.12(3H, s)	
4	6.67(2H, s)	3.62(1H, d) (J =3.9 Hz)	4.88(1H, d) (J =6.2 Hz)	3.90(3H, s) 3.82(3H, s) 3.46(3H, s)	2.57(3H, s)	4.25(1H, m, C(6)-H) 4.03(1H, s, C(8)-OH) 2.43(1H, d, C(6)-OH) (J =10.5 Hz)

a) All values are in δ units for $CDCl_3$ solution at 60 MHz with TMS as an internal standard. abbreviation: s=singlet, d=doublet, m=multiplet

Among the new alkaloids, **1** and **2** were found to be represented by the same formula ($C_{21}H_{27}O_6N$), and each of the IR spectra showed absorption band assignable to six-membered ketone. The above spectral data (Table I and II) suggested that **1** and **2** were closely related compounds, and this was further supported by close resemblance of fragmentation in the mass spectra. Thus, the rational formula for **1** and **2** was given as follows; $C_{15}H_{12}\cdot(C{=}O)\cdot(OCH_3)_4\cdot(NCH_3)\cdot(-O-)$.

The IR spectrum of **3** showed bands at 1730 cm^{-1} (six-membered ketone) and 1680 cm^{-1} (γ-lactam). Its NMR spectrum exhibited a signal assignable to N–CH_3 at lower field (δ 3.12)

7) H. Kondo, T. Nakamura, M. Fujii and T. Kato, *Itsuu Kenkyusho Nempo*, **1**, 5 (1950); H. Kondo and T. Watanabe, *ibid.*, **1**, 12 (1950); H. Kondo and K. Tanaka, *ibid.*, **4**, 6 (1953); **5**, 1 (1954); **6**, 4 (1955); **7**, 30 (1956); K. Tanaka, *ibid.*, **9**, 24 (1958); H. Kondo and K. Tanaka, *ibid.*, **9**, 33 (1958).
8) D.H.R. Barton, G.W. Kirby and A. Wiechers, *J. Chem. Soc.* (*C*), **1966**, 2313.
9) T. Ibuka, T. Konoshima and Y. Inubushi, *Tetrahedron Letters*, **1972**, 4001.

than those of **1** and **2**. From these findings, **3** should be a γ-lactam, and the rational formula, $C_{14}H_{10}\cdot(C{=}O)\cdot(OCH_3)_4\cdot(CONCH_3)\cdot(-O-)$ was given for **3**.

In contrast with **1**, **2** and **3**, the IR spectrum of **4** showed no carbonyl bands, but a sharp absorption band of hydroxyl groups at 3550 cm^{-1}. Its NMR spectrum showed a singlet at δ 4.03 due to a tertiary alcoholic proton and a doublet at δ 2.43 (J=10.5 Hz) ascribable to secondary alcoholic proton. These signals were disappeared by treatment with deuterium oxide. Therefore, the rational formula, $C_{16}H_{13}\cdot(OH)_2\cdot(OCH_3)_3\cdot(NCH_3)\cdot(-O-)$ was presented for **4**.

Mass spectral fragmentation patterns (Table III) of these bases (**1**, **2**, **3** and **4**) suggested that they were of hasubanan type[10] and most likely those bearing ketal or hemiketal moiety. Their base ion peaks well corresponded to the fragmentation which arose from loss of C ring from molecular ion.[10] Their closely related character was also discernible from the fragmentations.

TABLE III. Element-Table[a] of Stephamiersine (1), Epistephamiersine (2), Oxostephamiersine (3) and Stephasunoline (4)

Compd. No.	Relative intensity	*m/e* (obs.)	Error (millimass unit)	Elements
1	5	389.1798	−3.8	C21 H27 N1 O6
	48	244.1330	−0.6	C15 H18 N1 O6
	100	243.1276	1.7	C15 H17 N1 O2
	8	229.1078	−2.3	C14 H15 N1 O2
	48	213.1123	−2.9	C14 H15 N1 O1
2	4	389.1861	2.4	C21 H27 N1 O6
	44	244.1309	−1.8	C15 H18 N1 O2
	100	243.1239	0.8	C15 H17 N1 O2
	6	229.1057	−4.4	C14 H15 N1 O2
	35	213.1140	−1.2	C14 H15 N1 O1
3	16	403.1627	−0.1	C21 H25 N1 O7
	39	258.1103	−2.6	C15 H16 N1 O3
	100	257.1039	−1.1	C15 H15 N1 O3
	66	242.0816	0.0	C14 H12 N1 O3
	23	227.0915	−2.9	C14 H13 N1 O2
4	13	377.1842	0.5	C20 H27 N1 O6
	100	245.1423	0.8	C15 H19 N1 O2
	20	244.1329	−0.7	C15 H18 N1 O2
	16	230.1166	−1.4	C14 H16 N1 O2
	24	213.1125	−2.7	C14 H15 N1 O1

a) obtained on a Hitachi Datalyzer Hi-Resol. mass system; ion voltage 70 eV, temp. 130°

Heating either **1** or **2** in 1% methanolic sodium hydroxide under mild condition, gave the equilibrium mixture consisted of **1** and **2** in about 1: 3 ratio, though they remained unchanged in absence of the alkali. Therefore, **1** and **2** must be epimeric with respect to the position adjacent to ketonic function, and **2** was assumed to be the thermodynamically stable isomer. Oxidation of **1** with permanganate gave a lactam identical with **3** in every respect. Thus, **3** was proved to be a γ-lactam derivable from **1**.

Reduction of **2** with sodium borohydride gave a sole product, dihydroepistephamiersine (**5**),[11] mp 63°, mp 133° (bimorphism), $C_{21}H_{29}O_6N$, *m/e* 391 (M^+), $[\alpha]_D^{20}$ +75.4° ($CHCl_3$). Its

10) M. Tomita, A. Kato and T. Ibuka, *Tetrahedron Letters*, **1965**, 1019; *idem*, *Shitsuryo Bunseki*, **13**, 115 (1965).

11) According to Dr. A.J. van Wyk, University of Pretoria, this compound was isolated from *Stephania abyssinica* as a natural product and identified with authors' sample (private communication, dated May 28, 1974).

IR spectrum showed a hydroxyl band at 3520 cm^{-1}, and the NMR spectrum exhibited a doublet at δ 2.06 (J=9.5 Hz), which disappeared by treatment with deuterium oxide, due to a secondary alcoholic proton and a multiplet centered at δ 4.11 assignable to a proton on the hydroxyl-bearing carbon. The highly stereoselective reduction of **2** into **5** would involve the following process; the ketonic function of **2** would be attacked by hydride anion from the back-side of ketal ether bridge to give the product, **5**. The configuration of this hydroxyl group will be discussed later. Similar hydride delivery has been observed in the course of the reduction of ketonic function in the ketal and hemiketal hasubanan alkaloids.[12]

On treatment with hydrochloric acid in methanol under mild condition **5** was transformed to **4**, whose physical and spectral data were fully identical with an authentic sample. These reaction sequence revealed that **4** was a hemiketal alkaloid derivable from **2** through **5**. Thus the relationship of **1**, **2**, **3** and **4** was chemically substantiated.

By analogy with the above reduction of **2**, treatment of **1** with borohydride provided a sole product, dihydrostephamiersine (**6**), mp 143°, $C_{21}H_{29}O_6N$, $[\alpha]_D^{20}$ +102.2° ($CHCl_3$). Its IR spectrum showed a hydroxyl band at 3520 cm^{-1}, and NMR spectrum exhibited the signals of hydroxyl proton at δ 1.91 as doublet (J=11.5 Hz) which disappeared on deuterium oxide treatment and multiplet signals centered at δ 3.91 due to a proton on the hydroxyl-bearing carbon.

Acetolysis[3b] of **1** with acetic anhydride in a sealed tube gave a neutral compound (**7**), mp 75°, $C_{21}H_{20}O_7$, and the same treatment of **2** provided another neutral compound (**8**), mp 167°, $C_{22}H_{20}O_8$. The UV spectra (Fig. 1) of **7** and **8** showed the characteristic absorption curve of the phenanthrene type chromophore, and IR spectra (Table IV) showed acetate

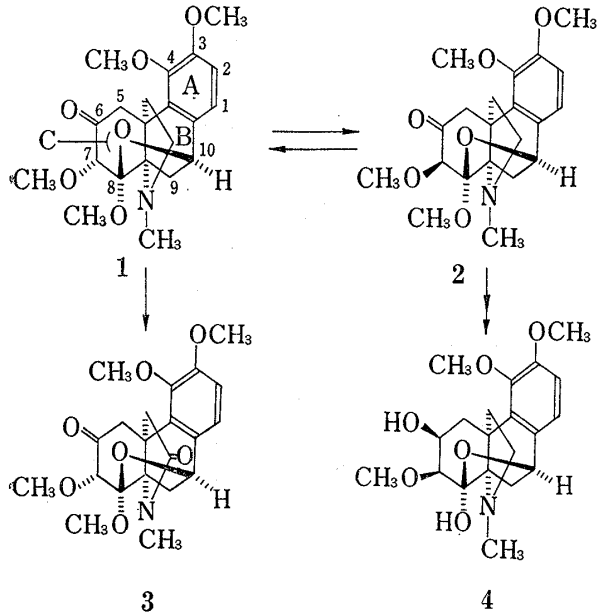


Chart 1

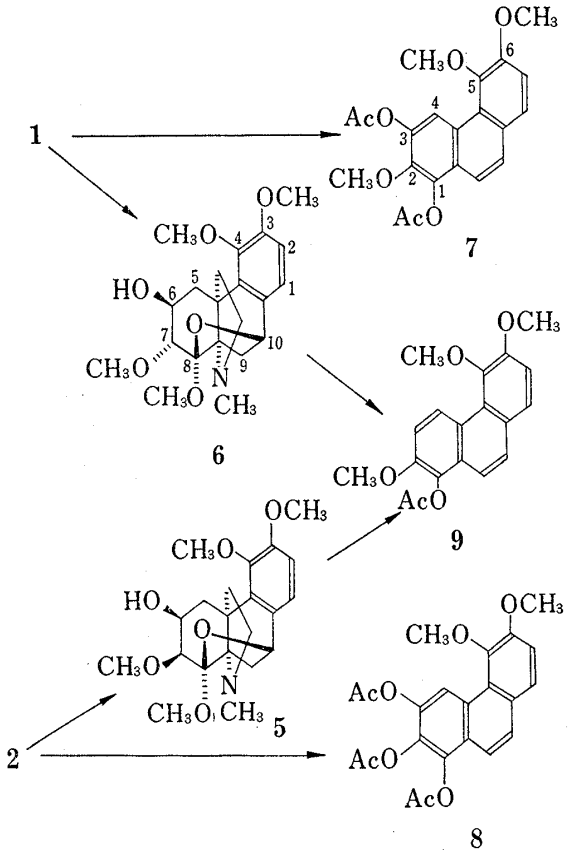


Chart 2

12) S.M. Kupchan and M.I. Suffness, *Tetrahedron Letters*, **1970**, 4978; S.M. Kupchan, M.I. Suffness, R.J. McClure and G.A. Sim, *J. Am. Chem. Soc.*, **92**, 5756 (1970); S.M. Kupchan, A.J. Liepa and T. Fujita, *J. Org. Chem.*, **38**, 151 (1973).

carbonyl bands. Acetolysis of either **5** or **6** afforded 1-acetoxy-2,5,6-trimethoxyphenanthrene (**9**), mp 139°, which was fully identified with an authentic sample.[13]

The NMR spectra (Table IV) revealed the presence of C-4 proton in the phenanthrenes (**7**, **8** and **9**) by signals for one proton intensity at relatively low field as singlets for **7** and **8**, or a doublet for **9**, indicating the substitution patterns, respectively. From the unambiguous structure of **9** coupled with the NMR data and from the interrelation of the starting materials, the structures of **7** and **8** were most reasonably assigned as 1,3-diacetoxy-2,5,6-trimethoxyphenanthrene (**7**) and 1,2,3-triacetoxy-5,6-dimethoxyphenanthrene (**8**), respectively. The assignment was further supported by the well known fact that on acetolysis of morphine-hasubanan series, an oxygen atom of a carbonyl group remains as acetoxyl group on the derived

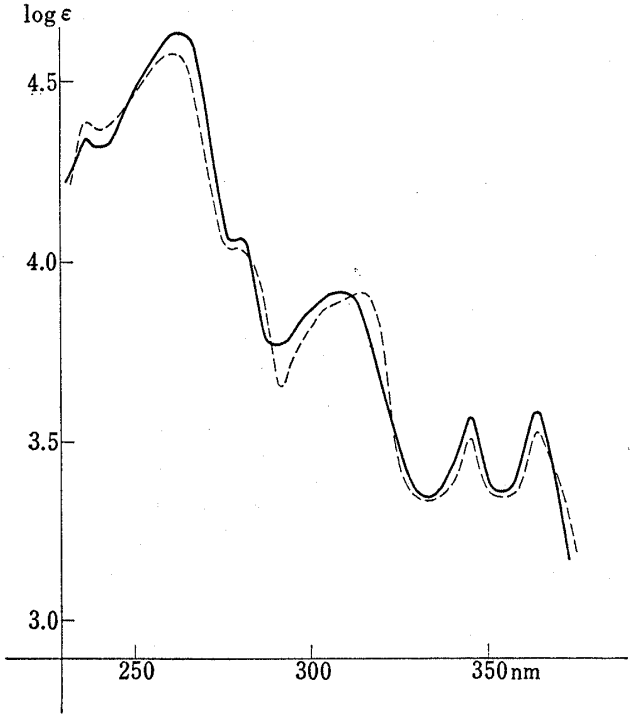


Fig. 1. Ultraviolet Absorption Spectra (in EtOH)

——— : 1,3-diacetoxy-2,5,6-trimethoxyphenanthrene (**7**)
--------- : 1,2,3-triacetoxy-5,6-dimethoxyphenanthrene (**8**)

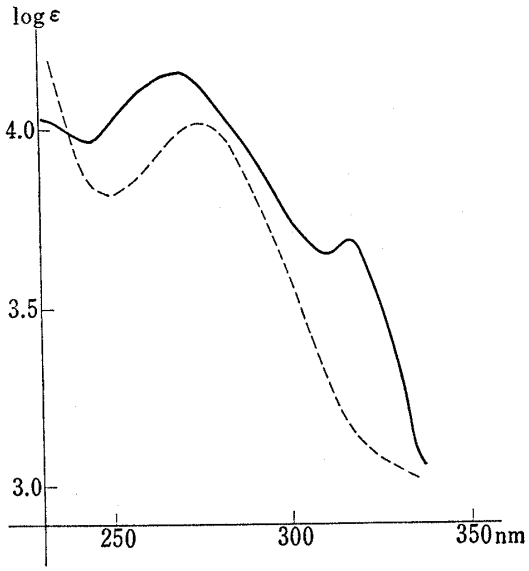


Fig. 2. Ultraviolet Absorption Spectra (in EtOH)

——— : compound **11**
--------- : compound **14**

Table IV. IR and NMR Spectral Data of Phenanthrene Derivatives

Compd. No.	IR $\nu_{max}^{CHCl_3}$ cm^{-1}	C(4)-H	NMR[a] OCH_3	OAc
7	1760	9.82(s)	3.97(3H, s)	2.45(3H, s)
			3.92(6H, s)	2.36(3H, s)
8	1775	9.47(s)	3.97(3H, s)	2.43(3H, s)
			3.93(3H, s)	2.33(6H, s)
9	1760	9.57(d)	4.03(3H, s)	2.46(3H, s)
		(J =8.3 Hz)	3.98(3H, s)	
			3.94(3H, s)	

a) All values are in δ units for $CDCl_3$ solution at 60 MHz with TMS as an internal standard.
abbreviation: s=singlet, d=doublet

13) M. Tomita, T. Ibuka and Y. Inubushi, *Tetrahedron Letters*, **1964**, 3617; M. Tomita, Y. Inubushi and T. Ibuka, *Yakugaku Zasshi*, **87**, 381 (1967).

phenanthrene nucleus, whereas an alcoholic hydroxyl group is eliminated by dehydration in the course of aromatization processes.[14]

The result of acetolyses of **1** and **2** together with the foregoing arguments disclosed that the ketonic function was situated at C-6 position of the hasubanan system, and remnant one oxygen and one methoxyl group should be assigned to a ketal ether and ketal methoxyl at C-8 position. The conversion of **2** into **4** also presented a chemical support to this ketal methoxyl assignment.

Furthermore, the NMR spectra of **1**, **2**, **3** and **4** exhibited doublet signals attributable to C-10 proton at δ 4.72—4.88 (J=6.5 Hz). In case of **2**, a nuclear Overhauser effect (NOE) (13%) was observed at this doublet (δ 4.82) by irradiating aromatic proton signal (δ 6.66), and the signals at δ 1.47 (doublet, J=10.5 Hz) and at δ 2.64 (double doublet, J_1=10.5 Hz, J_2=6.5 Hz) were assigned to C-9 methylene protons by double resonance technique. From these assignments, it follows that the ketal ether should link C-8 and C-10.

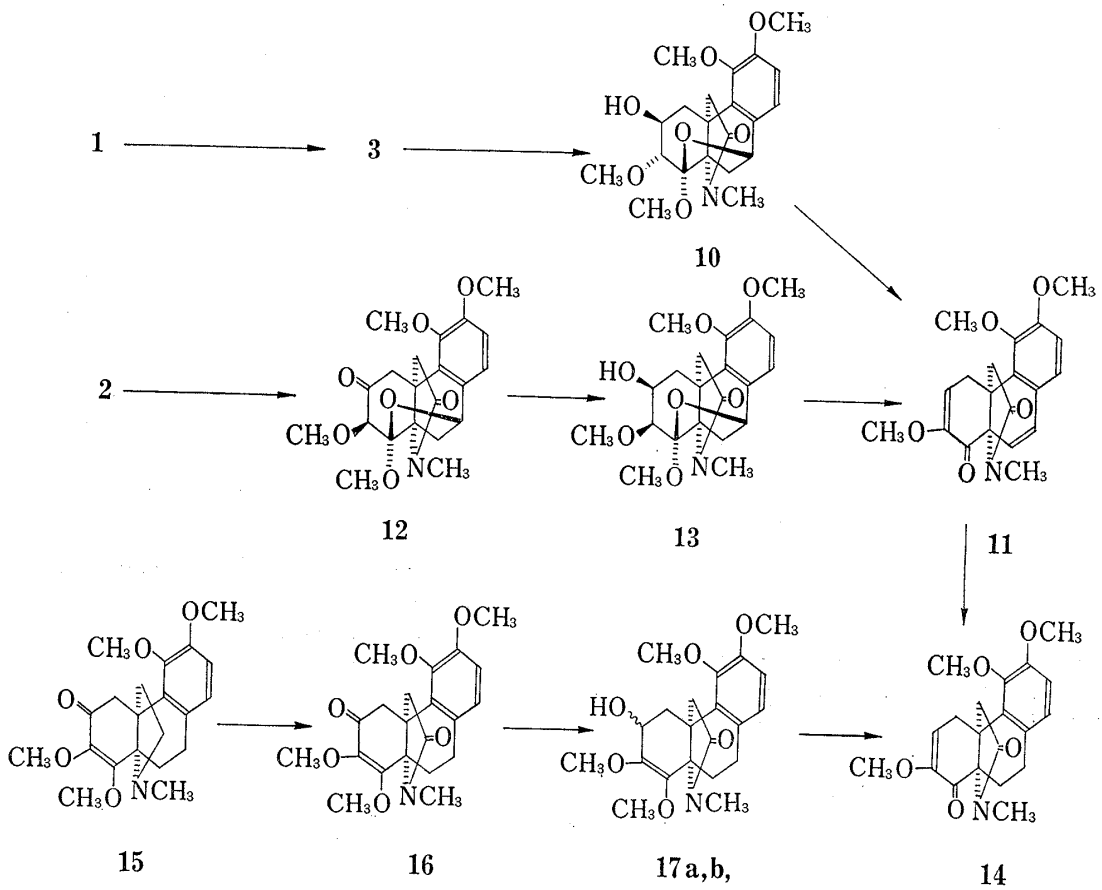


Chart 3

Though the above chemical and spectral evidences gave the two-dimensional formulas of **1**, **2**, **3** and **4**, these structures were verified by correlation to hasubanonine (**15**).[15]

Reduction of **3** with sodium borohydride gave dihydrooxostephamiersine (**10**), mp 236°, $C_{21}H_{27}O_7N$, *m/e* 405 (M^+). On treatment with acetic anhydride-perchloric acid in ethyl acetate, **10** was converted to a compound (**11**), mp 157°, $C_{20}H_{21}O_5N$, *m/e* 355 (M^+). Its UV spectrum (Fig. 2) showed an absorption curve of the styrene type chromophore, and the IR spectrum

14) E. Leete, *J. Am. Chem. Soc.*, **81**, 3948 (1959).
15) *a*) H. Kondo, M. Satomi and T. Odera, *Itsuu Kenkyusho Nempo*, **2**, 35 (1951); *b*) M. Tomita, T. Ibuka, Y. Inubushi, Y. Watanabe and M. Matsui, *Tetrahedron Letters*, **1964**, 2937; *idem*, *Chem. Pharm. Bull.* (Tokyo), **13**, 538 (1965).

exhibited the bands of conjugated ketone and double bond at 1680 cm^{-1} and 1640 cm^{-1}, respectively. The NMR spectrum revealed three methoxyl groups at δ 3.96, 3.86 and 3.62 as singlets, and C-6 olefinic proton at δ 5.96 as a double doublet (J_1=7.2 Hz, J_2=3.5 Hz), besides C-9 and C-10 olefinic protons at δ 5.72 and 6.80 as doublets (J=9.5 Hz). These spectral data suggested that **10** underwent the fission of the ketal ether followed by dehydration involving hydroxyl groups at C-6 and C-10. Therefore, **11** possesses C-9, C-10 double bond in conjugation with the benzene ring and C-6, C-7 double bond conjugated with the ketonic function. The above result gave a chemical support of the presence of ketal moiety between C-8 and C-10.

Oxidation of **2** with permanganate gave oxoepistephamiersine (**12**), mp 228°, $C_{21}H_{25}O_7N$, *m/e* 403 (M^+), which on reduction with sodium borohydride gave dihydrooxoepistephamiersine (**13**), mp 234°, $C_{21}H_{27}O_7N$, *m/e* 405 (M^+). On treatment with acetic anhydride-perchloric acid, **13** also afforded **11**.

Catalytic hydrogenation of **11** over palladized charcoal gave a compound (**14**), mp 171°, $C_{20}H_{23}O_5N$, *m/e* 357 (M^+), $[\alpha]_D^{23}$ −143° ($CHCl_3$). Its UV spectrum (Fig. 2) showed no absorption maximum at around 315 nm. Its IR spectrum exhibited bands at 1680 cm^{-1} and 1640 cm^{-1}, and the NMR spectrum revealed only one olefinic proton centered at δ 5.81 (double doublet, J_1=6.0 Hz, J_2=3.5 Hz). The above results demonstrated that **11** was hydrogenated at C-9, C-10 double bond to give **14**.

Reduction of 16-oxohasubanonine (**16**)[16] with sodium borohydride followed by alumina column chromatography gave C-6 epimeric alcohols, dihydro-16-oxohasubanonine-A (**17a**) and dihydro-16-oxohasubanonine-B (**17b**). The NMR spectra and chromatographic behavior of **17a** and **17b** indicated that hydroxyl group of the former has the quasi-axial configuration and that of the latter has the quasi-equatorial one, as with the case previously reported on dihydrohasubanonine.[15b] On treatment of either **17a** or **17b** with 1% hydrobromic acid gave a product identical with **14** in every respect.

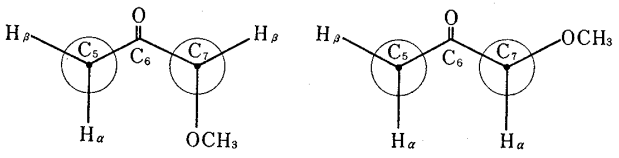
In view of the above facts, **1**, **2**, **3** and **4** possess the hasubanan skeleton, and the ketal ether or hemiketal ether bridge must be β-oriented, hence the methoxyl or hydroxyl group on C-8 and the hydrogen on C-10 must be α-oriented. Consequently, in case of **2** for example, C-9 methylene proton chemical shifts discussed earlier and their stereochemistry were assigned as follows: the higher field doublet (δ 1.47) is for α- and the lower field double doublet (δ 2.64) is for β-proton, respectively.

The absolute stereostructures of **1**, **2**, **3** and **4** were verified by the following NMR examination.[17] The NMR spectrum of **1** revealed C-5 methylene protons at δ 2.86 (β-equatorial proton, double doublet, J_1=11.5 Hz, J_2=1.5 Hz) and δ 3.67 (α-axial proton, doublet, J=11.5 Hz). The homonuclear internuclear double resonance (INDOR) spectra showed that this higher field proton (δ 2.86) couples with C-7 proton (δ 3.52, doublet, J=1.5 Hz) in a long range interproton coupling (4σ-bond coupling) according to W-rule.[18] While, **2** showed the signals of C-5 methylene protons at δ 2.99 (β-equatorial proton) and δ 3.18 (α-axial proton) as each doublet (J=11.5 Hz). Irradiation at the lower field proton (δ 3.18) gave a 6.5% NOE enhancement of C-7 proton signal (δ 4.27), but irradiation upon the higher field proton (δ 2.99) gave no signal enhancement of the C-7 proton signal. These NMR findings together with the foregoing results of the equilibrium reaction in alkali, disclosed the C-7 configuration of **1** and **2** as drawn in projection **1'** and **2'** (Fig. 3). Consequently, it has been assigned that

16) T. Ibuka, K. Tanaka and Y. Inubushi, *Tetrahedron Letters*, **1970**, 4811; *idem*, *Chem. Pharm. Bull.* (Tokyo), **22**, 782 (1974).

17) The spectra were taken on a Varian HA-100D spectrometer.

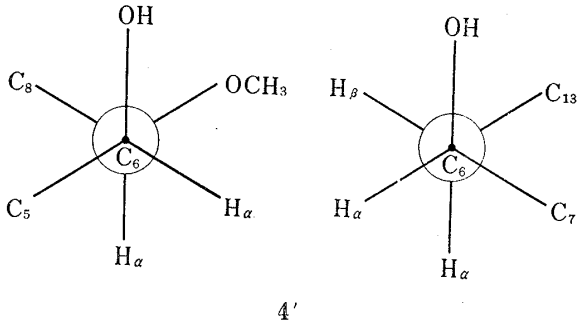
18) K.B. Wiber, B.R. Lowry and B.J. Nist, *J. Am. Chem. Soc.*, **84**, 1594 (1962); A. Rassat, C.W. Jefford, J.M. Lehn and B. Waegell, *Tetrahedron Letters*, **1964**, 233; L.M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd Edition, Pergamon Press, New York, 1969, pp. 334—341.



1′ 2′

Fig. 3. Projection Formulas of Stephamiersine (1) and Epistephamiersine (2) about C-5, C-6 and C-7 Substituents

1′: stephamiersine 2′: epistephamiersine



4′

Fig. 4. Projection Formulas of Stephasunoline (4)

The left view is along the C_6-C_7 axis and the right is along the C_6-C_5 axis.

C-7 methoxyl group of **1** has the α-axial configuration and that of **2** has the β-equatorial one.

The NMR spectrum of **4** exhibited the signals of C-5 methylene protons at δ 2.46 (J_1= 14.3 Hz, J_2=2.4 Hz) and δ 2.82 (J_1=14.3 Hz, J_2=3.8 Hz) as each double doublet, and C-7 proton signal (δ 3.62, doublet, J=3.9 Hz) enhancement (12%) was observed upon irradiation of the higher field methylene proton (δ 2.46). The NOE combined with the coupling constant values of C-5, C-6 and C-7 proton displayed the C-6 configuration of **4** as drawn in projection **4′** (Fig. 4). Thus, it has been determined that the C-6 hydroxyl group has a β-axial configuration and C-7 methoxyl group has a β-equatorial one.

The stereochemistry of C-6 hydroxyl groups in the foregoing highly stereoselective reduction products, **5**, **6**, **10** and **13** was elucidated by the coupling constant values on NMR. Each coupling constant values (2.3—2.9 Hz) between C-5 methylene protons and C-6 proton was found to be indicative of axial-equatorial or equatorial-equatorial coupling, and none was found to be for diaxial coupling. Therefore, the C-6 hydroxyl groups of **5**, **6**, **10** and **13** should have the β-axial configuration, respectively.

On the basis of the chemical and spectral evidences stated above, the constitution of stephamiersine (**1**), epistephamiersine (**2**), oxostephamiersine (**3**) and stephasunoline (**4**) was unambiguously represented as drawn in the formulas including their absolute stereostructures.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and uncorrected. UV, IR and mass spectra were taken on a Shimadzu MPS-50L, Hitachi EPI-G 3, Hitachi EPI-S 2, Hitachi R MU-7M and JEOL JMS-D 100 spectrometer. NMR spectra were measured on a Hitachi Perkin-Elmer R-20A, Hitachi Perkin-Elmer R-22 and Varian HA-100D spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard, and chemical shifts were given in δ value. The abbreviation, s, d, dd and m in the NMR spectra signify singlet, doublet, double doublet and multiplet. Optical rotations were measured on a Yanaco OR-10 and JASCO DIP-4 polarimeter. Column chromatography was effected using Brockmann neutral alumina activity II—III or Mallinckrodt silicic acid (100 mesh). Thin-layer chromatography (TLC) was performed on Aluminiumoxid G nach Stahl or Kieselgel G nach Stahl using indicated solvents and spots were detected by treatment with vapor of iodine and spraying Dragendorff's reagent.

Extraction and Fractionation of Alkaloids——The dried chipped stems and rizomes (79 kg) of *Stephania japonica* Miers collected in February 1970 at Bohnotsu-cho, Kagoshima Prefecture, were extracted three times with MeOH at reflux for 8 hr under mechanical stirring and the solvent was evaporated under reduced pressure. The residue (9 kg) was digested five times with an aqueous citric acid solution adjusted to pH 2—3 during 6 hr at 60—70°. The acid solution (90 liters) was shaken with $CHCl_3$ (total of 120 liters) to yield the "weak base fraction." The remaining acid layer was made alkaline to pH 9—10 with dil. NH_4OH and was shaken with $CHCl_3$ (total of 95 liters) to yield the "strong base fraction." i) The "weak base fraction" was shaken with 2% NaOH (total of 60 liters) and the residual $CHCl_3$ layer, after being washed with water and dried over $MgSO_4$, was evaporated to give a crude non-phenolic extract (1.2 kg). The aqueous layer was acidified with 5% AcOH, made alkaline with dil. NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was washed, dried over $MgSO_4$ and evaporated to give a crude phenolic extract (230 g). ii) The "strong base

fraction" was worked up by the same manner as in i) to give a phenolic (700 g) and a non-phenolic extract (1.8 kg).

Isolation of Alkaloids——i) Non-phenolic Bases from the "Weak Base Fraction": The crude extract (1.2 kg) was chromatographed over alumina column (5.5×98 cm) from benzene and eluted successively with benzene, benzene–ether (1: 1), benzene–AcOEt (1: 1), AcOEt, AcOEt–$CHCl_3$ (1: 1), $CHCl_3$, $CHCl_3$–EtOH (99: 1) and $CHCl_3$–EtOH (1: 1). a) The fractions eluted with benzene, benzene–ether (1: 1), benzene–AcOEt (1:1) and AcOEt were evaporated to dryness. The residues were combined and rechromatographed over silica gel column (5.5×95 cm) by eluting successively with $CHCl_3$, $CHCl_3$–EtOH (99:1) and $CHCl_3$–EtOH (1: 1). Elution with $CHCl_3$ and with $CHCl_3$–EtOH (99: 1) gave the mixture of stephamiersine (**1**), epistephamiersine (**2**) and oxostephamiersine (**3**) as a brownish solid which was crystallized from MeOH to give **3** as slightly yellow crystals. Recrystallization from MeOH gave 12.7 g of **3** as colorless prisms, mp 290°, 256° bimorphism). The methanolic mother liquor gave a mixture of **1** and **2** as a slightly yellow solid. Recrystallization from MeOH gave the mixture of **1** and **2** as colorless prisms,[19] which was dissolved in MeOH and 1% HNO_3 was added gradually with cooling. After filtration of **2** nitrate deposited at first, the mother liquor was allowed to stand at room temperature for a few hours to give **1** nitrate. The nitrates were recrystallized from MeOH, and **1** nitrate (mp 240° (decomp.)) and **2** nitrate (mp 212° (decomp.)) were obtained. They were worked up by the usual way to afford the free bases, **1** (mp 165° (MeOH), 2.1 g) and **2** (mp 98° (MeOH), 8.3 g). b) The eluate with AcOEt–$CHCl_3$ on the foregoing alumina column chromatography was evaporated to dryness and the residue was rechromatographed over silica gel column (3×42 cm) from $CHCl_3$ and elution with the same solvent gave 860 mg of metaphanine,[3] mp 232° (acetone–$CHCl_3$), $[\alpha]_D^{22}$ −42.7° (c=1.65, $CHCl_3$). c) The eluate with $CHCl_3$ on the foregoing alumina column chromatography was also rechromatographed over silica gel column (3.5×36 cm) from $CHCl_3$ and elution with the same solvent gave 1.2 g of stephanine,[4] mp 155—156° (acetone), $[\alpha]_D^{20}$ −88.3° (c=1.0, $CHCl_3$). The mother liquor of stephanine, after being evaporated to dryness, was rechromatographed over alumina column from benzene and elution with benzene and with AcOEt gave 280 mg of base-P (unidentified) as colorless prisms (MeOH), mp 195°. d) The fractions eluted from the foremost alumina column with $CHCl_3$–EtOH (99: 1) and $CHCl_3$–EtOH (1: 1) were combined with the mother liquors of the crystalline bases, and then the solvent was evaporated. The residue was rechromatographed over alumina column (3.5×38 cm) by eluting successively with benzene, AcOEt and $CHCl_3$. Elution with AcOEt gave 160 mg of base-X (unidentified) as orange-yellow pillars ($CHCl_3$), mp 316—318° (decomp.), and elution with $CHCl_3$ gave 420 mg of base-S (unidentified) as yellow pillars ($CHCl_3$), mp 270—272°.

ii) Phenolic Bases from the "Weak Base Fraction": The extract (230 g) was worked up by the multi-buffered extraction[20] (pH 4.8, 3.4 and 2.4) followed by chromatography over silica gel column from $CHCl_3$. Hypoepistephanine,[5] mp 256° (MeOH), $[\alpha]_D^{20}$ +181.1° (c=1.36, MeOH), 1.2 g was crystallized, but the mother liquor was not investigated further.

iii) Non-phenolic Bases from the "Strong Base Fraction": The extract (1.2 kg) was dissolved in $CHCl_3$ (10 liters) and the solution was shaken successively with MacIlvain buffer solution[19] (double strength) of pH 5.8, 4.6, 3.2 and 2.6. The residual $CHCl_3$ layer, after being washed with 2% Na_2CO_3 and water, was evaporated to dryness and the residue was extracted with 3% AcOH at 50—60°. Each extract was made alkaline with dil. NH_4OH and extracted with ether. The ethereal solutions were washed with water, dried over $MgSO_4$ and evaporated to dryness. a) The extract (485 g) from the buffer solution of pH 5.8 was chromatographed over alumina column (5×82 cm) from benzene and elution with the same solvent gave a crystalline solid. Recrystallization from MeOH gave 25 g of epistephanine,[6] mp 203—204°, $[\alpha]_D^{24}$ +184.8° (c=1.33, $CHCl_3$). The mother liquor of epistephanine, after being concentrated, was allowed to stand in a refrigerator overnight to afford a crystalline solid which was recrystallized from MeOH to give 350 mg of stebisimine,[8] mp 233°, $[\alpha]_D^{26}$ ±0° (c=0.97, MeOH). b) The extract (360 g) from the buffer solution of pH 4.6 was chromatographed over alumina column from benzene and elution with the same solvent gave additional amount of epistephanine (360 mg) and stebisimine (42 mg). c) The extract (318 g) from the buffer solution of pH 3.2 was chromatographed over alumina column (3.5×45 cm) by eluting successively with benzene, benzene–AcOEt (1: 1), AcOEt, $CHCl_3$ and $CHCl_3$–EtOH (9: 1). Elution with benzene and with benzene–AcOEt (1: 1) gave 280 mg of epistephanine, and the fractions eluted with AcOEt, $CHCl_3$ and $CHCl_3$–EtOH (9: 1) gave 18 g of protostephanine,[7] mp 73—74° (MeOH), $[\alpha]_D^{26}$ ±0° (c=1.46, MeOH). d) The extract (255 g) obtained from the buffer solution of pH 2.6 was chromatographed over alumina column from benzene and eluted with AcOEt, and then with $CHCl_3$. After the solvents were evaporated, the combined residue triturated with MeOH to give a crystalline solid which was recrystallized from the same solvent to afford 180 mg of stephasunoline (**4**) as colorless pillars. e) The extract (98 g) from the AcOH extraction was not investigated further.

19) This mixture, under the tentative name of XII-base was presented at 16th Annual Meeting of Pharmaceutical Society of Japan, Shizuoka, Nov. 1962; *cf.* Abstracts of Papers, p. 211.

20) Y. Watanabe, M. Uchiyama and K. Yasuda, *Yakugaku Zasshi*, 77, 807 (1957).

iv) Phenolic Bases from the "Strong Base Fraction": The extract (700 g) was dissolved in $CHCl_3$ (8 liters) and the solution was shaken with MacIlvain buffer solution[20] (double strength) of pH 5.0. The aqueous layer was made alkaline with dil. NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract, after being washed with water and dried over $MgSO_4$, was evaporated to dryness. The residue was dissolved in MeOH–ether and the solution was let stand in a refrigerator for several days to give a crystalline mass. Recrystallization from MeOH gave 38 g of hypoepistephanine. The $CHCl_3$ layer of the above buffer extraction was extracted with the buffer solution of pH 3.6, and the aqueous layer was worked up by the same manner as that described above to give 5.5 g of hypoepistephanine. The residual $CHCl_3$ solution was extracted with the buffer solution of pH 2.8, and the aqueous layer was worked up by the usual method to give 740 mg of stepinonine,[9] mp 280° (MeOH), $[\alpha]_D^{20}$ −28.3° (c=0.87, $CHCl_3$).

Stephamiersine (1) (**New Base**)——Colorless prisms (MeOH), mp 165°, $[\alpha]_D^{20}$ +33.0° (c=1.28, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{27}O_6N$: C, 64.76; H, 6.99; N, 3.60. Found: C, 64.82; H, 7.16; N, 3.63. Mass Spectrum *m/e*: 389 (M^+), 244, 243 (base peak), 229, 213. UV λ_{max}^{EtOH} nm (ε): 286 (2200). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (six-membered C=O). NMR: 6.67 (2H, s, aromatic H), 4.72 (1H, d, J=6.5 Hz, C(10)-H), 3.52 (1H, d, J=1.5 Hz, C(7)-H), 3.92, 3.82, 3.34, 3.31 (each 3H, s, 4×OCH_3), 2.64 (3H, s, NCH_3), 1.86 (1H, d, J=10.5 Hz, C(9)-Hα). Yield, 2.1 g. TLC: *Rf* 0.64 (Al_2O_3, $CHCl_3$). Hydriodide: mp 253° (decomp.) (MeOH). *Anal.* Calcd. for $C_{21}H_{27}O_6N\cdot HI\cdot 1/2H_2O$: C, 47.92; H, 5.55; N, 2.67. Found: C, 47.68; H, 5.39; N, 2.65. Nitrate: mp 240° (decomp.) (MeOH). *Anal.* Calcd. for $C_{21}H_{27}O_6N\cdot HNO_3$: C, 55.74; H, 6.24; N, 6.19. Found: C, 55.97; H, 6.51; N, 5.93.

Epistephamiersine (2) (**New Base**)——Colorless prisms (MeOH), mp 98°, $[\alpha]_D^{20}$ +64.1° (c=1.12, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{27}O_6N$: C, 64.76; H, 6.99; N, 3.60. Found: C, 64.95; H, 6.82; N, 3.60. Mass Spectrum *m/e*: 389 (M^+), 244, 243 (base peak), 228, 213. UV λ_{max}^{EtOH} nm (ε): 286 (2300). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (six-membered C=O). NMR: 6.66 (2H, s, aromatic H), 4.82 (1H, d, J=6.5 Hz, C(10)-H), 4.27 (1H, s, C(7)-H), 3.89, 3.76, 3.52, 3.45 (each 3H, s, 4×OCH_3), 2.63 (3H, s, NCH_3), 1.47 (1H, d, J=10.5 Hz, C(9)-Hα). Yield, 8.3 g. TLC: *Rf* 0.53 (Al_2O_3, $CHCl_3$). Hydriodide: mp 217° (decomp.) (MeOH). *Anal.* Calcd. for $C_{21}H_{27}O_6N\cdot HI$: C, 48.75; H, 5.46; N, 2.71. Found: C, 48.98; H, 5.34; N, 2.68. Nitrate: mp 212° (decomp.) (MeOH). *Anal.* Calcd. for $C_{21}H_{27}O_6N\cdot HNO_3$: C, 55.74; H, 6.24; N, 6.19. Found: C, 55.62; H, 5.98; N, 6.07. Methiodide: mp 181° (decomp.) (MeOH). *Anal.* Calcd. for $C_{21}H_{27}O_6N\cdot CH_3I$: C, 49.73; H, 5.69; N, 2.64. Found: C, 49.96; H, 5.43; N, 2.62. Oxime: mp 141° (EtOH). *Anal.* Calcd. for $C_{21}H_{28}O_6N_2$: C, 62.36; H, 6.98; N, 6.93. Found: C, 62.61; H, 5.82; N, 6.75.

Oxostephamiersine (3) (**New Base**)——Colorless prisms (MeOH), mp 290°, 256° (bimorphism), $[\alpha]_D^{27}$ +88.25° (c=1.87, $CHCl_3$). *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. Mass Spectrum *m/e*: 403 (M^+), 258, 257 (base peak), 242, 227. UV λ_{max}^{EtOH} nm (ε): 286 (2000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (six-membered C=O), 1680 (γ-lactam). NMR: 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×OCH_3), 3.12 (3H, s, NCH_3), 1.63 (1H, d, J=10.5 Hz, C(9)-Hα). Yield, 12.7 g. TLC: *Rf* 0.72 (Al_2O_3, $CHCl_3$). Monoxime: mp 265° (decomp.) (EtOH). *Anal.* Calcd. for $C_{21}H_{26}O_7N_2$: C, 60.28; H, 6.26; N, 6.70. Found: C, 60.37; H, 6.35; N, 6.73. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (γ-lactam).

Stephasunoline (4) (**New Base**)——Colorless pillars (MeOH), mp 233°, $[\alpha]_D^{20}$ +121.4° (c=1.44, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{27}O_6N$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.81; H, 7.33; N, 3.68. Mass Spectrum *m/e*: 377 (M^+), 246, 245 (base peak), 244, 230, 213. UV λ_{max}^{EtOH} nm (ε): 286 (2000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH). NMR: 6.67 (2H, s, aromatic H), 4.88 (1H, d, J=6.2 Hz, C(10)-H), 4.25 (1H, m, C(6)-H), 4.03 (1H, s, C(8)-OH), 3.90, 3.82, 3.46 (each 3H, s, 3×OCH_3), 3.62 (1H, d, J=3.9 Hz, C(7)-H), 2.82 (1H, dd, J=14.3, 3.8 Hz, C(5)-Hβ), 2.57 (3H, s, NCH_3), 2.46 (1H, dd, J=14.3, 2.4 Hz, C(5)-Hα), 2.43 (1H, d, J=10.5 Hz, C(6)-OH), 1.54 (1H, d, J=10.8 Hz, C(9)-Hα). Yield, 180 mg. TLC: *Rf* 0.34 (Al_2O_3, $CHCl_3$).

Equilibration of Stephamiersine (1) **and Epistephamiersine** (2)——i) A solution of **1** (100 mg) in 1% methanolic NaOH (5 ml) was refluxed for 30 min and the solvent was evaporated to dryness under reduced pressure. To the residue was added water (5 ml) and extracted with CH_2Cl_2. The CH_2Cl_2 extract, after being washed with water and dried over Na_2SO_4, was evaporated to give an amorphous solid (92 mg). TLC: *Rf* 0.64, 0.53 (Al_2O_3, $CHCl_3$). Judging from its NMR spectrum, this material was a mixture of **1** and **2** approximately in 1:3 ratio. The mixture was worked up *via* nitrate as described above to give 21 mg of **1** and 65 mg of **2**.

ii) A solution of **2** (100 mg) in 1% methanolic NaOH (5 ml) was refluxed and worked up in the same manner as in i) to afford a colorless solid (94 mg) which was judged from NMR spectrum to consists of **1** and **2** in 1:3 ratio. The separation of the mixture was performed *via* nitrate to give 23 mg of **1** and 64 mg of **2**.

iii) Refluxing **1** or **2** in MeOH for 2—4 hr resulted in complete recovery of the starting materials.

Oxidation of Stephamiersine (1) **to Oxostephamiersine** (3)——To a mixture of **1** (120 mg), $MgSO_4$ (120 mg), acetone (10 ml) and water (20 ml) was added dropwise a solution of $KMnO_4$ (120 mg) in acetone (15 ml) and water (20 ml), and the mixture was stirred for 3 hr at room temperature. The excess reagent was decomposed with $NaHSO_3$ in 5% H_2SO_4 and the solvent was evaporated under reduced pressure at room temperature. The residue was extracted with CH_2Cl_2 and the extract, after being washed successively with 2% HCl, 2% NaOH and water and dried over Na_2SO_4, was evaporated to give a crystalline solid (58 mg) which was

purified on silica gel column (5×0.5 cm) with $CHCl_3$ to afford a slightly yellow solid. Recrystallization from MeOH gave 38 mg of colorless crystals, mp 290°. On admixture of the product with an authentic sample of **3**, no depression of melting point was observed and the IR and the NMR spectra were fully identical.

Reduction of Epistephamiersine (2) to Dihydroepistephamiersine (5)——To a solution of **2** (180 mg) in MeOH–water (9: 1) (5 ml) was added sodium borohydride (60 mg) and stirred for 4 hr at room temperature, then the excess reagent was decomposed with 2% AcOH. The solvent was evaporated to dryness and 3% NH_4OH was added to the residue. The alkaline mixture was extracted with CH_2Cl_2 and the extract was washed with water, dried over Na_2SO_4 and evaporated to afford a colorless oil (160 mg), which was chromatographed over alumina column (2×20 cm) with benzene to give a slightly yellow solid. Recrystallization from MeOH gave 130 mg of **5** as colorless prisms, mp 63°, 133° (bimorphism). $[\alpha]_D^{20}$ +75.4° (c=1.84, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{29}O_6N$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.54; H, 7.52; N, 3.54. Mass Spectrum *m/e*: 391 (M^+), 246, 245 (base peak), 244, 230, 213. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH), NMR: 6.68 (2H, s, aromatic H), 4.86 (1H, d, J=6.5 Hz, C(10)-H), 4.11 (1H, m, C(6)-H), 3.89, 3.81, 3.52, 3.43 (each 3H, s, $4\times OCH_3$), 3.61 (1H, d, J=3.8 Hz, C(7)-H), 2.53 (3H, s, NCH_3), 2.06 (1H, d, J=9.5 Hz, C(6)-OH), 1.46 (1H, d, J=10.5 Hz, C(9)-H$_\alpha$).

Demethylation of Dihydroepistephamiersine (5) to Stephasunoline (4)——To a solution of **5** (100 mg) in MeOH (3 ml) was added conc. HCl (0.3 ml) and allowed to stand overnight at room temperature. Romoval of the solvent under reduced pressure at room temperature gave a brownish residue. The residue was made alkaline with 3% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract, after being washed with water and dried over Na_2SO_4, was evaporated to give a slightly yellow solid which was chromatographed over silica gel column (1×12 cm) with $CHCl_3$. The earlier eluate gave a crystalline solid which was rechromatographed from MeOH to give 52 mg of **4** as colorless pillars, mp 233°. This product was proved to be identical with an authentic sample of **4** by comparison of the IR and the NMR spectra and the optical rotation and mixed melting point determination.

Reduction of Stephamiersine (1) to Dihydrostephamiersine (6)——Sodium borohydride (45 mg) was added to a solution of **1** (138 mg) in MeOH–water (9: 1) (10 ml) and the mixture was stirred at room temperature for 3 hr, then the reaction mixture was treated with the similar manner as that in the reduction of **1** to give 118 mg of **6** as colorless prisms (MeOH), mp 143°, $[\alpha]_D^{20}$ +102.2° (c=1.14, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{29}O_6N$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.52; H, 7.39; N, 3.59. Mass Spectrum *m/e*: 391 (M^+), 246, 245 (base peak), 244, 230, 213. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH). NMR: 6.67 (2H, s, aromatic H), 4.77 (1H, d, J=6.5 Hz, C(10)-H), 3.91 (1H, m, C(6)-H), 3.90, 3.81, 3.46, 3.32 (each 3H, s, $4\times OCH_3$), 2.50 (3H, s, NCH_3), 1.91 (1H, d, J=11.5 Hz, C(6)-OH), 1.49 (1H, d, C(9)-H$_\alpha$).

Acetolysis of Stephamiersine (1) to 1,3-Diacetoxy-2,5,6-trimethoxyphenanthrene (7)——A mixture of **1** (165 mg), Ac_2O (3 ml) and conc. HCl (1 drop) was heated in a sealed tube at 170—180° for 10 hr, and after cooling the reaction mixture was poured onto ice-water and extracted with $CHCl_3$. The extract was washed successively with 3% HCl, 5% $NaHCO_3$ and water, and dried over Na_2SO_4. Evaporation of the solvent to dryness gave a brown oil which was chromatographed over silica gel column (1.3×18 cm) from $CHCl_3$ and elution with the same solvent gave a slightly yellow oil. Trituration of the oil with EtOH gave a crystalline solid which was recrystallized from EtOH to give 24 mg of **7** as slightly yellow pillars, mp 75°. *Anal.* Calcd. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24. Found: C, 65.89; H, 5.33. Mass Spectrum *m/e*: 384 (M^+). UV λ_{max}^{EtOH} nm (ε): 234, 260, 280, 304—308 (sh.), 345, 362. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (OAc). NMR: 9.82 (1H, s, C(4)-H), 3.97 (3H, s, OCH_3), 3.92 (6H, s, $2\times OCH_3$), 2.45, 2.36 (each 3H, s, $2\times OCOCH_3$).

Acetolysis of Epistephamiersine (2) to 1,2,3-Triacetoxy-5,6-dimethoxyphenanthrene (8)——A mixture of **2** (280 mg), Ac_2O (4 ml) and conc. HCl (1 drop) was worked up by the same manner as that in the acetolysis of **1** to give 38 mg of **8** as slightly yellow pillars (EtOH), mp 167°. *Anal.* Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 65.89; H, 5.33. Mass Spectrum *m/e*: 389 (M^+). UV λ_{max}^{EtOH} nm (ε): 258, 280, 306—314 (sh.), 346, 364. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775 (OAc). NMR: 9.47 (1H, s, C(4)-H), 3.97, 3.93 (each 3H, s, $2\times OCH_3$), 2.43 (3H, s, $OCOCH_3$), 2.33 (6H, s, $2\times OCOCH_3$).

Acetolysis of Dihydroepistephamiersine (5) and Dihydrostephamiersine (6) to 1-Acetoxy-2,5,6-trimethoxyphenanthrene (9)——i) A mixture of **5** (320 mg), Ac_2O (5 ml) and conc. HCl (1 drop) was heated in a sealed tube at 180° for 10 hr, and after cooling the reaction mixture was poured onto ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed successively with 3% HCl, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated to dryness. The residual oily gum was purified over silica gel column (0.5×8 cm) with $CHCl_3$ to afford a slightly yellow oil. Trituration of this oil with EtOH gave a crystalline solid which was recrystallized from EtOH to give 28 mg of **9** as slightly yellow pillars, mp 139°. *Anal.* Calcd. for $C_{19}H_{18}O_5\cdot1/6H_2O$: C, 69.30; H, 5.77. Found: C, 69.08; H, 5.73. Mass Spectrum *m/e*: 326 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (OAc). NMR: 9.57 (1H, d, J=8.3 Hz, C(4)-H), 4.03, 3.98, 3.94 (each 3H, s, $3\times OCH_3$), 2.46 (3H, s, $OCOCH_3$). On admixture of this compound with an authentic sample of **9** no melting point depression was observed and the IR spectra ($CHCl_3$) of two compounds were superimposable.

ii) A mixture of **6** (260 mg), Ac_2O (5 ml) and conc. HCl (1 drop) was treated with the similar manner as that in the procedure i) to give 25 mg of **9** as slightly yellow pillars (EtOH), mp 139°. This compound was identical with an authentic sample of **9**.

Reduction of Oxostephamiersine (3) to Dihydrooxostephamiersine (10)——To a solution of **3** (450 mg) in MeOH–water (9: 1) (80 ml) was added sodium borohydride (150 mg) and the mixture was stirred for 4 hr at room temperature, then the excess reagent was decomposed with 3% AcOH. The solvent was evaporated to dryness under reduced pressure, and water was added to the residue. The aqueous mixture was extracted with CH_2Cl_2 and the extract, after being washed with water and dried over Na_2SO_4, was evaporated to dryness to give a colorless oil. Trituration of this oil with MeOH gave a crystalline solid which was recrystallized from MeOH–ether (4: 1) to afford 380 mg of **10** as colorless prisms, mp 236°. *Anal.* Calcd. for $C_{21}H_{27}O_7N$: C, 62.21; H, 6.71; N, 3.46. Found: C, 62.09, H, 6.59; N, 3.48. Mass Spectrum *m/e*: 405 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1668 (γ-lactam). NMR: 6.76 (2H, s, aromatic H), 4.82 (1H, d, J=6.5 Hz, C(10)-H), 3.92, 3.83, 3.40, 3.36 (each 3H, s, 4×OCH_3), 3.71 (1H, d, J=3.2 Hz, C(7)-H), 2.97 (3H, s, NCH_3), 2.07 (1H, d, J=9.4 Hz, C(6)-OH), 1.62 (1H, d, J=11.0 Hz, C(9)-Hα).

Compound 11 from Dihydrooxostephamiersine (10)——A mixture of **10** (715 mg), AcOEt (20 ml), Ac_2O (2 ml) and 70% perchloric acid (0.5 ml) was allowed to stand for 18 hr at room temperature. A deep violet color of the mixture turned to dark brown slowly on standing. The reaction mixture was washed with 5% $NaHCO_3$, water and dried over Na_2SO_4. Evaporation of the solvent gave a deep brown oil which was chromatographed over alumina column (1.5×16 cm) from benzene and elution with benzene–AcOEt (9: 1) gave a slightly yellow oil (158 mg). The oil was crystallized on trituration with hexane and recrystallization from hexane–MeOH (4: 1) gave 125 mg of **11** as colorless prisms, mp 157°. *Anal.* Calcd. for $C_{20}H_{21}O_5N$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.76; H, 6.22; N, 4.13. Mass Spectrum *m/e*: 355 (M^+). UV λ_{max}^{EtOH} nm (ε): 265 (14000), 315 (5000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (conj. C=O, γ-lactam), 1640 (C=C). NMR: 6.86 (2H, s, aromatic H), 6.80 (1H, d, J=9.5 Hz, C(10)-H), 5.96 (1H, dd, J=7.2, 3.5 Hz, C(6)-H), 5.72 (1H, d, J=9.5 Hz, C(9)-H), 3.96, 3.86, 3.62 (each 3H, s, 3×OCH_3), 2.89 (3H, s, NCH_3).

Oxidation of Epistephamiersine (2) to Oxoepistephamiersine (12)——A solution of $KMnO_4$ (0.8 g) in water (40 ml) was added dropwise to a mixture of **2** (850 mg), $MgSO_4$ (600 mg), acetone (30 ml) and water (10 ml) with stirring under ice cooling, then the mixture was further stirred for 3 hr at room temperature. The precipitated MnO_2 and the excess reagent were dissolved with $NaHSO_3$–dil. H_2SO_4 solution and the solvent was evaporated under reduced pressure at room temperature. The residue was worked up by the same way as that in the oxidation of **1** to give 185 mg of **12** as colorless prisms (MeOH), mp 228°. *Anal.* Calcd. for $C_{21}H_{25}O_7N$: C, 62.52; H, 6.25; N, 3.47. Found: C, 62.79; H, 6.33; N, 3.46. Mass Spectrum *m/e*: 403 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (six-membered C=O), 1685 (γ-lactam). NMR: 6.73 (2H, s, aromatic H), 4.88 (1H, d, J=6.5 Hz, C(10)-H), 4.13 (1H, s, C(7)-H), 3.93, 3.84, 3.59, 3.49 (each 3H, s, 4×OCH_3), 3.15 (3H, s, NCH_3), 1.62 (1H, d, J=10.8 Hz, C(9)-Hα).

Reduction of Oxoepistephamiersine (12) to Dihydrooxoepistephamiersine (13)——Sodium borohydride (100 mg) was added to a solution of **12** (298 mg) in MeOH–water (9: 1) (20 ml), and the mixture was stirred for 4 hr at room temperature. The reaction mixture was treated with the similar manner as that in the reduction of **3** to give 272 mg of **13** as colorless prisms (MeOH–ether (4: 1)), mp 234°. *Anal.* Calcd. for $C_{21}H_{27}O_7N$: C, 62.21; H, 6.71; N, 3.46. Found: C, 62.11; H, 6.78; N, 3.53. Mass Spectrum *m/e*: 405 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH), 1670 (γ-lactam). NMR: 6.76 (2H, s, aromatic H), 4.93 (1H, d, J=6.5 Hz, C(10)-H), 4.09 (1H, m, C(6)-H), 3.92, 3.84, 3.60, 3.45 (each 3H, s, 4×OCH_3), 3.09 (1H, d, J=3.9 Hz, C(7)-H), 3.02 (3H, s, NCH_3), 2.23 (1H, d, J=9.3 Hz, C(6)-OH), 1.61 (1H, d, J=10.8 Hz, C(9)-Hα).

Compound 11 from Dihydrooxoepistephamiersine (13)——A solution of **13** (283 mg), AcOEt (15 ml), Ac_2O (2 ml) and 70% perchloric acid (0.3 ml) was allowed to stand for 18 hr at room temperature. The mixture was washed with 5% $NaHCO_3$, water and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave a brown oil which was worked up by the similar manner as that described before to afford 115 mg of **11** as colorless prisms (hexane–MeOH (4: 1)), mp 157°. This product was identical with **11** derived from **10** by comparison of their IR ($CHCl_3$) and NMR spectra and mixed melting point determination.

Catalytic Hydrogenation of 11 to Compound 14——A solution of **11** (98 mg) in MeOH (3 ml) was hydrogenated at an atmospheric pressure over 5% Pd-C (50 mg) at room temperature. After 20 min, the catalyst was filtered off, and the filtrate was evaporated to dryness. The residual oil (97 mg) dissolved in benzene was chromatographed over alumina column (2×16 cm) and elution with benzene–AcOEt (1: 1) gave a colorless solid. Recrystallization from EtOH–water (4: 1) gave 84 mg of **14** as colorless prisms, mp 171°, $[\alpha]_D^{23}$ −143° (c=1.52, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{23}O_5N$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.35; H, 6.59; N, 3.95. Mass Spectrum *m/e*: 357 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (γ-lactam, conj, C=O), 1640 (C=C). UV λ_{max}^{EtOH} nm (ε): 276 (9500). NMR: 6.80 (2H, s, aromatic H), 5.81 (1H, dd, J=6.0, 3.5 Hz, C(6)-H), 3.96, 3.86, 3.65 (each 3H, s, 3×OCH_3), 3.01 (3H, s, NCH_3).

Reduction of 16-Oxohasubanonine (16) to Epimeric Alcohols, 17a and 17b——To a solution of **16** (195 mg) in MeOH–water (9: 1) (20 ml) was added sodium borohydride (65 mg) and the mixture was stirred for 4 hr at room temperature. After the excess reagent was decomposed with 2% AcOH, the solvent was evaporated to dryness under reduced pressure and the residual oil was extracted with CH_2Cl_2. The extract was washed with water, dried over Na_2SO_4 and evaporated to give a colorless oil (186 mg) which was chromatographed over alumina column (1.5×18 cm) from benzene. Elution with the same solvent gave 76 mg of **17a** as an

amorphous solid. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1675 (γ-lactam), 1665 (C=C). NMR: 6.77 (2H, s, aromatic H), 4.28 (1H, m, C(6)-H), 3.91, 3.62 (each 3H, s, $2\times OCH_3$), 2.93 (3H, s, NCH_3). TLC: *Rf* 0.44 (Al_2O_3, $CHCl_3$). Further elution with benzene–EtOH (99: 1) gave a colorless solid (97 mg) which was recrystallized from MeOH to afford 82 mg of **17b** as colorless prisms, mp 79°. *Anal.* Calcd. for $C_{21}H_{27}O_6N$: C, 64.76; H, 6.99; N, 3.60. Found: C, 64.64; H, 6.86; N, 3.68. Mass Spectrum *m/e*: 389 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3425—3350 (OH), 1675 (γ-lactam), 1665 (C=C). NMR: 6.73 (2H, s, aromatic H), 3.85 (6H, s, $2\times OCH_3$), 3.83, 3.47 (each 3H, s, $2\times OCH_3$), 2.94 (3H, s, NCH_3).

Compound 14 from Dihydro-16-oxohasubanonine-A (17a)——A solution of **17a** (65 mg) in MeOH (3 ml) was heated with 3% HBr (1 ml) for 10 min at 60°. The solvent was evaporated to dryness and the residue was extracted with CH_2Cl_2. The extract, after being washed with water and dried over $MgSO_4$, was evaporated to give a colorless oil (64 mg) which was dissolved in benzene and chromatographed over alumina column (1.2×14 cm). Elution with benzene–AcOEt (1: 1) gave a colorless solid which was recrystallized from EtOH–water (4: 1) to afford 54 mg of **14** as colorless prisms, mp 171°, $[\alpha]_D^{25}$ −141° (c=1.26, $CHCl_3$). On admixture of this compound with **14** derived from **11** no melting point depression was observed and the IR spectra ($CHCl_3$) were superimposable.

Compound 14 from Dihydro-16-oxohasubanonine (17b)——To a solution of **17b** (42 mg) in acetone (3 ml) was added 3% HBr (0.5 ml) and the mixture was heated for 5 min at 60°. After the solvent was evaporated, the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was carried out by the similar manner as the described above to give 34 mg of **14** as colorless prisms, mp 171°. This product was identical with **14** derived from **17a** in every respect.

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