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OXOEPISTEPHAMIERSINE, A NEW HASUBANALACTAM ALKALOID FROM *STEPHANIA JAPONICA*[1]

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ABSTRACT.—A new hasubanalactam alkaloid, oxoepistephamiersine (**1**), was isolated together with known alkaloids, oxostephamiersine (**2**) and lanuginosine (**3**), from the petroleum ether extraction of the roots of *Stephania japonica*. The new alkaloid is the seventh hasubanalactam congener isolated from the *Stephania* genus.

Four hasubanalactam alkaloids[2] have already been isolated from the stems, roots, and leaves of *Stephania japonica* Miers (1-4). In the course of a reinvestigation of this native Japanese plant, a new hasubanalactam alkaloid, named oxoepistephamiersine (**1**), was isolated from a petroleum ether extract of the roots, together with two known alkaloids, oxostephamiersine (**2**) (1,2) and lanuginosine (**3**) (5,6).

RESULTS AND DISCUSSION

The dried and chipped roots (5.0 kg) of *S. japonica* were extracted with petroleum ether. After evaporation of the solvent, the residue was treated by the usual method, as described in the experimental section, to yield a non-phenolic extract. The extract (5.5 g) was subjected to silica gel column chromatography to give **1** (38.8 mg), **2** (29.3 mg), and **3** (31.2 mg).

The new alkaloid **1** was crystallized as light yellow prisms from MeOH, mp 227°, $C_{21}H_{25}NO_7$, $[\alpha]^{13}D$ +104.88°. Its ir spectrum exhibited bands characteristic of a six-membered ketone (1745 cm^{-1}) and a γ-lactam (1690 cm^{-1}), and its uv spectrum showed an absorption maximum at 284.5 nm. The hrms revealed a molecular ion peak at *m/z* 403.1630 (53.2%) with the most abundant peak at *m/z* 257.1063. The pmr and cmr data are summarized in Tables 1 and 2.

TABLE 1. Pmr Data for Oxoepistephaniersine (**1**) in $CDCl_3$

Proton[a]	Chemical shift[b]	Multiplicity (*J*, Hz)
C-1	6.76	d (8.46)
C-2	6.68	d (8.46)
C-7	4.13	s
C-9α	1.61	d (11.20)
C-9β	2.94	dd (6.37, 11.20)
C-10	4.87	d (6.37)
C-3 OCH_3	3.91[c]	s
C-4 OCH_3	3.83[c]	s
C-7 OCH_3	3.59[d]	s
C-8 OCH_3	3.49[d]	s
NCH_3	3.14	s

[a]Other signals were not assigned clearly.
[b]Values in δ scale relative to internal TMS.
[c,d]Asterisked assignments may warrant changing.

[1]Part 282 in the series: "Studies on Alkaloids of Menispermaceous Plants." Part 281: J. Kunitomo, Y. Murakami, M. Oshikata, M. Akasu, K. Kodama, N. Takeda, K. Hayada, M. Suzuki, A. Tatematsu, E. Kawabe, and H. Ishii, *Chem. Pharm. Bull.*, (in press).

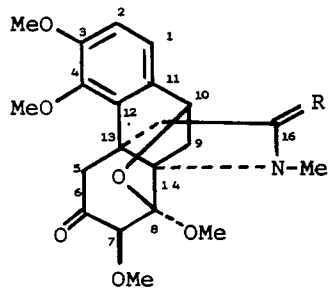
[2]The hasubanan skeleton bearing a carbonyl group at C-16 is called "hasubanalactam" [see Matsui and Watanabe (4)].

TABLE 2. Cmr Data for Oxoepistephamiersine (**1**) in $CDCl_3$[a]

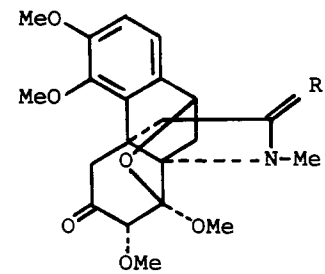
Carbon		Carbon	
1	120.39 (d)	12	129.22 (s)
2	111.45 (d)	13	46.76 (s)
3	153.49 (s)	14	74.99 (s)
4	148.34 (s)	15	46.00 (dd)
5	44.54 (dd)	16	173.60 (3)
6	200.74 (s)	C-3 OCH_3	55.86 (q)
7	88.53 (d)	C-4 OCH_3	60.47 (q)
8	105.71 (s)	C-7 OCH_3	59.55 (q)
9	33.21 (t)	C-8 OCH_3	52.28 (q)
10	76.01 (d)	NCH_3	28.44 (q)
11	133.61 (s)		

[a]The spectrum was recorded at 22.50 MHz under the same condition as described in Matsui *et al.* (7). Abbreviations (s, d, dd, t, and q) represent the multiplicity due to the direct ^{13}C-^{1}H coupling (^{1}J).

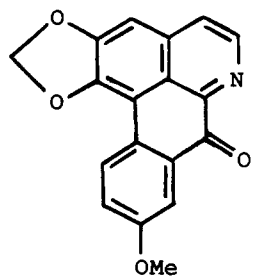
In general, the ir spectra of alkaloids of the hasubanalactam family exhibit a band around 1680 cm^{-1} due to a γ-lactam carbonyl group (1-4). Moreover, the pmr spectra of the family exhibit a down-field shift (~ +1.7 ppm) of the *N*-methyl protons compared with those of hasubanan alkaloids (1-4). The above spectral feature, as well as the cmr and mass spectral data (4,7), provides a reliable and practical method to distinguish the hasubananlactam from the hasubanan alkaloids.



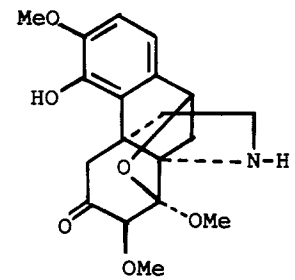
1 R=O
4 R=H_2



2 R=O
5 R=H_2



3



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Stradling *et al.* reported the effect of methoxyl groups on carbonyl ir absorption (8). These authors showed that an equatorial methoxyl group adjacent to the carbonyl group shifts the carbonyl absorption to higher frequency but that an axial epimer causes no appreciable shift. Thus, **1** showing a carbonyl absorption at 1745 cm^{-1}, was assumed to be an equatorial epimer, while an axial epimer (**2**) exhibited the band at 1725 cm^{-1}. On the other hand, as previously reported (1), the hasubanan alkaloids epistephamier-

sine (**4**) and stephamiersine (**5**) are epimeric at the C-7 position, with the C-7 methoxyl group of **4** equatorial (β) and that of **5** axial (α). In the pmr of **4** and **5**, the fact that the C-7 proton of **4** exhibited its resonance position down-field (δ 4.27) from that of **5** (δ 3.52) permits the former to be distinguished from the latter. This finding was also reported in the case of 6-dehydrostephuline (**6**) by Kupchan *et al.* (9). The same difference was observed for the hasubanalactam alkaloids as follows: **2** carrying a C-7 equatorial (β) proton reveals the signal at δ 3.62, whereas 16-oxoepistephamiersine prepared from **4** exhibits the signal down-field at δ 4.13 (1,2). These observations of the ir and pmr spectra of alkaloids of the hasubanalactam series with a ketonic function at C-6 position provide evidence for the stereochemistry at the C-7 position of **1**.

To confirm the structure deduced from spectral arguments, the following chemical modification was undertaken for **4**. Permanganate oxidation of **4** gave 16-oxoepistephamiersine, whose physical and spectral properties were identical with those of **1**. Based on the results presented here, the structure of **1** was established to be that drawn in the formula.

The known alkaloids, **2** and **3**, were separated efficiently on a silica gel column with $CHCl_3$. The former was obtained as colorless prisms, and the latter was crystallized as orange-yellow needles, which in $CHCl_3$ solution showed a green-yellow fluorescence in visible light and in acid solution gave a cherry-red coloration. The alkaloids **2** and **3** were identical with authentic samples.

It is interesting to note that the present extraction with petroleum ether gave the hasubanalactam and oxoaporphine alkaloids as major products in preference to the others (10).

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 $CHCl_3$. Pmr and cmr spectra were recorded in $CDCl_3$ on JEOL JNM-FX 90Q with TMS as internal standard and chemical shifts are reported in δ (ppm) units. Mass spectra were taken on a JEOL JNM-D 100 mass spectrometer using a direct inlet probe at 75 eV. Optical rotations were measured on a JASCO model DIP-140 polarimeter. Silica gel 60 (70-230 mesh) (E. Merck) was used for column chromatography. Tlc was performed on silica gel 60 F_{254} (E. Merck), and the spots 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 February 1983, by Mr. K. Shukuri. A voucher specimen is deposited in the Herbarium of the Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan.

EXTRACTION AND FRACTIONATION OF TERTIARY NON-PHENOLIC ALKALOIDS.—The dried and chipped roots (5.0 kg) were successively extracted with hot petroleum ether, and the solvent was removed under reduced pressure to yield 34 g of residue. The residue was digested with 5% aqueous citric acid, and the acid solution was made alkaline by addition of NH_4OH. The ammoniacal solution was extracted by shaking with 2% aqueous NaOH to remove phenolic bases, and the $CHCl_3$ layer was evaporated to dryness to give a tertiary nonphenolic extract (6.5 g). The latter extract (6.5 g) was chromatographed on a silica gel column (150 g, 3.6×33 cm) in C_6H_6, and eluted successively with $CHCl_3$, and then with increasing proportions of MeOH in $CHCl_3$. The extract (1.4 g) prepared from the $CHCl_3$ eluate (3.2 liters) was rechromatographed on a silica gel column (40 g, 2.5×22 cm) with $CHCl_3$. After fractions of 50 ml each had been collected, examined by tlc, and evaporated to dryness, the residues were crystallized from MeOH or $CHCl_3$. Fraction 11 and 12 gave **2** (29.3 mg), and fraction 16 gave the new alkaloid, **1** (38.8 mg). Later fractions 22 to 33, yielded **3** (31.2 mg).

OXOEPISTEPHAMIERSINE (**1**) (NEW ALKALOID).—Light yellow prisms, mp 227° (MeOH), $[\alpha]^{13}D$ +104.88° (*c* 1.0, $CHCl_3$). Uv λ max (ε) (EtOH) nm 284.5 (1940); ir ν max cm^{-1} 1745 (six-membered C=O), 1690 (γ-lactam). The hrms was consistent with empirical formula $C_{21}H_{25}NO_7$ (M^+, *m/z* 403.1630) and displays significant ions at *m/z* 257.1063 (base peak), 243.0836, 242.0799, and 227.0942. Yield; 38.8 mg. Pmr and cmr data, see Tables 1 and 2.

KNOWN ALKALOIDS.—Oxostephamiersine (**2**); colorless prisms, mp 313° (dec.) (MeOH),[3] $[\alpha]^{20}D$ +88.0° (*c* 1.0, $CHCl_3$). Ir ν cm^{-1} 1725 (six-membered C=O), 1680 (γ-lactam); ms *m/z* 403 (M^+), 257 (base peak). Yield; 29.3 mg. Lanuginosine (**3**); orange-yellow needles, mp 323° (dec.) ($CHCl_3$). Ir ν max cm^{-1} 1655; ms *m/z* 305 (M^+, base peak). Yield; 31.2 mg. Known alkaloids were identified by direct comparison (mmp, tlc, ir and pmr) with authentic samples.

OXIDATION OF EPISTEPHAMIERSINE (**4**) TO OXOEPISTEPHAMIERSINE (**1**).—In the mixture of Me_2CO, H_2O, and $MgSO_4$, **4** (80 mg) was oxidized by $KMnO_4$ under the same procedure as described in the literature (1). The reaction product, 16-oxoepistephamiersine, was identical with **1** by direct comparison (mmp, tlc, ir, and pmr).

LITERATURE CITED

1. M. Matsui, Y. Watanabe, T. Ibuka, and K. Tanaka, *Tetrahedron Lett.*, 4263 (1973).
2. M. Matsui, Y. Watanabe, T. Ibuka, and K. Tanaka, *Chem. Pharm. Bull.*, **23**, 1323 (1975).
3. M. Matsui, T. Kabashima, K. Ishida, T. Takebayashi, and Y. Watanabe, *J. Nat. Prod.*, **45**, 497 (1982).
4. M. Matsui and Y. Watanabe, *J. Nat. Prod.*, **47**, 465 (1984).
5. Y. Watanabe, M. Matsui, M. Iibuchi, and S. Hiroe, *Phytochemistry*, **14**, 2522 (1975).
6. J. Kunitomo, Y. Murakami, M. Oshikata, T. Shingu, M. Akasu, S.T. Lu, and I.S. Chen, *Phytochemistry*, **19**, 2735 (1980).
7. M. Matsui, Y. Watanabe, and T. Hinomoto, *J. Nat. Prod.*, **45**, 247 (1982).
8. S.S. Stradling and D.S. Tarbell, *J. Org. Chem.*, **29**, 1170 (1964).
9. S.M. Kupchan and M.I. Suffness, *Tetrahedron Lett.*, 4975 (1970).
10. Y. Inubushi and T. Ibuka, "The Alkaloids," vol. XVI, Ed. by R.H.F. Manske, New York: Academic Press Inc., 1977, pp. 393-398.

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