Pergamon

Tetrahedron Vol. 51, No. 13, pp. 3727-3736, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4020/95 $9.50+0.00

0040-4020(95)00117-4

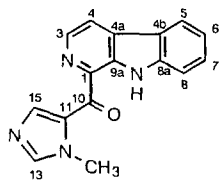
Four New β-Carboline Alkaloids Isolated from Two Okinawan Marine Sponges of *Xestospongia* sp. and *Haliclona* sp.[1)]

Motomasa Kobayashi,[a] Yin-Ju Chen,[a] Shunji Aoki,[a] Yasuko In,[b] Toshimasa Ishida,[b] and Isao Kitagawa*[a]

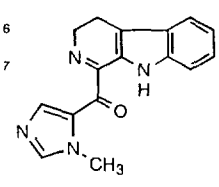
Faculty of Pharmaceutical Sciences, Osaka University,[a] 1-6, Yamada-oka, Suita, Osaka 565, Japan and Osaka University of Pharmaceutical Sciences,[b] 2-10-65, Kawai, Matsubara, Osaka 580, Japan

Abstract: Two new β-carboline alkaloids, xestomanzamine A (**1**) and xestomanzamine B (**2**), and a new manzamine-type alkaloid, manzamine X (**3**), were isolated as cytotoxic constituents from an Okinawan marine sponge of *Xestospongia* sp. Another new manzamine-type alkaloid, manzamine Y (**4**), was isolated from an Okinawan marine sponge of *Haliclona* sp. The structures of these alkaloids were elucidated on the bases of 2D-NMR and X-ray analyses.

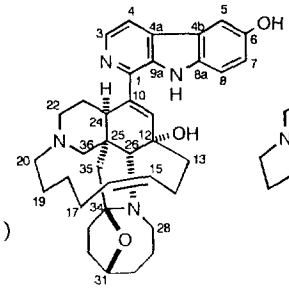
Manzamines are unique β-carboline alkaloids isolated from marine sponges and the structures are characterized by having an intricate nitrogen containing polycyclic system. In 1986, Higa and his group first reported manzamine A (**5**) as the major cytotoxic constituent of a marine sponge of *Haliclona* sp., which was collected at Manzamo, Okinawa.[2a)] Subsequent study of this particular marine sponge led them to the isolation and characterization of manzamines B (**6**), C (**7**), and D (**8**),[2b)] and afterwards manzamines E (**9**) and F (**10**)[2c)] were found from another sponge of *Xestospongia* sp. On the other hand, manzamines A (**5**) and F (**10**) were isolated from another marine sponge of *Pellina* sp.[2d)], while manzamines A (**5**), B (**6**), D (**8**), E (**9**), H (**11**), and J (**12**) were identified as the constituents of another marine sponge of *Ircinia* sp.[2e)] Very recently, two different manzamine-type alkaloids, 8-hydroxy-manzamine A (**13**)[2f)] and keramamine C (**14**)[2g)], have been isolated from two marine sponges of *Pachypellina* sp. and *Amphimedon* sp., respectively. On the whole, manzamine-type alkaloids have been isolated from six marine sponge species belonging to different genera. Thus, these findings have led us to presume that there may be participation of probably common microorganism(s) in the biosynthesis of these



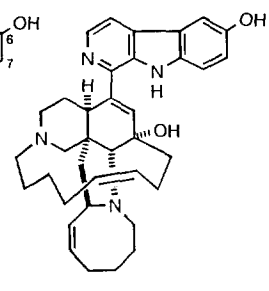
xestomanzamine A (1)



xestomanzamine B (2)



manzamine X (3)



manzamine Y (4)

Chart 1

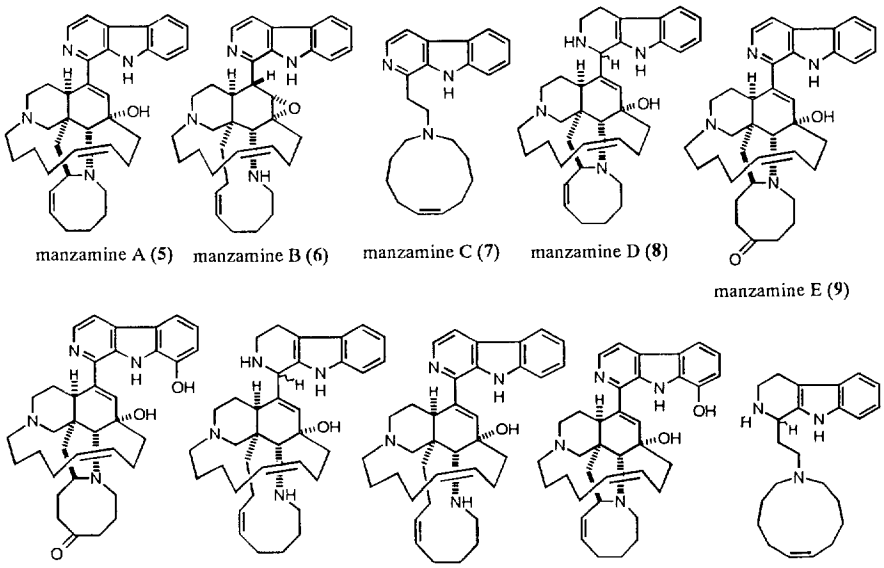


Chart 2

manzamine-type alkaloids.

In our continuing study of searching for new biologically active marine natural products, we have come across to find two different kinds of marine sponges, each containing manzamine-type alkaloids and inhabiting the close coral area. Thus, at the edge of the reef of Amitori Bay, Iriomote Island, Okinawa, we collected a marine sponge of *Haliclona* sp. while at the shallow water of the same Amitori Bay a marine sponge of *Xestospongia* sp. was collected and the both sponges have been shown to contain manzamine-type alkaloids. In anticipation of finding any indication of participation of microorganism(s) in the biosynthesis of manzamine-type alkaloids, we have analyzed in detail the chemical constituents of these two marine sponges. In this paper, we describe the chemical characterization of four new β-carboline alkaloids isolated from those sponges.

First, a marine sponge of *Xestospongia* sp., collected at the shallow water (-2 m), was extracted with acetone. The acetone extract was partitioned into an ethyl acetate-water mixture, and then the ethyl acetate phase was separated and evaporated under reduced pressure to provide the ethyl acetate extract. The TLC analysis showed that the ethyl acetate extract contained rich quantity of manzamine A (**5**) together with various concomitant minor alkaloids as detected by Dragendorff reagent. Referring to the separation procedure carried out by Higa and his group,[2b] the ethyl acetate extract was then partitioned into an *n*-heptane-CH_2Cl_2-CH_3CN mixture (50:15:35) (Chart 3). The crude alkaloidal fraction resulting from evaporation of the lower phase was dissolved in a small amount of MeOH and kept at 5°C for 24 h to precipitate manzamine A (**5**) as colorless crystals. The mother liquid, upon evaporation, gave an alkaloidal fraction, which was further separated by silical gel column chromatography and subsequent Sephadex LH-20 column chromatography to furnish three new alkaloids named xestomanzamine A (**1**), xestomanzamine B (**2**), and manzamine X (**3**), together with two known manzamine-type alkaloids, manzamine E (**9**) and

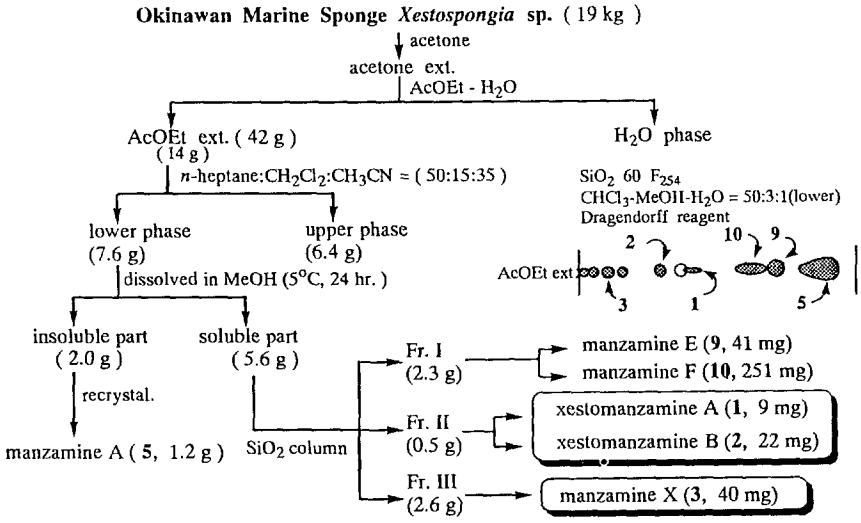


Chart 3

manzamine F (**10**).

Xestomanzamine A (**1**), obtained as yellow needles, showed a quasimolecular $(M+H)^+$ ion peak at *m/z* 277 in its FAB-MS and the molecular formula was defined as $C_{16}H_{12}N_4O$ by HR-FAB-MS. The ^{1}H-NMR and ^{13}C-NMR spectra of **1** showed the signals assignable to a β-carboline structure, which were confirmed by COSY and C-H COSY analyses. (Table I) The UV absorption maximum (λ_{max} at 395 nm) of **1** suggested that **1** has additional chromophore conjugated to its β-carboline moiety as judged from the λ_{max} at 348 nm of manzamine A (**5**). Furthermore, the ^{1}H- and ^{13}C-NMR spectra of xestomanzamine A (**1**) showed the signals characteristically ascribable to one carbonyl carbon (δc 184.2), one *N*-methyl group [δ 4.05 (3H, s), δc 35.2 (q)], and two singlet olefinic protons [δ 7.66 (1H, s), 8.93 (1H, s)]. The correlation spectroscopy carried out through the COLOC experiment of **1** showed several correlations between those signals as shown in Fig. 1. However, any correlation between proton(s) and the carbonyl carbon signal at δc 184.2 could not be observed. In order to shed light on these structural correlations and to confirm the chemical structure, xestomanzamine A (**1**), obtained as yellow needles from $CHCl_3$-MeOH,

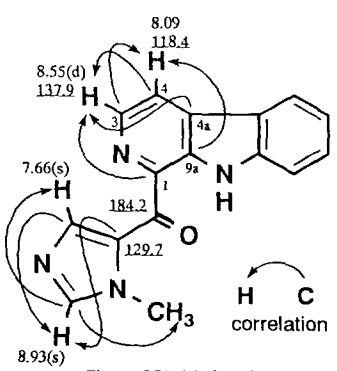


Fig. 1 COLOC Correlation for Xestomanzamine A (**1**)

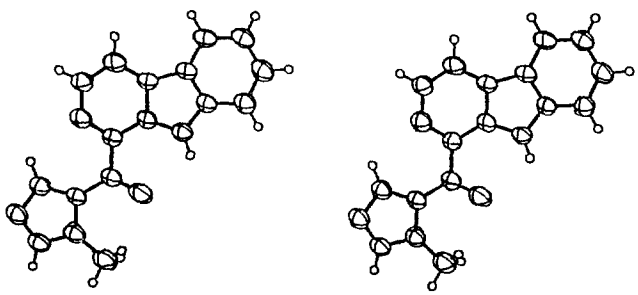


Fig. 2 Perspective Drawings of Xestomanzamine A (**1**)

was subjected to X-ray crystallographic analysis and the perspective drawings are depicted in Fig. 2. Consequently, the chemical structure of xestomanzamine A has been determined as **1** and all proton and carbon signals have been assigned as given in Table I.

Table I. NMR Data for Xestomanzamine A (**1**) and Xestomanzamine B (**2**) (* 270 MHz in $CDCl_3$, **67.8 MHz in $CDCl_3$, J values in Hz)

atom No.	1 δc**	1 δ*	2 δc**	2 δ*
1	136.4 (s)		155.8 (s)	
3	137.9 (d)	8.55 (d, J =5.0)	49.1 (t)	4.16 (dd, J =9.0, 9.0)
4	118.4 (d)	8.09 (d, J =5.0)	18.8 (t)	2.97 (dd, J =9.0, 9.0)
4a	131.5 (s)		118.0 (s)	
4b	120.6 (s)		124.7 (s)	
5	121.7 (d)	8.12 (d, J =8.2)	120.3 (d)	7.60 (d, J =7.9)
6	120.5 (d)	7.30 (dd, J =8.2, 6.2)	119.9 (d)	7.13 (dd, J =7.9, 7.0)
7	129.6 (d)	7.55 (dd, J =6.2, 7.3)	125.1 (d)	7.29 (dd, J =7.0, 7.3)
8	111.8 (d)	7.57 (d, J =7.3)	112.2 (d)	7.40 (d, J =7.3)
8a	140.8 (s)		136.9 (s)	
9a	136.5 (s)		125.1 (s)	
10	184.2 (s)		182.9 (s)	
11	129.7 (s)		126.3 (s)	
13	143.6 (d)	8.93 (s)	144.2 (d)	8.37 (s)
15	143.3 (d)	7.66 (s)	144.2 (d)	7.63 (s)
N-CH_3	35.2 (q)	4.05 (s)	35.2 (q)	3.99 (s)

HMBC (in DMSO-d_6) correlations of **2** : C-1 / H-3; C-3 / H-4; C-4 / H-3; C-4a / H-3; C-4b / H-4, H-6; C-5 / H-7; C-6 / H-8; C-7 / H-5; C-8 / H-6; C-8a / H-7; C-9a / H-4; C-11 / H-13; C-13 / H-15, N-CH_3; C-15 / H-13.

The FAB-MS of xestomanzamine B (**2**) showed $(M+H)^+$ ion peak at *m/z* 279 and the molecular composition was determined as $C_{16}H_{14}N_4O$ by the HR FAB-MS, which corresponds to two mass units more as compared with that of xestomanzamine A (**1**). It was a significant finding that the absorption pattern in the UV spectrum of **2** was quite different from that of **1**. The ^1H- and ^{13}C-NMR, COSY, and C-H COSY spectra of **2** indicated the presence of one 1,2-disubstituted benzene ring, one carbonyl group (δc 182.9), one *N*-methyl group [δ 3.99 (3H, s), δc 35.2 (q)], four olefinic quaternary carbons (δc 118.0, 125.1, 126.3, 155.8), two singlet olefinic protons [δ 7.63 (1H, s), 8.37 (1H, s)] and two methylene moieties [δ 2.97, 4.16 (both 2H, dd, J =9, 9 Hz), δc 18.8 (t), 49.1 (t)]. Furthermore, the HMBC spectrum (in d_6-DMSO) of **2** showed correlations among protons and carbons constituting the *N*-methyl imidazole ring. It also disclosed the presence of correlations between methylene protons and three quaternary carbons in the β-carboline moiety (between 3-H_2 and C-1, C-4, C-4a; between 4-H_2 and C-3, C-4a, C-9a) (Table I). Consequently, the chemical structure of xestomanzamine B (**2**) was presumed to be a 3,4-dihydro analogue of xestomanzamine A (**1**). This presumption was verified by a fact that xestomanzamine B (**2**) was gradually converted (at 21°C, for 20 d) presumably *via* air-oxidation to provide xestomanzamine A (**1**). Consequently, the structure of xestomanzamine B has been elucidated as **2**, that is 3,4-dihydroxestomanzamine A.

The third new alkaloid named manzamine X (**3**) showed $(M+H)^+$ ion peak at *m/z* 581 in its FAB-MS, and the molecular composition was determined as $C_{36}H_{44}N_4O_3$ by HR FAB-MS. The ^1H-NMR data for **3** resembled those of manzamine F (**10**) which has the same molecular composition as **3**, while the IR and ^{13}C-NMR spectra of **3** showed lack of a carbonyl moiety which is seen in **10**. The UV spectra of manzamine-type alkaloids mostly show two absorption maxima in the range of 300 and 400 nm. In the UV

spectrum of **3**, only one absorption maximum was observed at 378 nm, the pattern being alike to that, though the maximum at 355 nm, of manzamine F (**10**) which has an extra hydroxyl group at C-8 of the β-carboline ring. The COSY, C-H COSY, and HMBC spectra of **3** suggested that the extra hydroxyl group in **3** was located at C-6 of the β-carboline moiety (Table III). In comparison with the ^{13}C-NMR data for manzamine A (**5**), the carbon signals especially assignable to the central part carbons (C-10~C-26) of **3** were observed at similar chemical shifts to those carbon signals of **5** (Table II). So that, it was presumed that **3** had the same central part of **5**.

In order to determine the chemical structure of **3**, a yellow prismatic crystal of **3**, obtained from *n*-hexane-acetone, was subjected to X-ray crystallographic analysis and the perspective drawings thus obtained were as shown in Fig. 3. Accordingly, the chemical structure of manzamine X has been confirmed as **3** which comprises an inserted tetrahydrofuran ring in the lower part of the structure.

Table II. ^{13}C-NMR Data for Manzamine X (**3**), Manzamine Y (**4**), and Manzamine A (**5**)
(δc 67.8 MHz in $CDCl_3$, * ref. 2c)

atom-No.	3	4	5*	atom-No.	3	4	5*
1	142.9 (s)	143.3 (s)	143.6 (s)	17	26.1 (t)	25.0 (t)	24.9 (t)
3	137.9 (d)	137.0 (d)	137.5 (d)	18	26.3 (t)[a]	26.4 (t)	26.4 (t)
4	113.4 (d)	113.9 (d)	113.8 (d)	19	22.9 (t)	24.6 (t)	24.5 (t)[b]
4a	129.0 (s)	129.1 (s)	129.3 (s)	20	53.2 (t)	53.4 (t)	53.3 (t)
4b	122.3 (s)	121.7 (s)	121.1 (s)	22	49.8 (t)	49.2 (t)	49.1 (t)
5	106.7 (d)	106.0 (d)	120.9 (d)	23	32.5 (t)	33.4 (t)	33.5 (t)
6	150.3 (s)	149.8 (s)	119.2 (d)	24	39.3 (d)	40.8 (d)	41.0 (d)
7	118.5 (d)	118.4 (d)	127.9 (d)	25	45.4 (s)	47.0 (s)	46.9 (s)
8	112.3 (d)	113.3 (d)	112.8 (d)	26	75.2 (d)	78.0 (d)	78.0 (d)
8a	134.7 (s)	136.2 (s)	141.4 (s)	28	55.3 (t)	53.4 (t)	53.3 (t)
9a	134.3 (s)	134.0 (s)	133.3 (s)	29	28.0 (t)	26.4 (t)	26.2 (t)
10	139.8 (s)	141.3 (s)	141.2 (s)	30	26.8 (t)[a]	24.3 (t)	24.2 (t)[b]
11	136.6 (d)	134.9 (d)	135.1 (d)	31	79.6 (d)	28.3 (t)	28.3 (t)
12	69.4 (s)	71.2 (s)	71.3 (s)	32	40.7 (t)	142.4 (d)	142.3 (d)
13	41.7 (t)	39.2 (t)	39.1 (t)	33	36.9 (t)	123.6 (d)	123.5 (d)
14	21.8 (t)	20.8 (t)	20.6 (t)	34	103.9 (s)	57.1 (d)	57.0 (d)
15	128.5 (d)	126.9 (d)	126.8 (d)	35	51.4 (t)	44.7 (t)	44.7 (t)
16	132.3 (d)	132.8 (d)	132.8 (d)	36	66.5 (t)	70.3 (t)	70.3 (t)

a), b) These assignments may be interchaged in the same column.

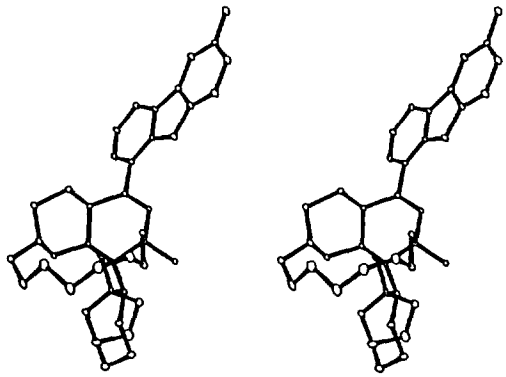


Fig. 3 Perspective Drawings of Manzamine X (**3**)

Table III. ^{1}H-NMR Data for Manzamine X (**3**), Manzamine Y (**4**), and Manzamine A (**5**)
(* δ 500 MHz in $CDCl_3$, J values in Hz, ** ref. 2c)

atom-No.	3 *	4 *	5 **	atom-No.	3 *	4 *	5 **
3	8.31 (d, J =5.1)	8.13 (d, J =4.8)	8.34 (d, J =5.1-5.3)	22	1.95 (m)	1.86 (m)	1.88 (m)
4	7.59 (d, J =5.1)	7.48 (d, J =4.8)	7.85 (d, J =5.1-5.3)		2.71 (m)	2.86 (m)	2.93 (m)
5	7.49 (d, J =2.5)	7.39 (d, J = 2.4)	8.08 (d, J =7.9)	23	1.51 (m)	1.74 (m)	1.78 (m)
6	----------	----------	7.23 (t, J =7.9)		1.93 (m)	2.86 (m)	2.95 (m)
7	7.13 (dd, J =2.5, 8.6)	7.08 (dd, J =2.4, 7.5)	7.52 (t, J =7.9)	24	3.00 (dd, J =12.0, 6.0)	2.55 (m)	2.55 (m)
8	7.26 (d, J =8.6)	7.54 (d, 7.5)	7.83 (d, J =7.9)	26	3.62 (s)	3.67 (s)	3.72 (s)
11	6.45 (s)	6.51 (s)	6.52 (s)	28	2.88 (dd, J =11.1 10.3)	3.21 (m)	3.27 (m)
13	1.68 (m)	2.02 (m)	1.75 (m)		3.34 (m)	3.98 (m)	4.03 (m)
	2.13 (m)		2.15 (m)	29	1.58 (m)	1.17-1.24 (m)	2.00 (m)
14	2.13 (m)	2.26 (m)	2.1-2.2 (m)		1.70 (m)	2.60 (m)	
	2.36 (m)			30	1.44 (m)	1.42 (m)	1.45 (m)
15	5.64 (m)	5.51 (m)	5.57 (m)		1.93 (m)	1.95 (m)	
16	5.53 (m)	5.51 (m)	5.57 (m)	31	4.55 (m)	2.26 (m)	2.30 (m)
17	1.70 (m)	1.57 (m)	1.60 (m)	32	2.40 (m)	6.20(m)	6.29(m)
	2.58 (m)	2.47 (m)	2.50 (m)		2.13 (m)		
18	1.44 (m)	1.17-1.24 (m)	1.20 (m)	33	1.44 (m)	5.30 (m)	5.39 (t)
		1.42 (m)	1.45 (m)	34	----------	4.89 (m)	4.94 (m)
19	1.43 (m)	1.42 (m)	1.45 (m)	35	2.34 (d-like, J =12.7)	1.83 (m)	1.85 (m)
	1.69 (m)	1.83 (m)	1.81 (m)		2.40 (d-like, J =12.7)	2.45 (m)	2.40 (m)
20	2.45 (m)	2.42 (m)	2.38 (m)	36	2.27 (d, J =12)	2.40 (m)	2.32 (m)
	2.67 (m)	2.55 (m)	2.58 (m)		3.14 (d, J =12)	2.86 (m)	2.88 (m)

HMBC Correlations of **4**:
H-3 / C-1, 4, 4a; H-4 / C-3, 4b, 9a; H-5 / C-4a, 6, 7, 8a; H-7 / C-5, 6, 8a; H-8 / C-6, 4b; H-11 / C-1, 24; H-13 / C-26; H-14 / C-13, 15, 16; H-15, 16 / C-14, 17; H-17 / C-18; H-20 / C-19, 22, 36; H-22 / C-20, 23; H-23 / C-24, 25; H-24 / C-22, 36; H-26 / C-11, 28, 36; H-31 / C-29; H-33 / C-31, 34, 35; H-35 / C-33; H-36 / C-25, 26, 35.

Another marine sponge of *Haliclona* sp., collected at the edge of the reef (-10 m), was also first extracted with acetone. The acetone extract was then fractionated in the same manner as carried out in the case of an above-described marine sponge of *Xestospongia* sp. (Chart 4). Here again, manzamine A (**5**) was obtained as the major constituent and silica gel and subsequent Sephadex LH-20 column chromatography of the crude alkaloid fraction provided a new manzamine-type alkaloid named manzamine Y (**4**) together with two known alkaloids, that is, manzamine B (**6**) and manzamine C (**7**).

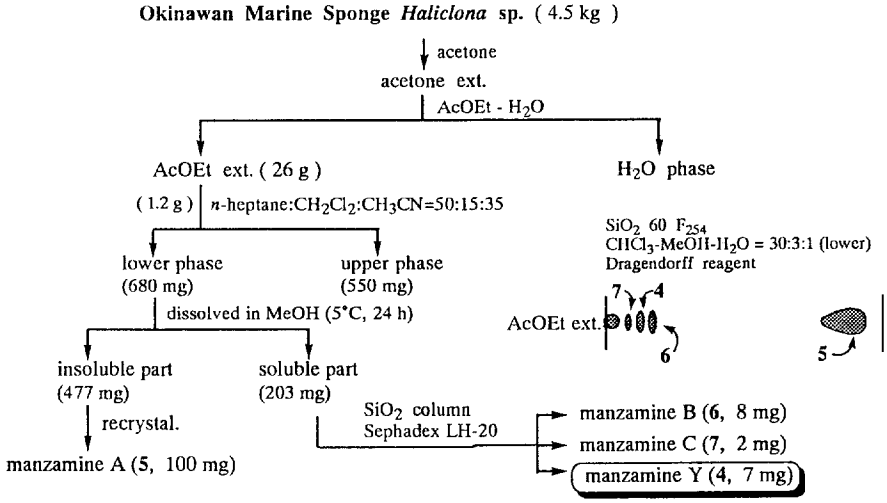


Chart 4

The FAB-MS of manzamine Y (**4**) showed $(M+H)^+$ ion peak at *m/z* 565 and the molecular composition $C_{36}H_{44}N_4O_2$ was determined by HR FAB-MS. The IR and UV spectra of **4** were alike to those of manzamine X (**3**), and the proton and carbon signals ascribable to the 6-hydroxy-β-carboline moiety were observed. In comparison of those physicochemical data for **4** with those for hitherto known manzamine-type alkaloids, manzamine Y (**4**) was presumed to be a 6-hydroxy analogue of manzamine A (**5**) and this presumption was substantiated by 2D-NMR analysis (COSY, C-H COSY, and HMBC) of **4**. All the proton and carbon signals were assigned as shown in Table II and III.

In 1992, Baldwin and Whitehead[3] proposed a hypothetical biogenetic pathway for manzamines A (**5**), B (**6**), and C (**7**), where manzamines were presumed to be biosynthesized from an intermediate composed of two dihydropyridine rings with an alkyl residue and a tryptophan. The proposal was based on the findings that several presumable intermediates in the biosynthetic pathway [*e.g.* ingenamine,[4a] keramaphidin B, [4b] xestocyclamine A,[4c] ircinal B,[2e] and manzamine J (**12**), [2c]] were isolated from marine sponges belonging to different genera. In regard to present manzamines X (**3**) and Y (**4**), manzamine Y (**4**) is presumed to follow manzamine A (**5**) *via* oxidation at the C-6 position. The tetrahydrofuran ring comprised in **3** is then presumed to be biosynthesized from **4** *via* initial allylic oxidation at C-31 in **4** and subsequent migration of the double bond ($\Delta^{32} \rightarrow \Delta^{33}$) and cyclization between hydroxyl at C-31 and C-34 as depicted in Fig. 4. On the other hand, a biogenetic pathway of xestomanzamines A (**1**) and B (**2**) is presumed as depicted in Fig. 5. That is, these alkaloids are presumed to be biosynthesized from an *N*-methyl histidine and a tryptamine units.

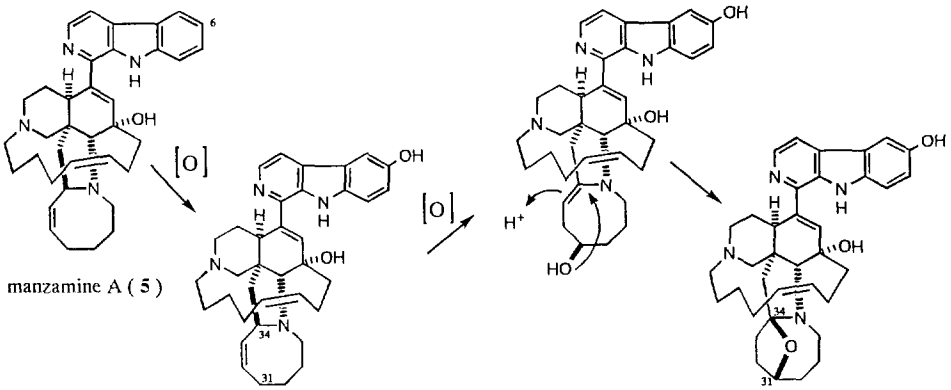


Fig. 4

Finally, it is quite interesting to point out that i) manzamine X (**3**) and manzamine Y (**4**) are the first example of 6-hydroxymanzamine-type alkaloids and ii) these alkaloids have been isolated from two marine sponges belonging to different genera, both collected at the close site in a coral reef. So that, it seems very likely that microorganism(s) may be participated in the biosynthesis of these manzamine-type alkaloids. In this connection, we have examined the inner tissue of these marine sponges under a scanning electron microscope. However, we have so far not yet been able to find any indication of microorganism(s) in these marine sponges. The study are currently in continuation.

Manzamines X (**3**), Y (**4**), and xestomanzamine B (**2**) exhibited weak cytotoxicities against KB cells with IC_{50} 7.9, 7.3 and 14.0 μg/ml, respectively.

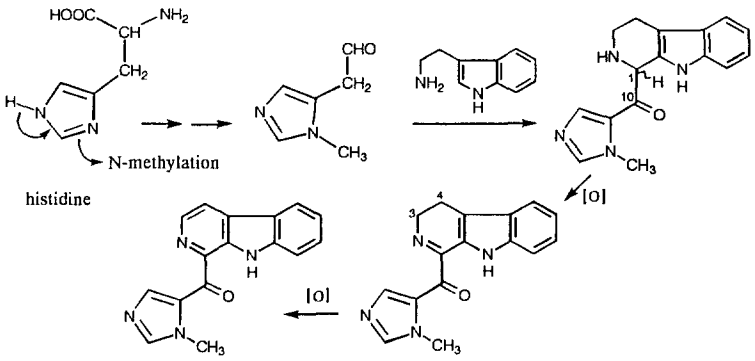


Fig. 5

Experimental Section

The IR spectra were obtained with a JASCO FT-IR 5300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL EX-270 (270 MHz) or GX-500 (500 MHz) spectrometer and with Me_4Si as the internal standard. The UV spectra were obtained with a Hitachi 330 spectrometer. The FAB-MS were recorded on a JEOL JMS SX-102 mass spectrometer. Melting points were determined on a Yanagimoto micro-melting point apparatus and recorded as read.

Isolation of Xestomanzamines A (1) and B (2), and Manzamines X(3), A (5), E (8), and F (9) from a Marine Sponge *Xestospongia* sp. The frozen sponge of *Xestospongia* sp. (19 kg), which was collected in June, 1992 at Iriomote Island, Okinawa, was initially steeped in acetone. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into an ethyl acetate-water mixture (1:1), and the ethyl acetate layer was taken and evaporated to give the ethyl acetate-soluble portion (42 g). The ethyl acetate-soluble portion (14 g) was then partitioned into an *n*-heptane-CH_2Cl_2-CH_3CN mixture (50:15:35). The residue (7.6 g) obtained by evaporation of the solvent from the lower layer was dissolved in MeOH and refrigerated at 5° C for 24 h. The resulting deposite of crystallized manzamine A (**5**, 1.2 g) was separated and the residue (5.6 g) obtained by evaporation of the mother liquid was separated by SiO_2 column ($CHCl_3$:MeOH=100:1) chromatography to give three Dragendorff-positive fractions [fractions I (2.3 g), II (0.5 g) and III (2.6 g)]. Fraction I (2.3 g) was subjected to SiO_2 column (n-hexane:AcOEt:acetone=7:1:1) and Sephadex LH-20 column ($CHCl_3$:MeOH=1:2) chromatography to give manzamine E (**9**, 41 mg) and manzamine F (**10**, 251 mg). Fraction II (0.5 g) was separated again by SiO_2 column ($CHCl_3$:MeOH:H_2O=150:3:1) and further purified by Sephadex LH-20 column ($CHCl_3$:MeOH =1:1) to afford xestomanzamines A (**1**, 9 mg) and B (**2**, 22 mg). Fraction III (2.6 g) was separated by SiO_2 column ($CHCl_3$:MeOH=30:1) to give crude manzamine X, which was further purified by recrystallization from *n*-hexane-acetone to give manzamine X (**3**, 40 mg).

Xestomanzamine A (**1**): yellow needles, mp 185-186°C ($CHCl_3$-MeOH). UV λmax (MeOH, nm (ε)): 221 (7400), 257 (1700), 300 (3900), 395 (1600). IR νcm^{-1} (KBr): 3427, 3075, 1612, 1211, 1128. ^{1}H-NMR

(270 MHz, $CDCl_3$, δ), ^{13}C-NMR (67.8 MHz, $CDCl_3$, δc): as shown in Table I. FAB-MS *m/z:* 277 $(M+H)^+$. HR FAB-MS *m/z*: Calcd for $C_{16}H_{13}N_4O$: 277.114. Found: 277.110.

Xestomanzamine B (**2**): yellow oil, UV λmax (MeOH, nm (ε)): 222 (27200), 270 (10300), 298 (16500), 388 (4500). IR cm^{-1} (KBr): 3451, 3110, 2926, 1641, 1190, 1130. ^{1}H-NMR (270 MHz, $CDCl_3$, δ), ^{13}C-NMR (67.8 MHz, $CDCl_3$, δc): as shown in Table I. FAB-MS *m/z*: 279 $(M+H)^+$. HR FAB-MS *m/z*: Calcd for $C_{16}H_{15}N_4O$: 279.125. Found: 279.125.

Manzamine X (**3**): yellow prisms, mp > 250°C (*n*-hexane-acetone). $[\alpha]_D$ +66.1° (*c*=1.93, $CHCl_3$, 19°C). UV λmax (MeOH, nm (ε)): 215 (29500), 300 (17000), 378 (4800). IR νcm^{-1} (KBr): 3290, 2930, 1640, 1562, 1462. ^{1}H-NMR (500 MHz, $CDCl_3$, δ), ^{13}C-NMR (67.8 MHz, $CDCl_3$, δc): as shown in Table II and III, respectively.

FAB-MS *m/z*: 581 $(M+H)^+$. HR FAB-MS *m/z*: Calcd for $C_{36}H_{45}N_4O_3$: 581.349. Found: 581.347.

Isolation of Manzamines Y (4), A (5), B (6) and C (7) from a Marine Sponge *Haliclona* sp. The frozen sponge of *Haliclona* sp. (4.5 kg, wet weight), also collected in June, 1992 at Iriomote Island, Okinawa, was extracted with acetone and the resulting extract was partitioned into an ethyl acetate-water mixture to give the ethyl acetate-soluble portion (26 g). The ethyl acetate-soluble portion (1.2 g) was partitioned into an *n*-heptane-CH_2Cl_2-CH_3CN mixture (50:15:35). The residue (680 mg) obtained by evaporation of the solvent from the lower layer was dissolved in MeOH and refrigerated at 5°C for 24 h. The resulting deposite of crystallized manzamine A (**5**, 100 mg) was removed and the residue obtained by evaporation of the solvent from the mother liquid was separated by SiO_2 column ($CHCl_3$→ $CHCl_3$: MeOH=100:1→ 30:1) and further purified by Sephadex LH-20 column ($CHCl_3$) to afford manzamine Y (**4**, 7 mg), manzamine B (**6**, 8 mg), and manzamine C (**7**, 2 mg).

Manzamine Y (**4**): yellow solid, $[\alpha]_D$ +33.2° (*c*=2.50, $CHCl_3$, 19°C). UV λmax (MeOH, nm (ε)): 215 (29500), 300 (11000), 378 (3000). IR νcm^{-1} (KBr): 3228, 2930, 1670, 1562, 1462, 1200. ^{1}H-NMR (500 MHz, $CDCl_3$, δ), ^{13}C-NMR (67.8 MHz, $CDCl_3$, δc): as shown in Table II and III, respectively. FAB-MS *m/z*: 565 $(M+H)^+$. HR FAB-MS *m/z*: Calcd for $C_{36}H_{45}N_4O_2$: 565.354. Found: 565.353.

Crystal Structure Analysis of Xestomanzamine A (1) and Manzamine X (3) The single crystals of **1** and **3** were obtained from $CHCl_3$-MeOH and *n*-hexane-acetone solvent system, respectively. All X-ray measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu Kα radiation (λ=1.5418 Å) and a 12kW rotating anode generator. Details of crystal data and intensity data collections were summarized as follows. Unit-cell dimensions were determined by a least-squares refinement using the setting angles of 25 carefully centered reflections in the range of $35°<2\theta<50°$. The weak X-ray reflectional intensities ($Fo<3\sigma(Fo)$) were rescanned to ensure good counting statistics. The stationary background counts were recorded on each side of the reflections. Four standard reflections were monitored for every 100 reflection intervals and showed no significant time dependence. The intensities were corrected for Lorentz and polarization effects, but not for absorption.

The structure was solved by direct method with MULTAN 87 program[5], and refined by the full matrix least-squares method with anisotropic temperature factors for non-hydrogen atoms using the program SHELX-76[6]. All hydrogen atoms were located on difference Fourier maps and refined with fixed isotropic temperature factors. All numerical calculations were carried out on a Micro Vax II computer at the Coputation Center, Osaka University of Pharmaceutical Sciences.

Stereoscopic molecular conformation of **1** and **3** are shown in Fig. 2 and 3, where the hydrogen atoms of **3** are omitted for the sake of clarity.

Crystal data of xestomanzamine A (**1**)[7]: $C_{16}H_{12}N_4O_1 \cdot CH_3OH$, M=308.342, monoclinic, space group $P2_1/a$, a=14.214 (6), b=10.759 (5), c=10.519 (2) Å, β= 111.76 (2)°, Z=4, Dx:1.371 g•cm^{-3}, μ(Cu-Kα)=7.21 radiation, F(000)=648, R_F=0.057 for 1962 contributing reflections. The coordinates of atoms were calculated.

Crystal data of manzamine X (**3**)[7]: $C_{36}H_{44}N_4O_3 \cdot H_2O$, M_r= 598.792, orthorhombic, space group $P2_12_12_1$, a=15.389(3), b=16.951(5), c=12.174 (2) Å, Z=4, Dx: 1.252 g•cm^{-3}, μ(Cu-Kα)= 6.17 radiation, R_F=0.077 for 2382 contributing reflections.

Oxidation of Xestomanzamine B (2) Xestomanzamine B (**2**, 3 mg) was kept in a microtube at room temp. (21°C) for 20 d. The resulting product was purified by Sephadex LH-20 column ($CHCl_3$:MeOH=1:1) to afford xestomanzamine A (**1**, 2.8 mg).

Acknowledgement

The authors are grateful to the Ministry of Education, Science, and Culture of Japan for financial support.

References

1) This paper constitutes Part XXXV of our studies on Marine Natural Products. Part XXXIV: Kobayashi, M.; Aoki, S.; Gato, K.; Matsunami, K.; Kurosu, M.; Kitagawa, I. *Chem. Pharm. Bull.*, **1994**, *42*, 2449-2451.

2) a) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.*, **1986**, *108*, 6404-6405; b) Sakai, R.; Kohmoto, S.; Higa, T.; Jefford, C. W.; Bernardinelli, G.*Tetrahedron Lett.*, **1987**, *28*, 5493-5496; c) Ichiba, T.; Sakai, R.; Kohmoto, S.; Saucy, G.; Higa, T.*Tetrahedron Lett.*, **1988**, *29*, 3083-3086; d) Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Tomotake, Y.; Matsuzaki, T. *Tetrahedron Lett.*, **1987**, *28*, 621-624; e) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sakai, T.; Kobayashi, J. *J. Org.Chem.*, **1992**, *57*, 2480-2483; f) Ichiba, I.; Corgiat, J. M.; Scheuer, P. J.; Borges, M. K. *J. Nat. Prod.*, **1994**, *57*, 168-170; g) Tsuda, M.; Kawasaki, N; Kobayashi, J. *Tetrahedron Lett.*, **1994**, *35*, 4387-4388.

3) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.*, **1992**, *33*, 2059-2062.

4) a) Kong, F.; Andersen, R. J.; Allen, T. M. *Tetrahedron*, **1994**, *50*, 6137-6144; b) Kobayashi, J.; Tsuda, T.; Kawasaki, N.; Matsumoto, K.; Adachi, T. *Tetrahedron Lett.*, **1994**, *35*, 4383-4386; c) Rodriguez, J.; Crews, P. *Tetrahedron Lett.*, **1994**, *35*, 4719-4722.

5) T. Debaerdemaeker, G. Germain, P. Main, C. Tate, M. M. Woolfson, MULTAN87, a system of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data: Universities of York, England, and Louvain, Belgium, 1987.

6) G. M. Sheldrick, SHELX76, Program for crystal structure determination, University of Cambridge, England, 1976.

7) Tables of atomic coordinates, temperature factors, bond lengths and angles have been deposited with the Cambridge Crystallographic Data Centre.

(*Received in Japan* 28 *December* 1994; *accepted* 3 *February* 1995)