required to reconstitute the polymeric blue carotenoprotein. However, some differences in the UV region of the absorbance spectrum of the reconstituted complex were found when it was compared with the native one (fig.). This indicates that although the structure of the reconstituted complex is fairly similar to that of the original one, since the interaction with the carotenoid occurs, the native conformation of the protein has not been fully recovered (probably the exposure of aromatic aminoacids has varied); nevertheless, the changes do not affect the carotenoid binding site to any marked extent.

Acknowledgments. This work was supported in part by a grant (Ref. 1931/82) from the Spanish 'Comisión Asesora para la Investigación Científica y Técnica'.

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0014-4754/86/091063-02$1.50 + 0.20/0

Hymenin, a novel α-adrenoceptor blocking agent from the Okinawan marine sponge *Hymeniacidon* sp.

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19 November 1985

Summary. A novel bromine-containing alkaloid, hymenin, has been isolated from the Okinawan marine sponge *Hymeniacidon* sp. as a potent α-adrenoceptor blocking agent and its structure determined to be **1** on the basis of the spectral data.
Key words. Sponge; hymenin; *Hymeniacidon* sp.; alkaloid; α-blocker.

α-Adrenoceptor blocking agents have been employed in basic research and in therapy under a wide variety of conditions. During our investigation on bioactive substances from marine invertebrates[1-4], we have examined the α-adrenoceptor blocking activity of 70% ethanolic extracts of various marine sponges collected at Okinawa, using isolated vascular smooth muscle. As a result, an orange marine sponge *Hymeniacidon* sp. has been found to possess a remarkable α-adrenoceptor blocking activity on the isolated rabbit aorta. In this communication, we report the isolation and structure elucidation of hymenin **1**, a novel α-adrenoceptor blocking constituent of *Hymeniacidon* sp.

Male albino rabbits (2–3 kg) were used. The procedure for preparing the isolated rabbit aorta and the technique of measurement of contractions were as previously described[5]. *Hymeniacidon* sp. was collected at Ishigaki Island, Okinawa, in June 1984. The methanol-toluene (3:1) extract of the sponge was partitioned between toluene and water. The aqueous phase was then extracted with chloroform, ethyl acetate and n-butanol. The butanol soluble material was passed through a silica gel column with chloroform-n-butanol-acetic acid-water (3:12:2:2) to afford an active fraction. This fraction was chromatographed on a Develosil ODS column with methanol-water (4:6) containing 0.05M acetic acid to yield hymenin **1** (0.005% wet wt) as an amorphous colorless solid.

The UV spectrum $\{\lambda_{max}^{MeOH}$ 274 nm (ε 9200)$\}$ and a positive color test of **1** with Echtrotsalz B suggest the presence of a substituted pyrrole chromophore[6,7]. The IR spectrum (KBr) showed an amide carbonyl band at 1680 cm^{-1}. The EI mass spectrum exhibited intense molecular ions at m/e 387, 389 and 391 (1:2:1), indicating that **1** is a dibrominated compound. The molecular formula $C_{11}H_{11}N_5OBr_2$ was established by high resolution FAB mass spectrometry (△ 1.2 mmu). The ^1H and ^{13}C NMR spectra of **1** were compared with those of oroidin **2** containing dibromopyrrole and guanidino moieties, which had been previously isolated from marine sponges of the genus *Agelas*[8-10].

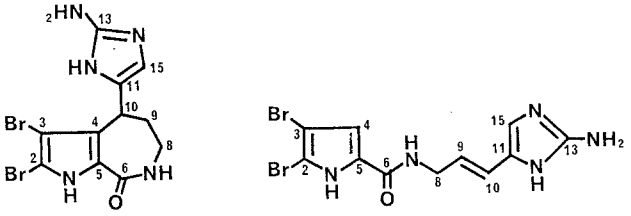
The detailed analyses of the ^1H and ^{13}C NMR spectra (table) revealed the structure (fig.) which was considered to result from an intramolecular cyclization at C–4 and C–10 of **2**. Thus, the ^{13}C chemical shifts of the pyrrole (C–2, δ 106.2; C–3, 100.7; C–5, 130.1) and amino imidazole (C–11, δ 124.5; C–15, 110.7; C–13, 147.6) of **1** correlated very well with those of the corresponding carbon atoms of **2** (C–2, δ 104.4; C–3, 97.8; C–5, 128.0; C–11, 124.7; C–15, 110.8; C–13, 147.5)[10]. The remaining carbon atoms of **1** (C–4, δ 123.8; C–9, 31.0; C–8, 36.4) were assigned as compared with the corresponding ^{13}C chemical shifts (C–4, δ 121.8; C–9, 29.0; C–8, 40.0) of debromo-hymenialdisine[11,12] having the similar ring system to **1**. The assignment of C–10 (δ 34.6) was established by proton selective decoupling experiments. In agreement with the ring system of **1**, extensive spin decoupling experiments revealed the unit $-CH=C-CH-CH_2-CH_2-NH$ (H–15, H–10, H–9, H–8 and NH–7), in which observations were made of the NOE (+1%) between H–10 and H–15. The chemical shift (δ 6.07) of H–10 was compatible with that (δ 6.24) of the corresponding proton of dihydrooroidin[13]. Additional support for the structure assigned derives from the EI mass spectrum of **1**, in which fragment ion peaks produced by expulsion of CH_2CH_2NHCO from M^+ were observed. Hymenin **1** contains an asymmetric center at C–10 $\{[\alpha]_D^{25} -15°$ (C = 0.5, MeOH)$\}$. The CD spectrum exhibited a positive Cotton effect with λ_{ext}^{MeOH} 242 nm (△ε +2.07); the stereochemistry remains to be assigned.

Hymenin appears to be closely related biogenetically to bromine-containing alkaloids such as oroidin[8-10], sceptrin[14] and keramadine[15] from marine sponges of the genus *Agelas*, mono-

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of hymenin **1**

Position	Proton	m	J (Hz)	Carbon	m
1	12.0[a]	brs[c]			
2				106.2[b]	s[c]
3				100.7	s
4				123.8	s
5				130.1	s
6				162.0	s
7	7.78	dd	2.1, 7.3,		
8	3.01 (3.12)[b]	ddd	7.3, 7.3, 14.0	36.4	t
	3.13 (3.24)	ddd	2.1, 9.8, 14.0		
9	1.84 (1.92)	ddd	4.3, 9.8, 14.0	31.0	t
	2.25 (2.27)	ddd	4.3, 7.2, 14.0		
10	3.92 (4.18)	t	4.3	34.6	d
11				124.5	s
12	7.48	brs			
13	5.41	brs		147.6	s
15	5.74 (6.07)	s		110.7	d

[a] δ in ppm, DMSO-d_6; [b] δ in ppm, MeOH-d_4; [c] Multiplicity in off-resonance decoupled spectrum.



1 2

Structures of hymenin **1** and oroidin **2**.

and dibromophakelin[16] from the marine sponge *Phakellia flabellata,* and hymenialdisine[11] from the marine sponge *Hymeniacidon aldis*. Hymenin **1** exhibits a potent α-adrenoceptor blocking activity. In the isolated rabbit aorta, the contractile response to norepinephrine (10^{-7}M) was abolished by **1** (10^{-6}M), whereas the responses to potassium chloride (4×10^{-2}M) and serotonin (10^{-6}M) were not affected by **1** (10^{-6}M). The only α-adrenoceptor blocking agent of marine origin which has previously been found is aaptamine from the marine sponge *Aaptos aaptos*[17]; the molecular skeleton of this substance is benzo-naphthyridine, and is therefore quite different from that of **1.** In addition, hymenin is an antibacterial agent, giving 12 and 13 mm zones of inhibition at a concentration of 10 μg/disc against *Bacillus subtilis* and *Escherichia coli,* respectively. Further clarification of the stereochemistry and the pharmacological properties of **1** is in progress.

Acknowledgments. We thank Dr T. Hoshino (Mukaishima Marine Biological Station, Hiroshima University) for his kind identification of the marine sponge, Dr N. Shoji (Institute of Pharmacy, Tokushima-Bunri University) and Dr M. R. Välchli (Japan Bruker) for the NMR measurements, Mr Z. Nagahama for his assistance in collecting the marine sponge, and Ms M. Hamashima of this institute for her technical assistance.

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0014-4754/86/091064-02$1.50 + 0.20/0

Aplysianin-A, an antibacterial and antineoplastic glycoprotein in the albumen gland of a sea hare, *Aplysia kurodai*

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Summary. Aplysianin-A, an antibacterial and antineoplastic factor in the albumen gland of the sea hare *Aplysia kurodai,* was isolated. It had a molecular weight of approximately 320 kD and consisted of subunits with a molecular weight of 85 kD. It contained 9.8% neutral sugar. Aplysianin A showed 50% inhibition of *Bacillus subtilis* growth at a concentration of 4 μg protein/ml and 50% lysis of murine MM46 tumor cells at 14 ng protein/ml. A partial identity of antigenic specificity of the purified specimen with an antineoplastic factor from *Aplysia* eggs was observed in immunodiffusion tests.
Key words. Antibacterial factor; antineoplastic factor; *Aplysia;* opisthobranch; albumen gland.

Sea hares, which are opisthobranch molluscs, have attracted the interest of many workers investigating chemical defense substances[2,3]. Most of these substances are low molecular weight compounds derived from algal diets. We found previously the presence of glycoproteins with antibacterial and antineoplastic activity in eggs and albumen glands of *Aplysia kurodai,* but not in mucous gland and other tissues[4,5]. The main active substance in eggs of *Aplysia* species was isolated as a glycoprotein[6]. Recently, we have found that the physiocochemical properties of the active factor in the albumen gland are different from those of the factor in the eggs laid. The latter is termed hereafter aplysianin-E, from the name of the suborder *Aplysiacea.* In this report, we describe the purification and characterization of the other active component, aplysianin-A, in the albumen gland of *A. kurodai.*

A. kurodai were collected from Lake Hamana, Shizuoka, in their spawning season (May and June), and from Okkirai Bay, Iwate, Japan in December 1984. Anterior genital mass, which is composed of the globular albumen gland and surrounding mucous gland, was removed from freshly dissected animals and was used for the extraction of active substances. Antibacterial activity was determined turbidometrically using *Bacillus subtilis,* as reported previously[4]. Antineoplastic activity of the purified aplysianin-A was measured in vitro using ^{51}Cr labeled murine MM46 cells[5].