tions) were observed (table). Mortality of embryos was similar in all groups and independent of both Cd concentration and incubation temperature (table).

Discussion. These observations indicate that under the experimental conditions explored, the survival of embryos does not seem to be noticeably affected by Cd. In contrast, the experimental embryos exhibit malformations, which are dose and temperature related. The fact that Cd toxicity may be influenced by temperature has been reported for other organisms[10, 13, 14]. In the case of *Bufo arenarum* embryos, high temperature may protect them against the teratogenic effects of Cd at lower concentrations, but at high concentrations of Cd the early malformations increase. These observations may be interesting because from an ecotoxicological point of view it is more probable that embryos will be exposed to low concentrations of Cd than to higher ones. In addition, as embryo development progresses, the initial protective effect of high temperature disappears, probably owing to a 'catch-up' phenomenon that occurs in the embryos treated at 20 °C. Considering hydropsy; this malformation may be related to a disturbance of the osmoregulatory mechanism by Cd[15]. Axial incurvations might be interpreted as being due to a displacement of calcium, as has been reported for fishes[16] and mammals[17]. The existence of cords and clusters of ciliated cells may be due to a disturbance in the differentiation of glandular cells which are intercalated between those with cilia.

The interference that Cd exerts on embryonic development seems to be related to its multiple effects upon enzymatic and structural proteins[18], and nucleic acids[18], as well as on the availability of essential elements[18,19] and energy-rich molecules[18, 19], with a consequent reduction of the performance of the embryo.

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2 Fellow of the CIC.

3 Member of the Carrera del Investigador Científico Tecnológico (CIC).

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A novel antagonist of serotonergic receptors, hymenidin, isolated from the Okinawan marine sponge *Hymeniacidon* sp.

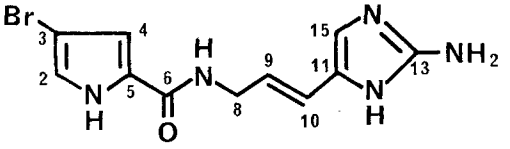
J. Kobayashi, Y. Ohizumi, H. Nakamura and Y. Hirata

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194 (Japan), and Faculty of Pharmacy, Meijo University, Nagoya 468 (Japan), 14 April 1986

Summary. A novel bromine-containing pyrrole compound, hymenidin, has been isolated from the Okinawan marine sponge *Hymeniacidon* sp. as a potent antagonist of serotonergic receptors and its structure elucidated using spectral data.
Key words. Sponge; hymenidin; *Hymeniacidon* sp.; bromopyrrole; antiserotonergic action.

In our studies on bioactive metabolites of marine organisms[2–5], extracts of numerous sponges collected in Okinawa were screened on the isolated rabbit aorta. The bioassay-directed purification resulted in isolation of a novel α-adrenoceptor blocking agent from the marine sponge *Hymeniacidon* sp.[6]. More recently, another fraction from an extract of the same sponge was found to inhibit markedly the contraction of the aorta induced by serotonin, but did not affect that induced by KCl or norepinephrine (NE). In this communication, we report the isolation and structure determination of **1**, a novel antagonist of serotonergic receptors, from the marine sponge *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[7]. The sponge *Hymeniacidon* sp., collected at Ishigaki Island, Okinawa, in June 1984, was stored at −20 °C until used. 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, successively. The butanol-soluble portion was subjected to 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 C_{18} reversed phase HPLC column (Develosil ODS, 5 μ, 10 × 250 mm) with methanol-water (2:3) containing 0.05 M acetic acid to yield hymenidin **1** (0.003% wet weight of the sponge) as an amorphous colorless solid.



1

Figure 1. Chemical structure of hymenidin **1**.

The UV spectrum {λ_{max}^{MeOH} 270 nm (ε 23,000)} and a positive color test of **1** with Echtrotsalz B (5-nitro-2-aminomethoxybenzene diazotate, Sigma) argue the presence of a substituted pyrrole chromophore[8,9]. The IR spectrum (KBr) showed an amide carbonyl band at 1675 cm^{-1}. The EI mass spectrum exhibited intense molecular ions ($C_{11}H_{12}N_5OBr$) at m/e 309 and 311 (1:1), and fragment ions at m/e 188 and 190 (1:1) and 171 and 173 (1:1) by loss of $C_6H_7N_3$ and $C_6H_{10}N_4$ from M^+, respectively. The detailed analysis of the ^1H NMR spectrum (DMSO-d_6, δ in ppm) revealed partial structure $-CO-NH-CH_2-CH=CH-$ (trans) [8.21 (H-7, exchangeable, brt, J = 5.5 Hz), 3.91 (H-8, t, J = 5.5 Hz), 5.83 (H-9, dt, J = 16.2 and 5.5 Hz) and 6.18 (H-10, d, J = 16.2 Hz)]. The ^1H NMR

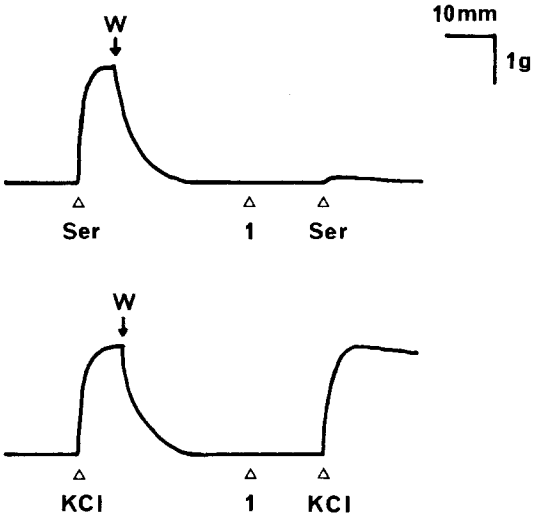


Figure 2. Effects of hymenidin **1** (10^{-5} M) on the contracture induced by serotonin (Ser, 10^{-6} M) and KCl (4×10^{-2} M) in the rabbit aorta. Drugs and **1** were applied at Δ. Ser and KCl were added 15 min after administration of **1**. Drugs were washed out twice (W) with fresh medium at arrows.

spectrum contained an exchangeable signal at 12.0 (H-1, brs) and signals for two aromatic protons at 6.86 (H-4, dd, $J_{2-4} = 1.2$ Hz and $J_{1-4} = 2.5$ Hz) and 6.94 (H-2, dd, $J_{4-2} = 1.2$ Hz and $J_{1-2} = 2.5$ Hz), indicating the existence of a 3,5-disubstituted pyrrole ring[10]. An aminoimidazole unit was suggested by the ^{13}C signals[11] at 125.2 (C-11), 147.8 (C-13) and 111.2 (C-15) and the ^1H signals at 12.0 (H-12, exchangeable, brs), 7.7 (13-NH_2, exchangeable, brs) and 6.40 (H-15, s)[12]. The connection of the pyrrole ring (C-5) to the amide carbonyl (C-6) was confirmed to be **1** (fig. 1) by the presence of the nuclear Overhauser effect (14%) between H-4 and H-7. Furthermore, the ^{13}C chemical shifts[11] of C-8 to C-15, correlating well with those of oroidin[6], supported the proposed structure of the olefinic part (C-8 ~ C-10) attached to the aminoimidazole ring (C-11 ~ C-15).

Hymenidin **1** appears to be closely related biogenetically to other bromopyrrole compounds found in marine sponges. Oroidin[13] is the 2-bromo form of **1**, while sceptrin[14] is a dimer of **1**. Hymenidin and oroidin are likely progenitors for bromine-containing sponge metabolites such as mono- and dibromophakellins[15] from *Phakellia flabellata*, dibromocantharelline and odiline[16] (identical with the compound named stevensine[12] later) from *Pseudaxinysaa cantharella* and hymenin[6] from *Hymeniacidon* sp. Hymenialdisine[17,18] from *Hymeniacidon aldis*, *Axinella verrucosa* and *Acanthella aurantiaca* contains a 2-bromopyrrole ring, differing from the other bromopyrrole metabolites found in marine sponges. Hymenidin exhibited a potent antiserotonergic activity. In the isolated rabbit aorta, the contractile response to serotonin (10^{-6} M) was abolished by **1** (1.5×10^{-5} M), whereas the responses to KCl (4×10^{-2} M) and NE (10^{-7} M) were not affected by **1** (fig. 2). From marine organisms, no antagonist of serotonergic receptors has yet been isolated apart from keramadine[10]. The antiserotonergic activity of **1** is almost equal to that of keramadine, although the structure of keramadine differs from that of **1** in the *cis* geometry of the double bond at C-9 and in the presence of a methyl group at N-12. Further clarification of the pharmacological properties of **1** is in progress.

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