LREIMS, m/e 249 (10%, $M^+ - CH_3$), ^1H NMR singlets at δ2.30 and 2.31 (3 H each), ^{13}C NMR signals at δ169.5 (s × 2) and 20.3 (q × 2), and an IR band at 1775 cm^{-1}). As expected for phenols, a bathochromic shift was observed in the UV spectrum upon addition of base (λ_{max} (MeOH, nm) 206 (ε 12 200), 223 (sh, ε 3800) and 289 (ε 3700) shifted to 209 (ε 16 500), 248 (ε 5800) and 303 (ε 5100)). Still to be accounted for are 10 protons, 4 carbons and 1 nitrogen. Based on the remaining ^{13}C and ^1H NMR data (δ 3.45 (6 H, s)/55.3 (q), 3.25 (2 H, t, J = 7.2)/27.5 (t) and 4.15 (2 H, t, J = 7.2)/(70.0 (t)), the final partial structure in **1a** must be N,N,N-dimethylethylamine where the nitrogen and β-carbon of the ethyl group are attached to ortho positions on the aromatic ring. Complete carbon assignments in the aromatic ring were made based on a long range C–H correlation NMR experiment (J = 10 Hz) which emphasizes three-bond coupling (H 8 and H 9 (δ 3.45) – C 2 (70.0), C 7a (139.5); H 2 (4.15) – C 8 and C 9 (55.3), C 3a (124.9); H 3 (3.25) – C 4 (112.5); H 4 (6.81) – C 6 (147.7), C 7a (139.5); H 7 (7.09) – C 3a (124.9), C 5 (149.5)). Assemblage of the partial structures suggested by these data yields 1,1-dimethyl-5,6-dihydroxyindolinium chloride as the proposed structure **1a**, and the corresponding diacetate as **1b**.

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α-Adrenoceptor blocking action of hymenin, a novel marine alkaloid

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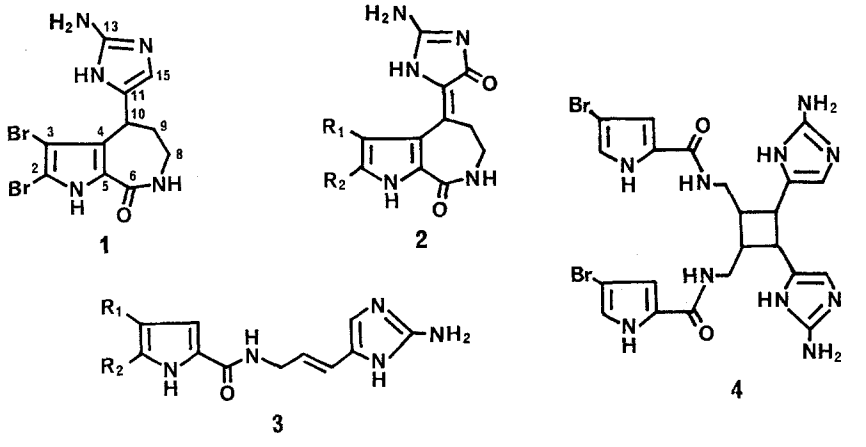
Summary. In the rabbit isolated aorta, hymenin (10^{-6} M), a novel marine alkaloid, caused a parallel rightward shift of the dose-response curve for norepinephrine without affecting that for histamine or KCl, suggesting that hymenin is a competitive antagonist of α-adrenoceptors in vascular smooth muscles.
Key words. α-Blocking action; hymenin; aorta; marine alkaloid; antagonist.

Marine organisms have proved to be a good source of compounds useful as tools for pharmacological, physiological and biological studies, since they act on specific sites in the cell membrane[1–4]. During our survey of marine natural products isolated by bioassay-guided purification, we have focused on compounds with α-adrenoceptor blocking activity because of their important role in basic and clinical pharmacology[5, 6]. Recently, a novel bromopyrrole compound, named hymenin (**1**), has been isolated as a potent α-adrenoceptor blocker from a marine sponge[7]. The present study was carried out to characterize the pharmacological properties of hymenin (**1**) and its related compounds (**2a, 2b, 3a, 3b** and **4**) as shown in figure 1.

Male Wistar rats (250–300 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The right carotid artery was cannulated for arterial blood pressure monitoring, and the blood pressure was continuously recorded by means of a pressure transducer on a polygraph. Drugs were administered via a cannulated right jugular vein. Male albino rabbits (2–3 kg) were killed by a blow on the head. The thoracic aorta was excised and mounted vertically in a 20-ml organ bath containing a Krebs-Ringer-bicarbonate solution of the following composition (mM): NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25.2, and glucose, 5.8, at pH 7.4, and were continuously gassed with 95% O_2 and 5% CO_2. The aorta was cut to form a helical strip as described previously[8]. Contractile force was recorded isometrically on a pen recorder. The following drugs were used in the present study: norepinephrine bitartrate (Sigma), histamine dihydrochloride (Wako Pure Chemical) and sodium



1 : Hymenin
2a: Hymenialdisine R_1 = H, R_2 = Br
2b: Debromohymenialdisine R_1 = R_2 = H
3a: Oroidin R_1 = R_2 = Br
3b: Hymenidin R_1 = Br, R_2 = H
4 : Sceptrin

Figure 1. Structures of hymenin (**1**) and its related compounds (**2a–4**) isolated from the sponge *Hymeniacidon* sp.

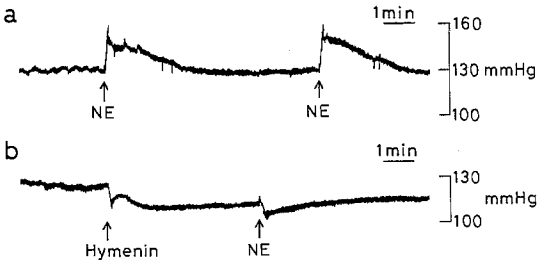


Figure 2. Effects of hymenin on blood pressure in rats. Hymenin (5 mg/kg) and norepinephrine (NE, 3 μg/kg) were administered via a jugular vein at arrows. *a* Control; *b* hymenin.

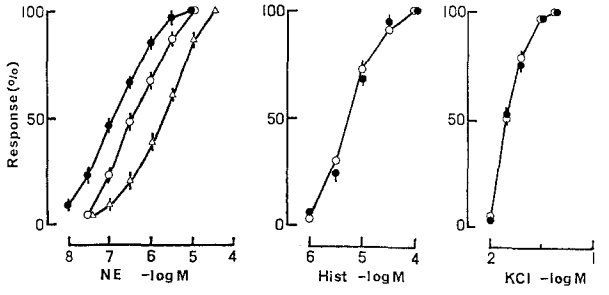


Figure 3. Log concentration-contractile response curves for norepinephrine (NE), histamine (Hist) and KCl in the isolated rabbit aorta in the presence or absence of hymenin. ●, Control; ○, hymenin at 10^{-6} M; △ hymenin at 10^{-5} M. NE was cumulatively added 15 min after the application of hymenin. The maximum response to each agonist is expressed as 100%. Symbols and vertical bars indicate means ± SEM (n = 4).

pentobarbital (Abbott Laboratories). Hymenin (**1**), hymenialdisine[9–11] (**2a**), debromohymenialdisine[10–12] (**2b**), oroidin[7,11,13,14] (**3a**), hymenidin[15] (**3b**) and sceptrin[16] (**4**) were isolated from the marine sponge *Hymeniacidon* sp. collected at Ishigaki island. These pyrrole compounds from the sponge were dissolved in dimethyl sulfoxide. All other drugs were dissolved in distilled water as required.

In rats (n = 3), hymenin (**1**) at 5 mg/kg caused a reduction of arterial blood pressure (15 ± 3 mm Hg) and its hypotensive effect lasted for 30 min or more (fig. 2). Pressure responses to norepinephrine (NE, 3 μg/kg) were depressed approximately 90% by hymenin (**1**) at 5 mg/kg.

As shown in figure 3, NE ($10^{-8} - 10^{-5}$ M), histamine ($10^{-6} - 10^{-4}$ M) or KCl ($10^{-2} - 4 \times 10^{-2}$ M) caused a concentration-dependent contraction of the aorta. The concentration-response curve for NE was shifted to the right in a parallel manner by treatment of the aorta with hymenin (**1**) at 10^{-6}–10^{-5} M, indicating competitive antagonism. But concentration-response curves for histamine and KCl were not affected by hymenin (**1**) even at 10^{-5} M. There were approximately 3-fold and 14-fold rightward shifts of the concentration-response curve for NE in the presence of hymenin (**1**) at 10^{-6}–10^{-5} M, respectively. Debromohymenialdisine (**2b**) at 10^{-4} M also brought about a rightward shift of the concentration-response curve for NE, whereas that for histamine or KCl was not modified. The PA_2 value was calculated by the method of van Rossum[17]. The PA_2 values of hymenin (**1**) and debromohymenialdisine (**2b**) were 6.14 ± 0.07 (n = 4) and 4.79 ± 0.17 (n = 4), respectively, in the aorta. In addition, the PA_2 value of phentolamine on the aorta was 7.80 ± 0.03 (n = 4) under the same condition. These data suggest that the inhibitory effects are probably specific for one agonist (NE).

After the hymenin-treated aorta had been washed out with fresh medium 4 times at 10-s intervals, the inhibitory effect of hymenin (**1**) was almost completely removed, indicating that the antagonism by hymenin (**1**) is reversible. In vascular smooth muscle, the NA-induced contraction is mediated through the α -adrenoceptor activation and is antagonized by specific α-adrenoceptor blocking agents[18]. These observations suggest that hymenin (**1**) and debromohymenialdisine (**2b**) possess α-adrenoceptor blocking activities in vascular smooth muscle. These results suggest that the hypotensive effect of hymenin (**1**) is related to α-adrenoceptor blockage.

On the basis of PA_2 values, hymenin (**1**) was 22 times more potent than debromohymenialdisine (**2b**) in the α-blocking activity. On the other hand, hymenialdisine (**2a**), oroidin (**3a**), hymenidin (**3b**) and sceptrin (**4**), which are compounds related to hymenin (**1**), did not affect the concentration-response curve for NE at concentrations of 10^{-5}–10^{-4} M. These results suggest that the presence of the seven-membered ring and the double bond between C-11 and C-15 of hymenin (**1**) may play important roles in the development of α-adrenoceptor blocking activity. Possibly, hymenialdisine (**2a**) and debromohymenialdisine (**2b**) possessing the double bond between C-10 and C-11 and the carbonyl group at C-15 have no or a little α-adrenoceptor blocking activity, while oroidin (**3a**), hymenidin (**3b**) and sceptrin (**4**), in which the seven-membered ring is opened, showed no α-adrenoceptor blocking activity but marked antiserotonergic activity[15], like keramadine[19] which is a bromopyrrole compound from a sponge of the genus *Agelas*. The bromine substitution at C-1 or C-2 on the pyrrole ring of hymenin (**1**) might also affect the α-adrenoceptor blocking activity; either increasing or decreasing the activity, depending on the position of bromine, since debromohymenialdisine (**2b**) without bromine showed the α-adrenoceptor blocking activity, but hymenialdisine (**2b**) with a bromine at C-2 lost the activity.

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