Cardiac Inhibitory Action of Constituents of the Marine Green Alga *Ulva pertusa*

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Abstract □ As part of a search for substances from marine organisms, which exhibit inotropic effects, the chemical constituents of the marine green alga, *Ulva pertusa* were investigated. The fractionated extract was tested for inotropic effects on the isolated guinea pig atria. The aqueous layer obtained from the acetone extract of fresh algae was concentrated, and the residue was extracted with methanol. The methanolic extract was fractionated by chromatography using mixtures of aqueous methanol. Elution with 50% aqueous methanol afforded material that had a significant negative inotropic effect. Further purification of this material by high-performance liquid chromatography (HPLC) using methanol–water (2:5) afforded crystalline adenosine, which was shown to be the active substance of *U. pertusa*, causing a negative inotropic action.

Keyphrases □ Cardioinhibitory effects—of extracts of the marine green alga (*Ulva pertusa*), adenosine □ *Ulva pertusa*—inotropic effects of extract fractions (adenosine), cardioinhibitory effects □ Adenosine—extracted from the marine green alga (*Ulva pertusa*), cardioinhibitory effects

The marine green alga, *Ulva pertusa*, is cultivated around the seashore in all parts of Japan, especially in the intertidal zone. This alga is also cultured *in vitro* (1). The green alga possesses only type I aldolase, which is also present in the animals and higher terrestrial plants, but not in bacteria which have the type II aldolase (2). It has been reported that a water-soluble fraction from the green alga had antimicrobial activity (3).

A screening program was designed to assess the biological activity of various marine organisms. In such screening tests, extracts of the marine green alga have produced cardioinhibitory action on the isolated guinea pig atria. A cardiotonic fraction (4) was extracted from a sea weed, *Undaria pinnatifida*, but the chemical nature of the active substance was not reported. Therefore, the purpose of this work was to investigate the chemical constituents and pharmacological properties of the active substance (causing the negative inotropic effect) of the green alga.

EXPERIMENTAL

Isolation—An acetone extract of *U. pertusa*[1] (5.6 kg) was concentrated under reduced pressure, washed successively with hexane (2 × 1.5 liters) and ethyl acetate (1.8 liters), and then concentrated *in vacuo* to dryness. The resulting brown powder was extracted with methanol to remove inorganic salts. After filtration the yellow methanolic solution was concentrated to give a light-brown oil (45 g), which showed a negative inotropic effect on the isolated guinea pig atria at concentrations $>10^{-4}$ g/ml. The crude oil was subjected to column chromatography on TSK-Gel G-3000 S[2] (220 ml). The column was developed successively with water (600 ml, fractions 1–6), aqueous methanol (80:20 200 ml, fractions 7 and 8), aqueous methanol (50:50, 200 ml, fractions 9 and 10), and methanol (400 ml, fraction 11). Fraction 9 (365 mg) was found to have inotropic activity; this fraction was purified by high-performance liquid chromatography (HPLC)[3] using a Megapack SIL C_8 column, a mobile phase of methanol–water (v/v 2:5), and a UV detector at 210 nm. The fraction corresponding to the major peak gave pale yellow crystals (46.3 mg, 8.3 × 10^{-4}% yield), which showed a negative inotropic effect at concentrations $>10^{-5}$ g/ml. Recrystallization from water gave colorless needles (32 mg): $C_{10}H_{13}N_5O_4$, mp 233–235°; UV[4] (methanol): 260 nm (ϵ 14,800); IR[5] (KBr): 1665, 1610, and 1580 cm^{-1}; ^{1}H-NMR[6] (deuterium oxide): δ 3.92 (3, m), 4.30–4.50 (2, m), 6.06 (1, d, J = 6 Hz), 8.16 (1, s), and 8.31 (1, s). The above data, as well as the chromatographic properties (TLC, HPLC) of the crystals, were in agreement with those of authentic adenosine[7].

Bioassay—The bioassay of fractions was performed on isolated guinea pig heart. The left atrium was separated from the rest of the heart and suspended in a 50-ml tissue bath containing physiological salt solution of the following composition (mM): NaCl, 120.3; KCl, 48; $CaCl_2$, 1.2; $MgSO_4$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25.2; and glucose, 5.5. The solution was bubbled continuously with a gas mixture of oxygen–carbon dioxide (19:1) and maintained at 30°, pH 7.4. Contractile response was recorded isometrically through a force-displacement transducer[8] and displayed on a polygraph[9]. The atrium was driven by an electrical stimulator[10] at a frequency of 2 Hz with square-wave pulses of 5 msec duration at 100% above threshold voltage. The atrium was allowed to equilibrate under 0.5-g tension for 60 min prior to beginning each measurement.

RESULTS AND DISCUSSION

Table I shows the negative inotropic effect of fractions extracted from the green alga *U. pertusa* on the guinea pig left atria driven electrically. The crude oil caused a negative inotropic effect at a concentration of 4 × 10^{-4} g/ml while fraction 9 (TSK-Gel filtration) showed an effect at 4 × 10^{-5} g/ml and the purified crystalline material at 10^{-5} g/ml. The potency of the purified crystalline material was essentially equivalent to

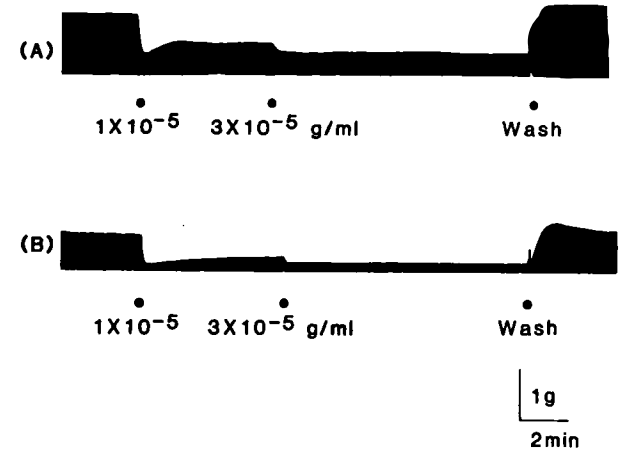

Figure 1—*Inhibitory effects of the final crystals (A) from* U. pertusa *and authentic adenosine (B) on the guinea pig atria driven electrically (2 Hz, 5 V, and 5 msec duration). The final crystals and adenosine at concentrations of 1 × 10^{-5} and 3 × 10^{-5} g/ml were applied to the muscle and then washed out of the solution.*

[1] Collected at Himaka-Jima, Aichi, Japan in August 1981. The alga was identified as *Ulva pertusa* (Chlorophyceae) by Professor W. Kida, Mie University, Japan. A voucher specimen (No. KY-15) representing material collected for this investigation is available for inspection at the Herbarium of Laboratory of Organic Chemistry, Department of Chemistry, Nagoya University, Nagoya, Japan.

[2] Toyo Soda Manufacturing Co., Ltd.

[3] JASCO TRI ROTAR-II liquid chromatograph.

[4] JASCO UVIDEC 510 spectrophotometer.

[5] JASCO Model IRS instrument.

[6] Varian HA-100D spectrometer.

[7] Kohjin Co., Ltd.

[8] Toyo Baldwin model T-7-30, Tokyo.

[9] Nihon Kohden model RM6000, Tokyo.

[10] Nihon Kohden model SEN7103, Tokyo.

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Table I—Inhibitory Effect of Adenosine and Fractions Extracted from *U. pertusa* on the Contractility of Isolated Guinea Pig Atria [a]

Substance	Concentration, g/ml	Inhibition, [b] %
Crude oil	4×10^{-4}	6.9
Fraction 9[c]	4×10^{-5}	40.4
Final crystals	1×10^{-5}	63.6
Adenosine	1×10^{-5}	69.0

[a] The guinea pig left atria was electrically driven with square pulses (2 Hz, 5 msec duration, and 5 V). [b] Inhibition was expressed by percent decrease in the systolic tension development of the atria. [c] Purified using column chromatography (TSK-Gel).

that of authentic adenosine, 1×10^{-5} g/ml. Figure 1 is representative of the negative inotropic effect of the purified crystalline material (1×10^{-5} and 3×10^{-5} g/ml) on the atria.

When the crystalline material was washed out of the tissue bath, the negative inotropic action of the agent disappeared. The contractility was restored to the control level within 1 min after washout, similar to the effect of the removal of adenosine reported by Baumann *et al.* (5). Furthermore, negative inotropic effects of the purified crystalline material and adenosine were not affected by the β-blocker propranolol (1×10^{-6} *M*) or the α-blocker phentolamine (1×10^{-6} *M*). Thus, the inhibitory action of the purified crystalline material is not mediated through the activation of adrenoceptors. In conclusion, the chemical and pharmacolgical results indicate that the cardioinhibitory substance isolated from the green alga *U. pertusa* is adenosine, which had not been found previously in marine organism.

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Degradation of Fenprostalene in Aqueous Solution

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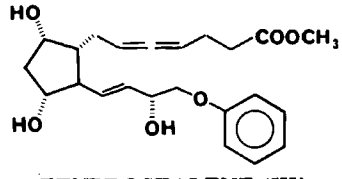
Abstract □ The degradation of the prostaglandin fenprostalene (III) was studied in aqueous solution. The reaction was both specific acid and base catalyzed. The only reaction found to occur was hydrolysis of the methyl ester at C-1. Activation energies for the acid- and base-catalyzed reactions were determined and are nearly identical to that for the hydrolysis of ethyl acetate, a model ester. A competing acid-catalyzed reaction of the C-1 free acid of III was found to be ~ 10 times slower than the hydrolysis of III.

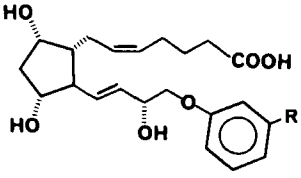
Keyphrases □ Degradation—fenprostalene, prostaglandins, high-performance liquid chromatography, kinetics, ester hydrolysis □ Fenprostalene—degradation, prostaglandins, high-performance liquid chromatography, kinetics, ester hydrolysis □ High-performance liquid chromatography—degradation of fenprostalene, kinetics, ester hydrolysis □ Ester hydrolysis—fenprostalene degradation, high-performance liquid chromatography, kinetics

The degradation of prostaglandin $F_{2\alpha}$ in aqueous solution is known to be acid catalyzed (1, 2). No evidence of degradation under alkaline conditions has been reported. Recently, the degradation of two $F_{2\alpha}$-type aryloxy prostaglandins (I and II) was reported by Jones *et al.* (3). The authors found that the degradation of these luteolytic agents in aqueous solution was acid catalyzed, and the rate of degradation as well as the type of products formed were dependent on the presence of oxygen in the solution. No degradation of these compounds was observed at pH values >5.

Fenprostalene (III)[1] is a new prostaglandin developed for use as an abortifacient and for estrus synchronization in cattle (4, 5). The likely degradation route of this F-type prostaglandin at high pH is hydrolysis of the C-1 methyl ester group. However, we were interested to see whether acid-catalyzed decomposition reactions analogous to those found for I and II (3) or $PGF_{2\alpha}$ itself (1, 2) might compete with acid-catalyzed hydrolysis of the ester moiety in III at low pH. Accordingly, we have studied the degradation of fenprostalene (III) and its C-1 free acid (IV) in aqueous solution as a function of pH.



FENPROSTALENE (III)



I: R = CF_3
II: R = Cl

EXPERIMENTAL

Materials—The fenprostalene (6) used was 99% pure by high-performance liquid chromatographic (HPLC) area normalization. The methanol was glass-distilled HPLC grade, and the water was purified through a filtration and ion exchange system[2]. All other chemicals were reagent grade quality.

Kinetics Methods—Buffer solutions contained 0.025 *M* total buffer,

[1] Fenprostalene is the generic name for methyl 7-[3,5-dihydroxy-2-(3-hydroxy-4-phenoxy-1-butenyl)cyclopentyl]-4,5-heptadienoate.

[2] Barnstead Nanopure System.

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