ii) 2,6-Bisacetanilino-4-picoline (**6**): A mixture of **7** (1 g) and acetic anhydride (0.8 g) was refluxed for 2 hr. The reaction mixture was then concentrated *in vacuo*, and the resulting residue was recrystallized from benzene to give **6** (0.6 g, 46%) as colorless needles, mp 148°. *Anal.* Calcd. for $C_{22}H_{21}N_3O_2$: C, 73.51; H, 5.89; N, 11.69. Found: C, 73.44; H, 5.76; N, 11.67. The IR and NMR spectra of this compound were identical with those of the compound obtained by desulfurization of **5a**.

5-Acetyl-13-methyl-5H,7H-bis[1,4]benzothiazino[3,2-*b*; 2',3'-*e*]pyridine (5b)—Compound **5a** (0.5 g) was dissolved in acetic acid (20 ml) with heating in a water bath. The resulting solution was cooled to room temperature and mixed with concentrated HCl (2 ml). After standing for 5 hr, the mixture was diluted with 20 ml of water, neutralized with 30% K_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to leave a crystalline mass, which was recrystallized from $CHCl_3$ -*n*-hexane to give **5b** (400 mg, 88.9%) as yellow needles, mp 298–300° (dec.). *Anal.* Calcd. for $C_{20}H_{15}N_3OS_2$: C, 63.66; H, 4.01, N, 11.14. Found: C, 63.44; H, 3.95; N, 11.14.

13-Methyl-5H,7H-bis[1,4]benzothiazino[3,2-*b*; 2',3'-*e*]pyridine (5c)—A mixture of **5a** (1.5 g), concentrated HCl (5.5 ml) and acetic acid (60 ml) was heated on a water bath for 15 min. The hydrochloride of **5c** formed in the reaction mixture was collected by filtration and washed with EtOH and water. Red needles. Yield, 1 g (83.3%). mp 191–193°. *Anal.* Calcd. for $C_{18}H_{14}ClN_3S_2$: C, 58.13; H, 3.79; N, 11.30. Found: C, 58.19; H, 3.53; N, 11.27. A suspension of the hydrochloride of **5c** (500 mg) in triethylamine (5 ml) was allowed to stand overnight. The product was collected by filtration, washed with water and recrystallized from benzene to give the base of **5c** (360 mg, 80%) as yellow needles, mp 219–221°. *Anal.* Calcd. for $C_{18}H_{13}N_3S_2$: C, 64.45; H, 3.91; N, 12.53. Found: C, 64.20; H, 4.91; N, 12.46.

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Prostaglandin $F_{2\alpha}$ from the Japanese Coastal Gorgonian, *Euplexaura erecta*¹⁾

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A biologically active substance with contracting activity towards isolated guinea-pig ileum has been isolated from the gorgonian *Euplexaura erecta* collected in Japanese coastal waters, and it was identified as prostaglandin $F_{2\alpha}$. This is the first report of the isolation of prostaglandins from gorgonians obtained outside the Caribbean area.

Keywords—*Euplexaura erecta*; prostaglandin $F_{2\alpha}$; Japanese coast; gorgonian; contracting activity; guinea-pig ileum; soft coral; thin-layer chromatography; mass spectrum

In 1969, Weinheimer and Spraggins³⁾ reported the surprising discovery of the occurrence of relatively large quantities of two unusual prostaglandins in air-dried specimens of a soft coral, the gorgonian *Plexaura homomalla*, occurring in coastal waters off Florida. These prostaglandins, (15*R*)-PGA₂ (**1**, 0.2% of the dried weight) and its acetate methyl ester (**2**, 1.3%), differ from the previously known prostaglandins isolated from mammalian sources in having the “nonmammalian” *R* configuration at the allylic hydroxyl center.

- 1) A preliminary report of this work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April, 1976, Abstracts, II, p. 263.
- 2) Location: 2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan.
- 3) A.J. Weinheimer and R.L. Spraggins, *Food-Drugs Sea, Proc. Conf. Drugs Sea*, 2nd, 1969, 311 (1977); *idem*, *Tetrahedron Lett.*, 1969, 5185.

Subsequently, a number of prostaglandins have been isolated from gorgonians by several groups⁴⁾ and some species were found to contain 15S isomers, having the "mammalian" configuration. However, gorgonians containing prostaglandins have so far only been found in the Caribbean area.

We report here the isolation of a biologically active substance from a Japanese coastal gorgonian and its identification as $\text{PGF}_{2\alpha}$ (3). This finding is the first demonstration that gorgonians containing prostaglandins are not limited to the species in the Caribbean area.

In the course of research aimed at the isolation of biologically active substances from marine natural products, a water solution of a methanolic extract of *Euplexaura erecta* (Japanese name: Ohgi-hutoyagi) collected at Sagami Bay in Japan, was passed through an Amberlite XAD-2 column, which was then eluted with methanol. The methanol eluate showed significant contracting activity with isolated guinea-pig ileum strips. In this bioassay, histamine, acetylcholine, bradykinin, $\text{PGF}_{2\alpha}$, etc., show contracting effects, while papaverine, paragraine,⁵⁾ etc., have relaxing activities.

Fractionation of the methanolic extract was carried out to isolate the active substance, as shown in Chart 2, following the activity by means of the above bioassay.

4) a) W.P. Schneider, R.A. Morge, and B.E. Henson, *J. Am. Chem. Soc.*, **99**, 6062 (1977); b) W.P. Schneider, G.L. Bundy, F.H. Lincoln, E.G. Daniels, and J.E. Pike, *ibid.*, **99**, 1222 (1977), and references cited therein.

5) Y. Komoda, S. Kaneko, M. Yamamoto, M. Ishikawa, A. Itai, and Y. Iitaka, *Chem. Pharm. Bull.* (Tokyo), **23**, 2464 (1975).

The contracting activity found in the methanolic extract was concentrated successively in the ethyl acetate layer of an ethyl acetate–water partition, and in the acidic fraction obtained by treatment of the ethyl acetate layer with 5% sodium bicarbonate solution.

The acidic fraction was subjected to two successive preparative thin-layer chromatography (TLC) runs on silica gel. The first preparative TLC was carried out using a plate with a layer of 2.0 mm thickness. After development and removal of solvents by evaporation, the plate was covered with a glass plate except for one strip, exposed to iodine vapor, and then divided into six zones according to the visualized bands. The contracting activity was found only in the fraction which was obtained by extracting the adsorbent in the zone of $R_f=0.08-0.25$, which showed three spots on analytical TLC. This active fraction was further purified by a second preparative TLC with plates having a 0.25 mm layer, giving the active substance (AS) which showed essentially a single spot on analytical TLC.

The contracting activity on guinea-pig ileum strips and the acidic nature of AS suggested that it might be a prostaglandin (s), and this was supported by the finding that the R_f value (0.17) of AS was identical with that of $\text{PGF}_{2\alpha}$. This identification was confirmed as followed.

The methylated residue obtained by treatment of AS with diazomethane was purified by preparative TLC on silica gel, affording an active substance methyl ester (AS-Me), which had the same R_f value as $\text{PGF}_{2\alpha}$ methyl ester. AS-Me was treated with bis(trimethylsilyl)-trifluoroacetamide, and the mass spectrum of the resulting product (AS-Me-TMS) was identical with that of the trimethylsilyl derivative of $\text{PGF}_{2\alpha}$ methyl ester (M^+ and all fragment ions).

Therefore, it was concluded that the active substance contained in *E. erecta* was $\text{PGF}_{2\alpha}$. Schneider *et al.*^{4b)} have also isolated traces of $\text{PGF}_{2\alpha}$ from *P. homomalla* (var. S) from the Cayman Islands.

In the case of *P. homomalla*, various other prostaglandins have been isolated as their free acids and/or methyl esters in relatively high yields. However, no prostaglandins other than $\text{PGF}_{2\alpha}$ have so far been found in the acidic or neutral fractions (Chart 2) of *E. erecta*.

Experimental

Bioassay—A strip of ileum (about 20 mm in length) was prepared from isolated ileum of a male or female guinea-pig (250–500 g), and a load (4 g) was applied at 37° in a Magnus bath (10 ml) containing Mg^{2+} -free Tyrode's solution through which air was blown. The isometric contractions obtained on addition of samples dissolved in water to the Magnus bath, were recorded with a Nihon-Kohden SB-1T force-displacement transducer connected to a Nihon-Kohden RJG-3024 rectifier.

Extraction of *Euplexaura erecta*—Samples (775 g) of *E. erecta* (Japanese name: Ohgi-hutoyagi) were collected in Nov., 1974 at Shimoda, Sagami Bay. They were cut into small pieces (1–3 cm in length) and extracted with methanol at room temperature for 24 hr. The mixture was filtered and the residue was extracted twice with fresh methanol under the same conditions. The filtrates were combined and concentrated *in vacuo* at 35–40°, giving a methanolic extract (24.5 g).

Treatment of the Methanolic Extract with Amberlite XAD-2—The methanolic extract (50 mg) was dissolved in water (10 ml) and passed through an Amberlite XAD-2 column (5 ml). The column was washed with water (12 ml) and then eluted with methanol (25 ml). On bioassay, the methanol eluate (3 mg) showed significant contracting activity, while the combined water eluate had no effect.

Partition of the Methanolic Extract—The methanolic extract (24.2 g) was partitioned between ethyl acetate (100 ml) and water (50 ml). The aqueous layer was washed with ethyl acetate (4 × 20 ml). The combined ethyl acetate layer, which showed contracting activity on bioassay, was extracted with 5% sodium bicarbonate solution (50 ml, then 4 × 20 ml), washed with water, dried over anhydrous sodium sulfate, and evaporated down to afford a neutral fraction (1.63 g), which was devoid of biological activity. The combined sodium bicarbonate layer was washed with ethyl acetate (10 ml), acidified with 2N hydrochloric acid, and extracted with ethyl acetate (50 ml, then 4 × 20 ml). The combined ethyl acetate layer was washed, dried, and evaporated down as before, giving an acidic fraction (54 mg) which showed biological activity.

Preparative TLC of the Acidic Fraction—The acidic fraction (52 mg) was dissolved in ethyl acetate, applied to a pre-coated silica gel plate (E. Merck, 2.0 mm × 20 cm × 20 cm), and developed with benzene–dioxane–acetic acid (20:10:1). The plate was covered with a glass plate except for one strip, exposed to iodine vapor and divided into six zones based on the visualized bands. The adsorbent of each zone was

scraped off and extracted with 10% methanol-ethyl acetate. The contracting activity was found only in the fraction with $R_f=0.08-0.25$. This active fraction showed three major spots on analytical TLC developed with the same solvent system.

Preparative TLC of the Active Fraction—The active fraction was further purified by preparative TLC on eight silica gel plates (E. Merck, 0.25 mm $\times$ 20 cm $\times$ 20 cm) using the procedure described above. Each plate was divided into five fractions, and bioassay of these fractions showed that only one of them contained the active substance (AS), which corresponded to the middle of the three major spots of the above active fraction. AS had the same R_f value (0.17) as PGF_{2 α} .

Preparation of the Active Substance Methyl Ester (AS-Me)—A solution of AS in methanol (1 ml) was treated with an ethereal solution of CH₃N₂ at room temperature. The solvent was evaporated off after 1 min. The residue was purified by preparative TLC on silica gel, developing with benzene-dioxane (5:4), to afford AS-Me, which had the same R_f value (0.22) as authentic PGF_{2 α} methyl ester.

Trimethylsilylation of AS-Me and Mass Spectrometry—A methanolic solution of AS-Me was transferred to a probe for mass spectrometry and dried sufficiently *in vacuo*. The residue was treated with one drop of bis(trimethylsilyl)trifluoroacetamide at room temperature for 1 hr under a dry atmosphere. The resulting AS-Me trimethylsilyl derivative (AS-Me-TMS) was used directly to obtain a mass spectrum (Hitachi RMU-7M mass spectrometer; ionizing voltage, 70 eV; sample heating temperature, 105°). The spectrum was identical (M^+ 584 and all fragment ions) with that of an authentic PGF_{2 α} -Me trimethylsilyl derivative obtained from PGF_{2 α} -Me by the same procedure.

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Effect of Ginseng Saponin on Serine Dehydratase Activity in Rat Liver

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Treatment of rats with saponin (fraction 5) from the roots of *Panax ginseng* C.A. MEYER caused a decrease in the activity of hepatic serine dehydratase (EC 4.2.1.13). Maximum decrease in the enzyme activity was observed 2 hr after the administration of the ginseng saponin, and it was found that this response depended on the amount of fraction 5 administered to rats. In contrast, when the animals were starved, ginseng treatment resulted in an increased level of the enzyme.

Keywords—*Panax ginseng* C.A. MEYER; serine dehydratase; rat liver; enzyme activity; saponin; starvation

As reported previously,²⁾ treatment of rats with saponin from the roots of *Panax ginseng* C.A. MEYER produced an increase in the activity of hepatic pyruvate kinase (EC 2.7.1.40) in rats fed on a laboratory pellet chow. In contrast, when glycolysis occurred on a major scale, *i.e.*, on feeding a high carbohydrate diet, ginseng treatment resulted in decreased pyruvate kinase activity. These results suggest that the ginseng saponin may influence the direction of liver carbohydrate metabolism through its effects on enzyme systems.³⁻⁵⁾ From this point of view, the present paper describes the effects of ginseng saponin on the activity of serine dehydratase (EC 4.2.1.13), which catalyzes the degradation of serine to pyruvate and ammonia.

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5) T. Yokozawa, K. Kanai, M. Takefuji, and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **24**, 3202 (1976).