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Prostaglandin (15S)-PGA_2 derivatives in the gorgonian *Plexaura homomalla* (Esper), forma *kükenthali* Moser[1]

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Summary. The lipid fraction, 17%, of air-dried *Plexaura homomalla* forma *kükenthali,* collected in the Caribbean at Puerto Rico, yielded 50% of its weight as the mammalian prostaglandin (15S)-PGA_2 methyl ester. The freeze-dried gorgonian yielded (15S)-PGA_2 largely as the acetate of the methyl ester. (15S)-PGA_2 was also obtained from material collected at St. Croix and at South Caicos. Field observations indicate that *Plexaura kükenthali* may be a species separate from *Plexaura homomalla* and that it is abundant on some shallow water reefs in the Caribbean. Prostaglandins could not be detected in the lipid fraction of eggs isolated from *Plexaura homomalla* (Esper) forma *homomalla.*
Key words. Gorgonia; *Plexaura homomalla;* prostaglandins.

In 1969, Weinheimer and Spraggins[2] reported the isolation of derivatives of the 'non-mammalian' prostaglandin, 15-epi PGA_2 or (15R)-PGA_2, from the gorgonian *Plexaura homomalla* (Esper) forma *homomalla*[3] collected off the Florida Keys. A few years later derivatives of the prostaglandins identical with those derived from mammalian sources, (15S)-PGA_2 and PGE_2, were obtained from *Plexaura homomalla* collected at Grand Cayman[4].

Bayer[5] has raised the question whether the collection in which (15R)-PGA_2 was found might have been a mixture of the typical *homomalla* and the *kükenthali* forms of the gorgonian, and suggested that the *kükenthali* form should be investigated for its prostaglandins. Our first observations at Puerto Rico and St. Croix indicated that the *kükenthali* form is very abundant on some shallow water reefs, that it occurs side by side with the 'true' *Plexaura homomalla,* and that the living gorgonians differ in a number of ways. It is likely that *Plexaura kükenthali* is a species separate from *Plexaura homomalla.* Preliminary surveys, in 1974, indicated the *kükenthali* form to be rich in prostaglandins, established in 1978 as derivatives of the mammalian (15R)-PGA_2.

Kung and Ciereszko[6] found eggs in the gorgonian *Pseudopterogorgia americana* (Gmelin) to be very rich in lipids in which the saturated wax cetyl palmitate made up some 70% of the total lipid. In contrast, the eggs obtained from *Plexaura homomalla* forma *homomalla* were found to contain much unsaturated lipid leading us to suspect the presence of prostaglandins. We therefore analyzed the egg lipids of *homomalla* for PGA_2, with negative results.

Materials and methods. Specimens of *Plexaura homomalla* forma *kükenthali* were collected in the Caribbean Sea, on San Cristobal Reef off La Parguera, Puerto Rico, at Tague Bay, St. Croix, US Virgin Islands, and at South Caicos, in December 1976 and January 1978. Specimens collected at Puerto Rico were deposited in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, D.C., as vouchers. The live *Plexaura kükenthali* had appearance and texture clearly different from those of live *P. homomalla.* Some specimens were frozen fresh and freeze-dried shortly after collection, before they were discolored. Others were hung on a line to drip dry. Drying was completed with a hot air blower. The South Caicos sample was preserved in alcohol. Air and freeze dried samples were ground and extracted with methanol-chloroform (1:1) or with n-hexane in a Soxhlet extrator. Lipid content of the dried cortex approached 17%. The lipid extracts were analyzed by chromatographic and spectrophotometric methods.

Eggs were obtained, at Puerto Rico, in December 1976, from freshly collected *P. homomalla* by placing them for an hour in fresh water and then transferring them to sea water. Exposure to fresh water softened the cortex of the gorgonian so that the cortex could be stripped off the skeleton easily, releasing eggs which floated. Eggs were removed from the surface of the sea water by skimming with a fine sieve and washing with sea water. The eggs were preserved in ethanol and later extracted with ethanol, ether and chloroform to yield an unsaturated lipid extract. No prostaglandin could be detected in the lipid extract by chromatographic analysis[7].

Results and discussion. The dark brown lipid extract of air-dried, *P. kükenthali* showed on thin-layer chromatography[7] a strong UV absorbing spot, R_f 0.61, like that of authentic (15S)-PGA_2 methyl ester. On gas chromatography (6 ft. 1% OV-17 at 220°) the largest peak eluted had a retention time of 5.1 m, corresponding to that of (15S)-PGA_2 methyl ester. The UV spectrum in 95% EtOH showed strong absorption around 218 nm which shifted to 278 nm on addition of 45% KOH to the

cell, corresponding to the isomerization of PGA_2 methyl ester to PGB_2 methyl ester.

A 2.0 g sample of the total lipid extract was chromatographed on 200 g of Merck silica gel, eluting with a total of 4 l of a gradient of 25→75% ethyl acetate in Skellysolve B, collecting 100 ml fractions. Fractions 13–18, combined on the basis of thin-layer chromatography, contained 1.02 g of (15S)-PGA_2 methyl ester with proton NMR identical with that of mammalian derived material. The amount of PGA_2 methyl ester from crude extract of *P. kükenthali* is about the same, about 50%, as that obtained from *P. homomalla*[4]. To further confirm configuration at C-15, a 200 mg sample from fraction 13–18 above was converted to PGB_2 methyl ester as follows. It was dissolved in 5 ml toluene, 10 drops of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) were added and the mixture was stirred at room temperature. After 72 h the solution was diluted with ethyl acetate, washed with 1 N HCl, then saturated salt solution, dried with Na_2SO_4 and evaporated. The brown residue was chromatographed on silica gel, eluting with 60% ethyl acetate-Skellysolve B, to give 109 mg of material with a proton NMR spectrum identical with that of (15S)-PGB_2 methyl ester derived from mammalian sources. Rotations were determined for a solution, 20 mg in 2 ml ethanol, at several wavelengths (Perkin-Elmer No. 241 polarimeter): $[\alpha]^{25}_{589} = +24.1°$, $[\alpha]^{25}_{578}$, $[\alpha]^{25}_{546} = +30.1°$, $[\alpha]^{25}_{436} = +65.2°$.

An authentic sample, 200 mg, of (15S)-PGA_2 from mammalian sources was treated as above in toluene with DBU, and the isomerized acid was esterified with ethereal diazomethane and chromatographed for PGB_2 methyl ester. The purified (15S)-PGB_2 methyl ester showed the following rotations (20 mg in ethanol): $[\alpha]^{25}_{589} = +22.7°$, $[\alpha]^{25}_{578} = +23.9°$, $[\alpha]^{25}_{546} = 28.3°$, $[\alpha]^{25}_{436} = 61.3°$.

Other specimens of *P. kükenthali* collected at different locations were extracted with chloroform-methanol (1:1) to give lipid extracts which were compared with samples of PGA_2 acetate methyl ester and PGA_2 methyl ester. Freeze-dried samples from Puerto Rico and from St. Croix showed mostly PGA_2 acetate methyl ester, and a little PGA_2 methyl ester on thin-layer chromatography. A Puerto Rico sample which was frozen, then thawed before freeze-drying, showed only a trace of the acetate along with the PGA_2 methyl ester. Evidently enzymatic hydrolysis took place on thawing the frozen sample. A South Caicos sample preserved in isopropanol also gave mostly the methyl ester. Schneider et al.[4] report the presence of an esterase in *Plexaura homomalla* which splits the prostaglandin acetate methyl ester to the prostaglandin methyl ester.

Although air-dried *P. kükenthali* resembles air-dried *P. homomalla*, it is unlikely that the two would be confused in the field by an experienced collector, as living specimens can be distinguished easily by their appearance. Dr Tom K. B. Karns who collected the specimens of *P. homomalla* used by Spraggins[22] for the initial isolation of (15R)-PGA_2 selected only the true *P. homomalla* which differs from *kükenthali* in its color, texture of cortex, growth habit and attachment. Live *P. kükenthali* has a greyish color with a tinge of violet, is less robust than *homomalla*, less strongly attached to its substrate, and 'bleeds blue' shortly after removal from the sea. It is likely that *Plexaura kükenthali* is a species separate from *P. homomalla*. In Puerto Rico and St. Croix they occur side by side in the same shallow water environments.

Another plexaurid which has been confused with *P. homomalla* is an as yet undescribed species from which we have obtained three cembranoid derivatives not present in *P. homomalla*, and which does not yield significant amounts of prostaglandins[8]. Morse et al.[9] have demonstrated the occurrence of a prostaglandin-endoperoxidase in both forms of *Plexaura homomalla, (homomalla* and *kükenthali)*, as well as in a number of other species of gorgonians which do not contain prostaglandins.

Following the discovery of the occurrence of prostaglandins in *P. homomalla*[2,4] they have been sought in other marine organisms. Prostaglandin-like substances have been detected in 5 of 17 species of Cuban gorgonians[10] and in a variety of animals collected in Japan[11]. Prostaglandins PGE_2 and $PGE_{2\alpha}$ have been isolated from the red alga *Gracileria lichenoides*[11]. More recently, new prostaglandin derivatives have been obtained from soft corals, PGF derivatives from *Lobophyton depressum* (Red Sea)[13] and claviridenone-A, -B, and -D from *Clavularia viridis* (Okinawa)[14]. A series of prostanoids bearing chlorine at C-10 and a tertiary hydroxyl at C-12 was isolated from the octocoral *Telesto riisei* by Yu and Scheuer[15].

Gerhart[16] has recently shown that both (15R)-PGA_2 and (15S)-PGA_2 induce vomiting in fishes and learned aversion to pellets containing PGA_2. One of the few predators commonly found on *P. homomalla* is the flamingo tongue snail, *Cyphoma gibbosum* Linnaeus, which we found to contain PGB_2, rather than its less stable and more biologically active isomer PGA_2, when found feeding on *P. homomalla*[17]. Our interest in the chemistry of *P. homomalla*, at the University of Oklahoma, was stimulated by the observation that its secretions caused distress in spiny lobsters[18]. It appears that both (15R)-PGA_2 and (15S)-PGA_2, may serve as chemical defenses in the marine environment.

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