PROSTAGLANDINS OF MARINE ORGANISMS

S. V. Isai and O. D. Korotchenko

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This review on the prostaglandins (PGs) of marine organisms covers the literature up to 1979, inclusive, and that of 1980 in part. The main attention is directed to methods for isolating and identifying the PGs.

Interest in the prostaglandins (PGs) is so high that the available literature is no longer limited to short reviews of various questions in this field. In recent years several foreign monographs [1-6] and that of I. S. Azhgikhin [7] have appeared.

It is known that marine organisms are rich sources of highly active compounds. As a result of intensive investigations, substances possessing a broad action spectrum have been isolated from them [8-13].

Many publication have appeared on the amounts of PGs in various human tissues [14-16], while investigations connected with the search for PGs and their precursors in marine organisms are few. We have succeeded in finding only four review papers on the PGs of marine organisms [17-20]. These are mainly reports of conferences or symposia.

Interest in the PGs of marine organisms is not weakening; the search is proceeding and is revealing sources other than the corals of PGs of marine origin. We have considered it desirable to generalize literature information on the PGs of marine organisms. In discussing each paper we give the qualitative and quantitative characteristics of the PGs and their derivatives that have been isolated if such information is given in the original source.

The majority of publications on the PGs of marine organisms include consideration of *Plexaura homomalla* – primitive animals of the type of the coelenterates. A symposium has been devoted to the all-sided study of this organism [19].

In 1969, Weinheimer and Spraggins reported the isolation of several PGs from *P. homomalla* [21]. They succeeded in obtaining from the air-dry cortex, by extraction with hexane followed by chromatography on silicic acid, PG isomers with a yield of 0.2-1.3%, which is 100 times greater than the concentration of PGs found in mammals. By using practically the whole set of physicochemical methods, including optical rotation and circular dichroism, these authors established the presence of 15-epi-PGA_2 and its diester (acetate/methyl ester). They showed for the first time that these PGs have the (15R) configuration of the substituent [19]. Unfortunately, in physiological tests the 15-epi-PGA_2 did not show the properties characteristic for mammalian PGs.

In 1972 a paper by Schneider et al. appeared [22] which reported PG esters of the A_2 and E_2 groups in the same species of coral as that from which PGs had first been isolated by Weinheimer and Spraggins. It must be mentioned that in this case the *P. homomalla* was collected in two regions of the Carribbean Sea and the PGs were isolated from the frozen raw corals and not from the air-dry cortex as in Weinheimer's work. After the isolation of the PGs and the determination of their structure, it was found that in addition to the compounds detected previously [21] there were others, differing by the configuration of the substituent at the 15th carbon atom. For a more accurate designation of the absolute configuration, including the asymmetric center, use was made of the Cahn–Ingold–Prelog rule. The configuration of the substituent at the 15th carbon atom is denoted with the aid of the R, S terminology [23].

All known mammalian PG derivatives have the S configuration at the 15th, asymmetric, carbon atom [24]. However, the PGs isolated from the coral *P. homomalla* possessed the (15R) configuration of the substituent. At the present time, it is known that some forms of *P.*

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homomalla contain PG derivatives of the S configuration, identical with those isolated from mammals, as well as PGs of the R configuration [22]. In some species of soft corals, especially the gorgonians, PGs of the (15R) and of the (15S) configurations are found simultaneously. The amounts of PGs isolated by Schneider et al. from *P. homomalla* were 1.4% for (15S)-PGA_2 and 0.4% for the methyl ester of (15S)-PGA_2 (on the dry weight). In addition, 0.06% of crystalline (15S)-PGE_2 was isolated and it was shown this PG was identical with mammalian PGE_2 in both its physical and its biological properties.

In the same year, 1972, in the chromatographic purification of the (15S)-PGA_2 from *P. homomalla*, a new PG derivative was isolated and identified — the 5-trans isomer of PGA_2 [25]. From its chromatographic behavior it is less polar than PGA_2, and its amount ranged between 5 and 15% depending on the PGA_2 present. With an intensification of the investigation of *P. homomalla* extracts, it was found that in the presence of small amounts of free acids (unfortunately, the authors do not state precisely which), the 5-trans isomer exists mainly in the form of the 15-acetate/methyl ester. At the same time, it is not clear whether this isomer is formed biosynthetically from endogenous trans-5-arachidonic acid or by the transformation of cis-5-PGA_2.

Bundy et al. have confirmed that the PGs from *P. homomalla* may be starting points for the synthesis of PGE_2 and $PGF_{2\alpha}$ [26]. For these purposes PGs both with the R configuration of the substituent at the 15th carbon atom and with the S configuration, which are readily obtained, for example, from a crude hexane or chloroform—methanol (2 : 1) extract of *P. homomalla* followed by chromatography on a column of silica gel, TLC, etc., are suitable [21]. After the reduction of the mixture of isomeric epoxy derivatives of PGA_2, a 56% yield is obtained of PGE_2 15-acetate methyl ester, the hydrolysis of which gives crystalline PGE_2 (with 90% yield), identical in all respects with PGE_2 from mammals. The reduction of PGE_2 with sodium tetrahydroborate leads directly to $PGF_{2\alpha}$.

In 1972, Light and Samuelsson, continuing an investigation of *P. homomalla*, became interested in the gorgonians as potential sources of PG synthetases [27]. To isolated the PGs, these authors made use of the system generally employed for obtaining the total lipids in various cases, i.e., a mixture of chloroform and methanol (2 : 1). In the course of their investigation they confirmed Weinheimer and Spraggins's results [21] and showed that in addition to the (15R)-PGA_2 and its acetate/methyl ester identified previously, and also (15R)-PGE_2, *P. homomalla* contained the methyl esters of (15R)-PGE_2 and of (15R)-PGA_2. All the structural indices of the PGs and their derivatives were obtained with the aid of gas chromatography in association with mass spectrometry. The amount of (15R)-PGE_2 in the tissue of *P. homomalla* was 0.1-0.2% (on the dry weight), but no quantitative estimate of the methyl esters of (15R)-PGE_2 and (15R)-PGA_2 was made.

Mention must be made of the work of Miyares Cao et al. [28] on the determination of PG-like substances in various species of Cuban coral. They analyzed seven species of gorgonian corals, in five of which they found PG-like substances, while, of several species of *Plexaura*, only in *P. homomalla* did they record significant amounts of PGs. After the preparation of extracts from the individual species of goronians, they were fractionated into classes with the aid of thin-layer chromatography. The preparatively separated zones corresponding in chromatographic behavior to the PGs were subjected to pharmacological studies, using various organs of a number of animals for this purpose. Such biotesting has so far permitted the authors to speak of the presence of PGE_2 only in corals of the species *P. homomalla*.

In 1975-1976, one of the authors of the present review, working in Miyares Cao's laboratory (Republic of Cuba) showed the presence of $PGF_{2\alpha}$ in two subspecies of *P. homomalla* (*typica* and *kükentahli*) and also in *P. flexuosa*. Various solvents were used for extraction, but $PGF_{2\alpha}$ was detected only in the case of chloroform—ethanol (1 : 1). The $PGF_{2\alpha}$ was characterized by TLC and by biotesting. For TLC the ethyl acetate—methanol—water (8 : 2 : 5; organic phase) system was used, with a standard sample of $PGF_{2\alpha}$ as marker. Biotesting was carried out both on the total extracts and also on the zones isolated preparatively (in the case of *P. homomalla kükentahli*). The tests were performed on rabbit stomach and rat uterus. On the kymogram, very intense responses were obtained in comparison with authentic samples of $PGE_{2\alpha}$ and PGE. The results of the investigations have been presented to the Cuban Academy of Sciences.

In 1977, a paper by Schneider et al. appeared [29] in which we found confirmation of our results on the detection of $PGE_{2\alpha}$ in *P. homomalla*. These authors, making use of several methods of extraction with various solvents, isolated eight previously known PG derivatives, including $PGF_{2\alpha}$. However, the amount of the latter was so small that they limited themselves

to its chromato-mass-spectrometric characterization. In the same year, workers at Havana University isolated from *P. homomalla* and identified PGA_2 and its methyl ester and acetate [30]. The structures of the compounds isolated were determined with the aid of TLC and IR spectroscopy and were confirmed by comparison with synthesized PGA_2 derivatives. Analysis with eight mixtures of solvents showed that the best separation was achieved in the benzene—ethyl acetate—acetic acid (75 : 15 : 1) system.

Papers by Corey et al. [31, 32] in the study of the biosynthetic pathways for the PGs in *P. homomalla* are interesting. In one of them [31], an obvious link between the biosynthesis and metabolism of coral PGs and the symbiotic algae isolated from *P. homomalla* is shown. It is characteristic that these algae are found only in the one species of *Plexaura*. Among the fatty acids detected in a homogenate of the algae there was about 0.7% of arachidonic acid. A homogenate of the algae contained no PG synthetase, as was shown by the absence of any conversion of eicosatrienoic acid added to the homogenate. It is assumed that the C_{20} fatty acids synthesized in the algae are rapidly transferred into the cells of the coral where PGs of type A are synthesized from them. Corey considers that in the coral the biosynthesis of PGA_2 takes place by a pathway different from that in mammals [32]. It was shown that, in the coral, biosynthesis of PGA_2 does not include the formation of the endoperoxides PGH_2 and PGG_2 which are intermediates in the biosynthesis of mammalian PGs.

We have already mentioned that the isolation of the pGs of the A_2 group from *P. homomalla* has permitted a passage to obtaining primary PGs, i.e., PGs of groups E and F, from the same source [26]. Investigations in this directions have continued and some of them have been the subject of patenting. Without dwelling in detail on a discussion of these investigations, we may mention only thatthey relate mainly to Schneider's work on the preparation of PGE_2, $PGF_{2\alpha}$, and their isomers from *P. homomalla* [29, 33-35].

Such ardent attention to *P. homomalla* can be explained by the fact to the workers concerned it was important to study it under laboratory conditions in order to understand why precisely this species of gorgonians produces appreciable amounts of PGs. Consequently, it is not fortuitous that the symposium devoted to the study of this organism was held in 1974. Of the 13 lectures presented at the symposium [19], only two or three related to the chemistry of the PGs and the others dealt with the most diverse questions of the biology of *P. homomalla*: ecology, anatomy, histology, sex cycle and reproduction, and cultivation. Not all participants in the symposium were of one mind on the necessity for the marine cultivation of *P. homomalla*. On the one hand, some experiments have shown that *Plexaura* is an ideal organism for cultivation, and on the other hand, a representative of the well-known firm of Upjohn, Dr. Babcock, stated that the company has available several methods for the industrial synthesis of PGs. In addition, a new synthetic PG has been obtained the activity of which is 400 times greater than the activity of the natural PGs. Consequently, Babcock concluded, it is undesirable to take up so much time with questions connected with *P. homomalla*. This opinion is not indusputable.

In 1980, a paper by Domingues et al. [36] appeared on the isolation of PGs from *P. homomalla* living in the coastal waters of Venezuela. The authors isolated various PG derivatives, in a total amount of about 3.5% (on the dry weight), but did not detect PGE_2 and $PGF_{2\alpha}$. Thus, at the present time 24 PGs and various derivatives of them have been isolated from *P. homomalla* and characterized. In spite of the fact that synthesis of the PGs from *P. homomalla* has been established [26], interest in this organism has not ceased.

In addition to investigations on *P. homomalla*, those on other marine materials have begun. These studies are based on various plans.

Thus, Bito [37] has published a paper on the accumulation of PGs by various tissues of mammals and of marine vertebrates and invertebrates. Labeled PGs were used. It was shown that in all tissues of bivalve mollusks and in the majority of tissues of a number of other marine invertebrates an accumulation of [^{3}H]-PGs, particularly PGA_1, took place. When kept in an aquarium, some invertebrates were capable of accumulating labeled [^{3}H]-PGs from sea water. According to Bito's results, in marine vertebrates, just as in land mammals, there is a specificity in the accumulation of PGs, while in marine invertebrates there is no such specificity: Accumulation takes place in all tissues. This author has put forward the hypothesis that the accumulation of prostaglandin activity is connected with the tissues responsible for the transport of PGs through the cell membranes. He did not determine the chemical nature of the product accumulated but suggested that it must be either free PGs or closely related metabolites.

A review paper by Weeks [24] on the proceedings of a conference on PGs held on September 24-28, 1972, in Vienna gives the results of an investigation of a number of insects and of marine invertebrates as the subject of a search for a biosynthetic source of PGs. The gills of the carp and of the lobster prove to be particularly active in the conversion of PG precursors, polyunsaturated fatty acids, into PGs. The hypothesis was put forward of a role of PGs in salt-water metabolism.

Lomova et al. [38] have found combined PGs in developing egg cells of the sea urchin. The main method of evaluating the PGs was biotesting. The presence of PGs was established in both unfertilized sea urchin egg cells and also in the fertilized cells present in various stages of embryogenesis. The total amount of PGs in the egg cells was not constant but varied according to the stage of their development. Under the influence of these compounds the arterial pressure in rabbits was lowered and the horn of the rat uterus responded by a contraction when the PGs were added to physiological solution. In order to confirm the presence of combined PGs in the extracts used by the authors, some of the samples were studied by preparative TLC. After the isolation of the individual PGs they were subjected to biotesting. It was found that the activity of the PGs in relation to the hypotensive effect was retained but amounted to only half the initial activity.

The Japanese scientist Nomura and his colleagues have reported the presence of PGs in fish testes [39]. They used flounder, tuna, and salmon for the investigation. Two methods were used for isolating PGs from the fractions: Ethereal extracts were obtained by Samuelsson's method [40], and the chloroform extracts by the method of Light and Samuelsson [27]. PGE_2 and $PGF_{1\alpha}$ were isolated and identified by TLC, UV spectroscopy, gas—liquid chromatography, and bioanalysis. The investigators considered that the possibility of the presence of PGs in fish as the same as in mammals, but the majority of fish are egg-layers and the mechanism of their reproduction is different, and therefore, possibly, fish do not need large amounts of PGs.

A series of papers by the same authors then followed. PGE_2 was isolated from the intestinal tract of the shark and identified [41]. The methods used for the extraction and identification of the PGs were the same as in the preceding investigation. It was shown that the concentration of PGE_2 in the gastro-intestinal tract was approximately 2.5 μg per 1 g of raw tissue. Simultaneously with the identification of the PGs, the fatty acid composition of the tissue was determined. Arachidonic acid made up about 17% of the total amount of fatty acids while the amount of $C_{20:3}$ and $C_{20:5}$ acids did not exceed 2.4%. No PGF_1 was detected, which is in harmony with the opinion of some workers that the gastro-intestinal tract is an important section for the production of PGEs. The authors explain the absence of PGE_1 and PGE_3 by the presence of only small amounts of eicosatrienoic and eicosapentaenoic acids.

In 1976, a paper by Nomura and Ogata on the distribution of TGs in the animal kingdom, especially in marine animals, was published [42]. It gave a semiquantitative estimate of 22 species of marine vertebrates and invertebrates by bioanalysis. Organisms such as sea-squirts, mussels, scallops, raw-fish, crabs, sea anemones, and some species of fish (shark, carp) were also analyzed. The PG extracts obtained by Samuelsson's method were purified by column chromatography. The fundus of the rat stomach was used for the bioanalysis of the PG fractions. The concentration of PGs was expressed in nanograms per 1 g of wet tissue relative to standard PGE_2. The distribution of PGs in many tissues was studied. It was found that the PGs were concentrated mainly in such tissues as the gastro-intestinal tract, the heart, the gills, and the air sac, while in the majority of other tissues PGs were represented in considerably smaller concentrations. The next two papers by Nomura and Ogata had a biosynthetic plan. They were interested in the formation of PGs from dihomo-γ-linolenic acid in marine carp, crabs, and some mollusks in comparison with the biosynthesis of the PGs in a rabbit medullary layer homogenate [43]. The investigation was performed with a label. The labeled PGs formed were partially identified by TLC. In general, the yields of the PGs were low for marine organisms: The conversion of the acid into PGs was less than 10%, while in the case of the rabbit such conversion into PGE amounted to 17%. In a study of individual tissues, a moderate PG synthetase activity was found in the gills and mantle, while the activity in the gonads was very low. In addition, the authors identified the PGs of four species of bivalve mollusks by the TLC method [44]. A semiquantitative bioanalysis was given (the fundus of the rat stomach was used). The biotransformation of the $8,11,14\text{-}C_{20:3}$ acid into PGE_1 and $PGF_{1\alpha}$ was detected in various molluscan tissues.

Morse and his colleagues have shown that on the addition of hydrogen peroxide to sea water a synchronous spawning takes place in some species of mollusks [45]. The authors con-

sider that peroxides activate PS endoperoxide synthetases. The PG endoperoxides, in their turn, are the direct precursors of the PGs. In the opinion of these authors, the phenomenon detected may find a number of practical applications, especially in therapeutic practice.

Studies of PG synthetases in marine organisms are continuing [46].

In 1979, Komoda et al. reported on the isolation of $PGF_{2\alpha}$ from the soft coral *Euplexaura erecta*, which lives in the coastal waters of Japan [47]. The PG-like material was extracted with methanol at room temperature for a day. The methanolic extract, previously purified by passage through Amberlite, showed a considerable activity on strips of guinea-pig ileum. In the further purification of the methanolic extract by fractionation and preparative TLC, a substance was obtained having a R_f value on chromatogram identical with that of $PGF_{2\alpha}$ and possessing activity on bioanalysis. The subsequent identification consisted in obtaining derivatives and recording the mass spectrum. This investigation demonstrated for the first time that gorgonians containing PGs are not limited to species from the basin of the Caribbean Sea.

Very recently, Carmely et al. have isolated from the soft coral *Lobophyton depressum* living in the Red Sea four PGs belonging to the F series. These are (15S)-$PGF_{2\alpha}$ 11-acetate methyl ester and its 18-acetoxy derivative and the free carboxylic acids corresponding to them [48]. The structures of the compounds isolated have been established mainly on the basis of spectral data.

While the detection of PGs in *Plexaura homomalla* was fortuitous, this is the first example of a directed search for and isolation of PGs from a soft coral of a different family (Alcyonacea). One of the PGs, (15S)-$PTF_{2\alpha}$ 11-acetate methyl ester was isolated in the crystalline form from a methylene chloride extract of the coral, and its 18-acetoxy derivative in the form of oil from the same extract. The free acids were isolated from a crude ethyl acetate extract. The authors give no information on the quantitative yield of PGs. They encountered difficulties in the interpretation of the spectral results because of the low degree of resolution of the ^1H NMR spectra of the PGs, which are very similar at first sight to the spectra of glycerides containing hydroxy and/or oxo carboxylic acids, and because of the high percentage of glycerides present in many marine organisms and, particularly, in soft corals. In view of this, the authors consider that it is most convenient to detect PGs by biotests.

In their laboratory, the authors of the present review have also used the method of biotesting in total PGs in extracts in an investigation of 40 species of marine invertebrates from the Sea of Japan [49]. Here the main method of isolation was extraction by ether with acidification [50]. The results obtained show that the bulk of the organisms studied had the same activity in comparison with the standard samples of PGs. Five of the species analyzed, belonging to various types showed a higher activity at considerably lower concentrations (20-40 times) than for the other species.

Subsequently, the PG extracts of some echinoderms (*Stichopus japonicus*) were fractionated into classes by preparative TLC with the visualization of the zone by specific reagents. The zones corresponding to PGE_2 and $PGF_{2\alpha}$ revealed biological activity [51].

In addition to papers on the study of the PGs of marine animals investigations on their detection in marine algae have also begun to appear [52].

Thus, from a consideration of the available literature on the PGs of marine organisms it follows that the fixation of the raw material, its extraction, and the preservation of the samples are extremely important in the isolation of PGs.

Speaking of extraction, it is difficult to single out any one method. Depending on the solvent used for the fixation of the initial raw material the subsequent procedure for the isolation of the PGs may be a multistage one. Many authors give their preference to a simpler method of extraction (Samuelsson's method) [40] and use a modification of it in their investigations.

If we turn to the first studies on the isolation of the PGs, it can be observed that attention was focused on the necessity of acidification in extraction [40]. However, it was subsequently found that PGs are also isolated with the use of the method of extraction which is traditional in the chemistry of the lipids (with a mixture of chloroform and methanol) [27].

Thus, in one of the very latest papers on the isolation and chemical conversion of PGs from *P. homomalla*, Schneider et al. used five different extractions, including hydrolysis in water. They showed that when frozen coral tissue was used they obtained only the 15-acetate methyl ester of PGA_2 while if the coral was kept at room temperature in water or methanol a mixture of PGA_2 and its methyl ester was obtained [29].

Wide use is made of reverse-phase partition chromatography [50], and also of separation on the lipophilic Sephadex LH-20 [53], for the purification and separation of individual PGs.

To purify large amounts of PGs from marine materials we have used successfully both Amberlite XAD-2 and also other adsorbents. Their advantage is mainly that we avoid the use of large volumes of solvents and of the multistage extraction procedures that are traditional for the isolation of the PGs. In a recent paper by Japanese authors [47] it has been shown that the methanolic extract of a soft coral does not lose its bioactivity if Amberlite XAD-2 is used for purification.

Speaking of the keepability of the sample, it must be mentioned that the authors of many of the investigations that have been cited have reported the form of raw material with which they worked. We have detected $PGF_{2\alpha}$ only when freshly-trapped animals or those frozen at $-20°C$ for a short time (on the order of a day) were used for extraction. This does not, it is true, apply to the PGs of subgroup A, the storage of which in the form of extracts permits work to be carried on with them for years. Thus, at the present time we are using *P. homomalla* collected in 1976 in the form of chloroform—ethanol extracts, acidified ethanol extracts, and air-dry powder. Our results on 40 species of marine invertebrates also indicates that total PG extracts exhibit activity on biotesting only for a relatively short time. After a month the activity is lost completely (biotesting on the horns of the rat uterus).

Thus, the literature of the PGs of marine organisms that has been considered indicates an unweakening interest by the scientists of various countries in the search for new sources of PGs and in other questions in the PG field.

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