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Biosynthesis of 8-*R*-HPETE and Preclavulone-A from Arachidonate in Several Species of Caribbean Coral. A Widespread Route to Marine Prostanoids.

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Summary: The Caribbean coral species *Plexaura homomalla, Plexaura nina, Plexaura flexuosa, Pseudopterogorgia americana, Muriceopsis flavida,* and *Eunicea asperula* have all been found to convert arachidonate to 8-*R*-HPETE (**4**) and preclavulone-A (**3**) demonstrating that this route to marine prostanoids is widespread among such coral.

Two striking developments in the field of organic natural products have been the discovery of prostanoids in primitive marine organisms and the more recent finding that the marine pathway of biosynthesis is totally different from the mammalian endoperoxide pathway. The Caribbean coral *Plexaura homomalla* is a rich source of prostaglandin A_2 (PGA_2) methyl ester acetate (**1**) (up to 2% of dry weight) or the 15-epimer (depending on subspecies)[1,2] made by a pathway which appears to differ from the endoperoxide route.[3] The Pacific coral *Clavularia viridis* produces a different family of prostanoids, typified by clavulone I (**2**),[4] evidently from the common intermediate preclavulone-A (PC-A) (**3**). The biosynthesis of **3** has recently been shown to occur via initial 8-lipoxygenation of arachidonate to give **4** (8-*R*-HPETE) and subsequent conversion, presumed to be via allene oxide **5** and pentadienyl cation **6**, directly to **3**.[5,6] Subsequently it was demonstrated that the Caribbean coral *Pseudoplexaura porosa*, reported earlier to biosynthesize 8-*R*-HPETE, also can biosynthesize PC-A from arachidonate via **4**.[7] The results taken together were strongly suggestive of generality of marine prostanoid biosynthesis by the 8-HPETE → allene oxide **5** → pentadienyl cation **6** → PC-A pathway and the biosynthesis of PGA_2 in *Plexaura homomalla* in parallel fashion. We report herein experiments with a variety of other gorgonian coral (generally arborescent in appearance) which confirm the generality of PC-A biosynthesis from arachidonate, even when no prostanoids can be detected in these coral by conventional analytical methods such as HPLC analysis. Of special interest is the observation of 8-HPETE and PC-A biosynthesis in *Plexaura homomalla* and the closely related species *Plexaura nina.*

The samples of soft coral used in this work were collected off Key Largo FL and Grand Bahama island in February and May, 1987. Color photographs of each coral were made *in situ* before collection to assist identification.[8] More rigorous classification was made by microscopic analysis of calcareous sclerites according to Bayer.[9] The specific findings for each species of coral are summarized below. In general enzyme preparations for biosynthetic studies were obtained by homogenization of *ca.* 10 g of frozen coral (electric blender for *ca.* 30 sec) in 100 ml of ice cold buffer (pH 8.0 50 mM Tris containing 1 M sodium chloride) and centrifugation at *ca.* 5000 g for 5 min. Radiolabeled arachidonate (45 μg, 100,000 cpm in 55μl of ethanol) was added to 5 ml of the clear supernatant and the mixture was mixed and incubated for 2.5 h at 23°C. After extractive isolation and esterification by ethereal diazomethane products were analyzed by HPLC (silica gel, 16 : 1 hexane *t*-butylmethyl ether as eluent) and measurement of radioactivity. Final

identification was made by scaling up incubations with unlabeled arachidonate to obtain sufficient product for HPLC, 500 MHz ^{1}H NMR, FT-IR, UV and mass spectral measurements and comparison with synthetic standards. Conversion of arachidonate to 8-*R*-HPETE was also studied using an acetone powder preparation[7] from the various corals. The synthesis of 8-*R*-HPETE and its conversion to PC-A were found to occur in each case where PC-A formation was observed from arachidonate. In no instance could 8-HETE, 8-HPETE or PC-A be detected by HPLC in fresh extracts of the coral indicating that steady state *in vivo* concentrations of these eicosanoids must be very low.

1. ***Plexaura homomalla:***

Homogenate preparations converted arachidonate to a mixture of PC-A and the 9,12-*trans* isomer of PC-A ("*epi*-PC-A," **7**), ratio *ca.* 3 : 1 in 10% yield. 8-*R*-HPETE was converted to the same mixture of PC-A and *epi*-PC-A in 23% yield. An acetone powder preparation from *P. homomalla* transformed arachidonate rapidly under 60 atm[10] of O_2 into 8-*R*-HPETE (20% yield, 100% ee). The optical rotation of PC-A methyl ester was found to be essentially zero which is indicative of a racemate since the rotation of totally synthetic chiral PC-A methyl ester is known to be 132°.[11] We presume that the enzyme contained in our homogenate is unable to control chirality in the oxidopentadienyl cation cyclization, perhaps due to subtle changes in tertiary structure of the extracted enzyme from that which exists *in vivo*. That the conversion of 8-*R*-HPETE into PC-A is enzymic was shown by control experiments in which enzyme preparations that had been heated for 2 h to 70°C and cooled were found not to convert 8-*R*-HPETE to PC-A. Another interesting finding was that two dehydro PC-A methyl esters **8** having 12-*E*, 14-*E* and 12-*Z*, 14-*E* olefinic geometry could be isolated by -78° acetone extraction of frozen coral and HPLC purification. Although the same mixture **8**[12] can be prepared by treatment of PGA_2 methyl ester acetate by DBU in benzene at 23°C for 5 min, **8** is not formed by this route under conditions of storage, extraction or isolation and thus must be regarded as a natural constituent of *Plexaura homomalla*. It is interesting that addition of HOH to **8** under enzyme control could in principle lead to PGA_2, and further that **8** could be formed by oxidation of PC-A.

To date we have not been able to demonstrate bioconversion of 8-*R*-HPETE or PC-A to PGA_2 or derivatives in *P. homomalla*, however work along these lines is continuing.[13,14]

2. ***Plexaura nina:***

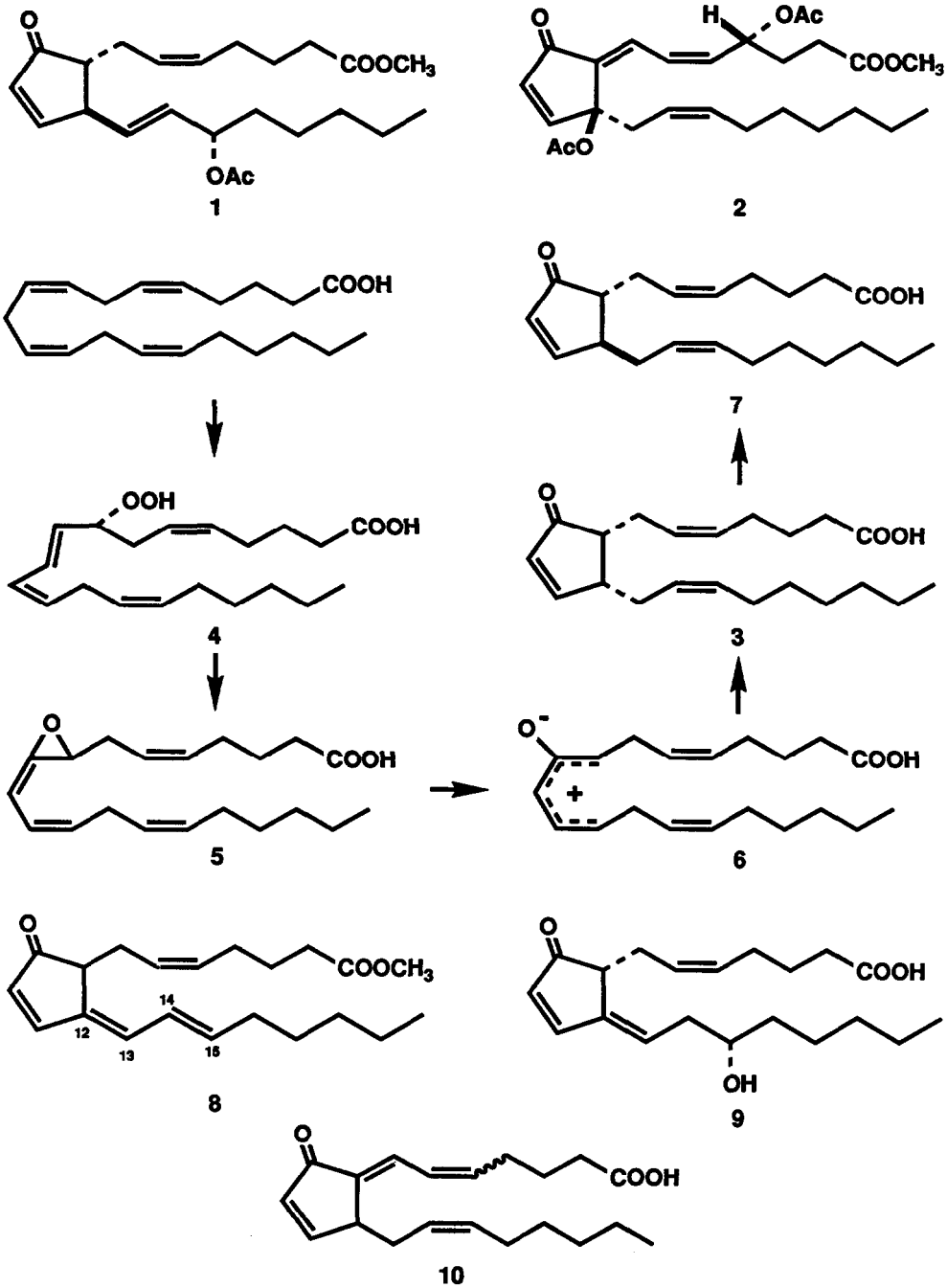
Both homogenate and acetone powder prepared from *P. nina* converted arachidonate to a mixture of 8-*R*-HPETE and PC-A. As expected 8-*R*-HPETE was also converted to PC-A. Extracts of *P. nina* aqueous homogenate contained a new prostanoid (in addition to PGA_2) which is less polar than PGA_2 and which has been purified and identified as **9** (λ_{max} 280 nm) from ^{1}H NMR, IR and MS data.

3. ***Plexaura flexuosa:***

Biosynthetic experiments performed with this coral using acetone powder also indicated the conversion arachidonate → 8-*R*-HPETE → PC-A. At 60 atm of O_2[10] the yield of 8-*R*-HPETE from arachidonate was 32%. The yield of PC-A's from 8-*R*-HPETE was 20% as a 3.4 : 1 mixture of PC-A and *epi*-PC-A. Further, the homogenate was found to convert PC-A in 5% yield to **10**, a new eicosanoid (UV_{max} 224, 282, 292, 302 nm) of the clavulone type. HPLC analysis of extracts of *P. flexuosa* did not reveal detectable amounts of **10**.

4. ***Pseudopterogorgia americana:***

The homogenate from this common beige and purple "sea plume" converted arachidonate to 8-*R*-HPETE and thence to a mixture of PC-A and *epi*-PC-A. Yields directly from arachidonate were 1.2% for 8-*R*-HPETE and 6.6% for the PC-A's.


O
COOCH3
OAc
1
H
OAc
AcO
2
COOH
7
OOH
4
3
5
6
O-
+
8
12
13
14
15
9
OH
10

5. ***Muriceopsis flavida:***

The homogenate from this common purple "sea whip" converted arachidonate to 8-*R*-HPETE (1%) and a mixture of the epimeric PC-A's (3.1%) and also converted 8-*R*-HPETE to the PC-A's. No eicosanoids could be detected in the extracts of *M. flavida.*

6. ***Eunicea asperula:***

The homogenate from *Eunicea asperula* transformed arachidonate into 8-*R*-HPETE (4%), PC-A (5.2%) and *epi*-PC-A (2.1%). PC-A methyl ester isolated from *E. asperula* was found (after HPLC purification) to show no optical rotation and thus is racemic. The homogenate converted 8-*R*-HPETE to PC-A in 30% yield.

In summary, each of the six coral listed above are capable of converting arachidonate to 8-*R*-HPETE (**4**) and thence to PC-A (**3**) and *epi*-PC-A (**7**). This brings the total to eight species, all very diverse, which exhibit biosynthesis of these eicosanoids from arachidonate. Although the biological and biochemical function of these marine eicosanoids is still unclear, research on this point is clearly in order. We hope that additional work will illuminate these issues and also define the pathway of biosynthesis of PGA_2 in *P. homomalla,* especially with reference to the intermediacy of PC-A.[15]

REFERENCE AND NOTES

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7. E. J. Corey and S. P. T. Matsuda, *Tetrahedron Letters,* **28**, 4247 (1987).
8. It is a pleasure to acknowledge the assistance of Robert McCredie, Barry King, David Milner and Steven Broadhurst of the Syntex Corporation, Grand Bahama in the collection of samples and underwater photography. Permission to collect coral was kindly granted by the Hon. Colin Higgs, Director of Fisheries for the Bahamas. Each coral sample was frozen on dry ice within a few seconds after removal from the sea and maintained at -78°C thereafter.
9. F. M. Bayer, *The Shallow-water Octocorallia of the West Indies Region.* Martinus Nijhoff, The Hague, 1961. Using a stereoscopic microscope and scalpel, air-dried coral specimens were dissected into cortical surface, cortex, and axial sheath. A small fragment from each region was placed on a glass slide and covered with a drop of sodium hypochlorite (undiluted Chlorox) to remove organic material. The resultant sclerites were examined under low magnification and compared with Bayer's diagrams with regard to shape, size, and color. We thank Dr. Bayer for a specimen of *P. nina.*
10. See E. J. Corey and R. Nagata, *J. Am. Chem. Soc.,* **109**, 8107 (1987). Higher pressure of O_2 increases rate of lipoxygenation and yield of 8-*R*-HPETE.
11. An enantioselective total synthesis of PC-A has recently been reported from these laboratories. E. J. Corey and Y. B. Xiang, *Tetrahedron Letters,* **29** (1988).
12. Characterized by 500 MHz ^1H NMR, infrared, ultraviolet (λ_{max} 312 nm in hexane) and mass spectra.
13. Encouraged by our work on the cationic marine route to eicosanoids workers in another laboratory have embarked on similar research. See, A. R. Brash, S. W. Baertschi, C. D. Ingram, and T. M. Harris, *J. Biol. Chem.,* **262**, 15829 (1987).
14. Negative results of experiments on prostanoid biosynthesis are common in our experience over many years. Only a fraction of coral samples show PGA_2 synthesizing ability and the PGA_2 synthase activity of these is rapidly lost even at low temperatures.
15. This research was assisted financially by the National Institutes of Health.

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