tremendous potential of cmr spectroscopy as a structural tool in organometallic chemistry.

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The Role of the Symbiotic Algae of *Plexaura homomalla* in Prostaglandin Biosynthesis

Sir:

The startling announcement by Weinheimer[1] that *Plexaura homomalla* contains A-type prostaglandins in high concentration (up to 1.8% by weight) has instigated a variety of studies on this interesting species of "soft" coral.[2] We have reported recently that under suitable conditions enzymic conversion of 8,11,14-eicosatrienoic acid or arachidonic acid to PGA_1 or PGA_2, respectively, can be effected by homogenates of *Plexaura homomalla*.[3] Unfortunately, the enzyme preparations which were obtained did not bring about sufficiently high conversion to be preparatively useful. Our initial studies did not touch on the question of whether prostaglandin biosynthesis occurs in the coral cells or in the algae which coexist with *Plexaura homomalla* in a symbiotic relationship. This report deals with the isolation and culture of the algae to determine what contributions, if any, they make to prostaglandin biosynthesis. There are two possible roles for the algae to play: the synthesis and subsequent release of PGA_2 to the coral or the synthesis and release of a fatty acid precursor, arachidonic acid. The former role coupled to a method for culturing the algae could lead to a highly useful process for the production of PGA_2. The latter possibility, which seems quite reasonable in view of the widespread occurrence of C_{20} polyunsaturated acids in marine algae,[4] would be of special interest in connection with the nature of the biochemical events in the symbiosis.

Plexaura homomalla, collected off New Providence, Bahamas, was transported to Cambridge in sea water at 20°.[5] Examination revealed that the algae were found as single cells imbedded in the tissue of the coral. To free the cells, the coral was scraped with a razor under sterile sea water at 4°. The minced tissue was a viscous slime which was filtered through four layers of cheese cloth. The filtrate was centrifuged at 1500*g* for 4 min to collect the algae. After several washings of the cells by suspension in fresh sea water followed by centrifugation, the cells, in a minimum of sea water, were layered on a discontinuous sucrose gradient consisting of 20, 30, and 40% layers. After 7 min of centrifugation at 1600*g*, the algae were found at the 30–40% interface and in the 40% layer. These fractions were combined and washed with sterile sea water. To remove other contaminating cells, this pellet was applied to a continuous 20–40% sucrose gradient. After 3 min at 900*g*, the algae formed a green band midway down the gradient. This fraction was collected and washed repeatedly with sterile sea water. From 15 g of coral approximately 50–100 mg of clean algae cells were obtained.

These cells were transferred to flasks containing sterile Gonyaulox medium.[6] The algae grew best at 24–26° when exposed to alternating 12-hr light and dark periods. The algae first formed green clusters and finally a thin film covering the bottom of the flasks. Agitation of the mixtures under incubation did not enhance growth. It is possible that the algae are dependent on the coral for nutrients since growth was not as pronounced in the medium F/2[7] which contained fewer vitamins than the Gonyaulox medium.

To determine the fatty acid distribution, 5 g of packed algae cells were repeatedly extracted with 1:1 $CHCl_3$–MeOH and with ethyl acetate until all color was removed from the cells. The combined organic extracts were concentrated and then hydrolyzed under argon using 1 *N* aqueous alcoholic potassium hydroxide at 25°. After removal of the neutral material, the acids were esterified with ethereal diazomethane at 0°. This material, after separation by preparative tlc (two developments using 25% $CHCl_3$–hexane), yielded 16 mg of fatty acid methyl esters.

This mixture was analyzed with a gas chromatograph equipped with an electron capture detector using a 6-ft glass column packed with 12% DEGS at 165°. To confirm assignment of the components, a gas chromatograph–mass spectrometer combination was employed.[8] As can be seen in Table I, 39% of the esters were C_{16}, 54% were C_{18}, and only 1% were C_{20} fatty acids. Since arachidonic acid constitutes only 0.7% of the mixture, it would appear that if the algae is the source of the arachidonic acid used by the coral, it is not stored in quantity by the algae but passed on directly to the coral.

Table I

Fatty acid	% of total fatty acids	Fatty acid	% of total fatty acids
14:0	0.3	18:2	12
16:0	2	18:3	42
16:1	3	20:3	0.3
16:2	12	20:4	0.7
16:3	22		

In order to test for prostaglandin biosynthesis in the algae, it was necessary to rupture the cells. Significantly, a more vigorous procedure was found to be required than that needed to prepare the enzymically

(1) A. J. Weinheimer and R. L. Spraggins, *Tetrahedron Lett.*, 5186 (1969).
(2) See, *e.g.*, (a) W. P. Schneider, R. D. Hamilton, and L. E. Rhuland, *J. Amer. Chem. Soc.*, **94**, 2122 (1972); and (b) R. J. Light and B. Samuelsson, *Eur. J. Biochem.*, **28**, 232 (1972).
(3) E. J. Corey, W. N. Washburn, and J. C. Chen, *J. Amer. Chem. Soc.*, **95**, 2054 (1973).
(4) See (a) R. F. Lee and A. R. Loeblich III, *Phytochemistry*, **10**, 593 (1971); (b) G. R. Jamieson and E. H. Reid, *ibid.*, **11**, 1423 (1972).
(5) The total elapsed time between collection in the sea and isolation of the algae was ~7 hr. At no time before use was the coral exposed to air.
(6) M. Fogel and J. W. Hastings, *Arch. Biochem. Biophys.*, **142**, 310 (1971).
(7) R. R. L. Guillard and J. H. Ryther, *Can. J. Macrobiol.*, **8**, 229 (1962).
(8) We wish to thank Professor Klaus Biemann of MIT for making available the mass spectrometer.

active homogenate from the whole coral. Approximately 200 mg of wet algae cells in 12 ml of a pH 8.0 buffer (0.05 *M* tris and 1 *M* NaCl) was passed twice through a French press at 12,000 psi. Two milliliters of this homogenate was used for the incubation. After 40 min at 28°, none of the 20 μg of the tritiated eicosatrienoic acid had been converted to PGA_1. These are the standard conditions for conversion of eicosatrienoic acid to PGA_1 by homogenates of *Plexaura homomalla*.[3] It was also found that no appreciable amounts of A prostaglandins could be detected in the whole algal cells by extraction with ether–ethyl acetate and the analysis of the concentrated extracts. It is quite clear from these results that the prostaglandin synthetase is contained in the coral cells, not in the algae.

Although the results of the above investigation are disappointing from the viewpoint of possible large scale *in vitro* biosynthesis of prostaglandins, they raise several interesting points for further research, especially in the completely undeveloped area of the biochemistry of symbiotic relationships between coral and their algal guests. Also still to be determined are the factors which govern the rates of arachidonic acid synthesis, transport from algae to coral, and conversion to prostaglandins.[9]

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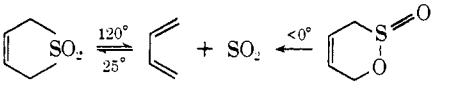
Decomposition of 3,6-Dihydro-1,2-oxathiin 2-Oxides to Sulfur Dioxide and 1,3-Dienes. A $_{\pi}4_s + {}_{\pi}2_s$ Cycloreversion

Sir:

The concerted addition of sulfur dioxide to 1,3-dienes to give 2,5-dihydrothiophene 1,1-dioxide is well known.[1] The reverse of this reaction has also been studied in detail[2] and has been classified as one of a family of symmetry allowed cheletropic reactions.[3] The stereospecific elimination of SO_2 from episulfones[4] and 2,7-dihydrothiepin 1,1-dioxides[5] is an additional example of this type of fragmentation reaction.

We would like to report that 3,6-dihydro-1,2-oxathiin 2-oxides fragment into SO_2 and 1,3-dienes. This transformation is stereospecific and is apparently the first example of the formation of SO_2 and 1,3-dienes *via* a $_{\pi}4_s + {}_{\pi}2_s$ cycloreversion (retro-Diels–Alder). It occurs with remarkable ease, at least 150–200° below that of the cheletropic reaction producing the same products (eq 1). The $_{\pi}4_s + {}_{\pi}2_s$ cycloaddition of SO_2 to 1,3-dienes is apparently not observable because of the presence of the more favorable process leading to 2,5-dihydrothiophene 1,1-dioxides. *N*-Sulfenylamines (RN=S=O)[6] and sulfines (R_2=C=S=O)[7] which have considerable structural analogy to SO_2 readily take part in $_{\pi}4_s + {}_{\pi}2_s$ cycloadditions.

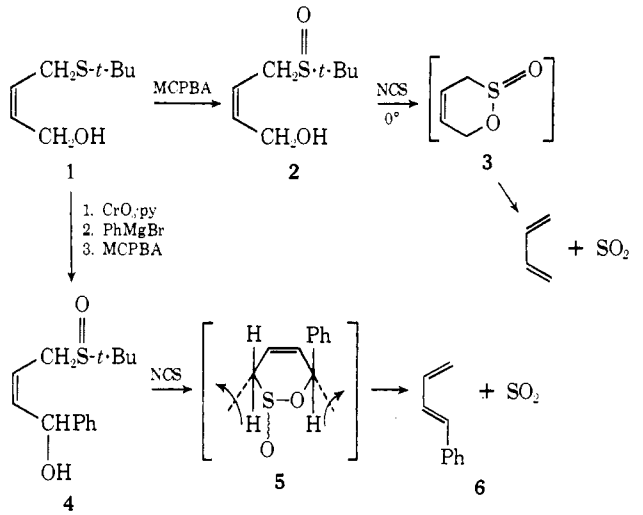


(1)

The ease of the above $_{\pi}4_s + {}_{\pi}2_s$ cycloreversion is illustrated by the following observations. Reaction of the cis hydroxy sulfoxide **2**[8,9] with *N*-chlorosuccinimide in CH_2Cl_2 at 0°[10] led to immediate SO_2 evolution and formation of 1,3-butadiene (>90% vpc). 3,6-Dihydro-1,2-oxathiin 2-oxide (**3**), the expected product of the reaction of **2** with NCS,[10] is therefore unstable and decomposes rapidly to SO_2 and 1,3-butadiene even at 0°. In contrast, the rapid formation of these two products from the isomeric 2,5-dihydrothiophene 1,1-dioxide requires temperatures above 120°.[1]

The stereospecific nature of the 3,6-dihydro-1,2-oxathiin 2-oxide decomposition was shown by the conversion of the cis hydroxy sulfoxide **4**, prepared as shown in Scheme I, to isomerically pure *trans*-1-phenyl-1,3-butadiene (**6**).[11] In the fragmentation of the intermediate **5** to diene and SO_2 a steric effect superimposed on the disrotatory motion at C_3 and C_6 would account for the formation of only **6**.[12] The formation of isomerically pure **6** from **5**, which was undoubtedly a mixture of diastereomers,[13] indicates that the sulfur–oxygen stereochemistry may be of negligible importance in determin-

Scheme I



(1) S. D. Turk and R. L. Cobb in "1,4-Cycloaddition Reactions," J. Hamer, Ed., Academic Press, New York, N. Y., 1967, Chapter 2.
(2) (a) W. L. Mock, *J. Amer. Chem. Soc.*, **88**, 2857 (1966); (b) S. D. McGregor and D. M. Lemal, *ibid.*, **88**, 2858 (1966).
(3) R. B. Woodward and R. Hoffmann, "The Conservation of Orbital Symmetry," Academic Press, New York, N. Y., 1970, pp 152–163.
(4) For a review see N. H. Fischer, *Synthesis*, 393 (1970).
(5) (a) W. L. Mock, *J. Amer. Chem. Soc.*, **89**, 1281 (1967); (b) *ibid.*, **91**, 5682 (1969); (c) *ibid.*, **92**, 3807 (1970).
(6) G. Kresze, ref 1, Chapter 13.
(7) B. Zwanenberg, L. Thijs, J. B. Broens, and J. Strating, *Recl. Trav. Chim. Pays-Bas*, **91**, 443 (1972).
(8) All new numbered compounds gave correct analyses.
(9) Prepared from *cis*-2-butene-1,4-diol *via* the monotetrahydropyranyl ether, mesylation thereof, followed by reaction with *tert*-butyl mercaptide, deprotection and oxidation.
(10) N. K. Sharma, F. Jung, and T. Durst, *Tetrahedron Lett.*, 2863 (1973); F. Jung, N. K. Sharma, and T. Durst, *J. Amer. Chem. Soc.*, **95**, 3420 (1973).
(11) Vpc analysis of the product and comparison with a mixture containing both the cis and trans isomer (O. Grummitt and F. J. Christoph, *J. Amer. Chem. Soc.*, **73**, 3479 (1951)) indicated that 6 was >99.5% isomerically pure.
(12) A similar argument can be used to explain the formation of pure *trans*-1,3-pentadiene in the decomposition of 2-methyl-2,5-dihydrothiophene 1,1-dioxide.
(13) Cyclization of a diastereomeric mixture of **7b** and of one isomer of **7b** gave the same diastereomeric mixture of oxathiin 2-oxide (**8b**). This shows that the cyclization is not stereospecific and that, in general, a diastereomeric mixture can be expected.