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Identification of Prostaglandins in the Gorgonian, *Plexaura homomalla*

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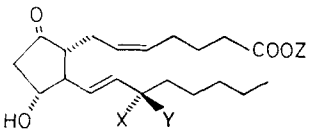
Hexane extracts of the air-dried cortex of the gorgonian *Plexaura homomalla* (Esper) were found to contain (15*R*)-hydroxy-9-oxo-prost-5,10,13-trienoic acid [*i.e.* (15*R*)-prostaglandin A_2] and its 15-acetate methyl ester in 0.2% and 1.3% yield from the cortex by Weinheimer and Spraggins in 1969. Chloroform-methanol extracts of this organism have now been shown to contain these two compounds as major prostaglandin constituents, but also a more complex mixture of prostaglandin derivatives including 11α,(15*R*)-dihydroxy-9-oxo-prost-5,13-dienoic acid [*i.e.* (15*R*)-prostaglandin E_2] and the methyl esters of (15*R*)-prostaglandin E_2 and (15*R*)-prostaglandin A_2. (15*R*)-Prostaglandin E_2 was identified by its chromatographic behavior, by the chromatographic behavior and mass spectrum of its *O*-methyloxime, methyl ester, trimethylsilyl ether derivative, and by oxidative ozonolysis to fragments of established structure. The configuration of the 15-hydroxyl group was determined in the cleavage fragment 2-hydroxyheptanoic acid by gas-liquid chromatography of its methyl ester (−)-menthyloxycarbonyl derivative. Similar degradations of material with chromatographic properties equivalent to prostaglondin A_2 and prostaglandin E_2 yielded 2-hydroxyheptanoate with the *S* configuration, demonstrating the presence of small amounts of (15*S*)-prostaglandins in the lipid extract.

Weinheimer and Spraggins [1] reported the isolation of two prostaglandins, 15-acetyl-(15*R*)-prostaglandin A_2 methyl ester (I) and (15*R*)-prostaglandin A_2 (III), from hexane extracts of the air-dried cortex of the gorgonian *Plexaura homomalla* (Esper). The two substances were present in rather high concentrations, 1.3% and 0.2% of the cortex dry weight, respectively. These C-15 epimers of prostaglandin A_2 (IV) were devoid of the blood-pressure-lowering effect of prostaglandin A_2. Their function in the gorgonian tissue is unknown, but their occurrence is very interesting since the concentration of prostaglandins normally found in various mammalian tissues is much smaller [2]. They are also of interest as potential synthetic precursors to biologically active prostaglandins of the A_2, E_2 (VII), or $F_{2\alpha}$ type [3] because they can be obtained in large quantities.

Trival names. Prostaglandin E_2, 11α-(15*S*)-dihydroxy-9-oxoprost-5,13-dienoic acid; prostaglandin $F_{2\alpha}$, 9α,11α,-(15*S*)-trihydroxy-prost-5,13-dienoic acid; prostaglandin A_2, (15*S*)-hydroxy-9-oxo-prost-5,10,13-trienoic acid; (15*R*)-prosttaglandin E_2, 11α, (15*R*)-dihydroxy-9-oxoprost-5,13-dienoic acid; (15*R*) · prostaglandin A_2, (15*R*)-hydroxy-9-oxo-prost-5,10,13-trienoic acid; methyl acetyl (15*R*)-prostaglandin A_2, methyl (15*R*)-acetoxy-9-oxo-prost-5,10,13-trienoate.

Definition. C-value, the "theoretical" number of carbon atoms in the fatty acid chain obtained from the retention time by using a calibration curve of logarithm of retention time *versus* number of carbon atoms in normal fatty acids.

Our own interest in the gorgonians originated in their potential use as source of the prostaglandin synthetase. A prerequisite for such studies was detailed knowledge on the gorgonian prostaglan-

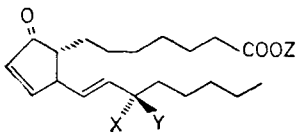


I. Methylacetyl-(15*R*)-prostaglandin A_2 where X = H, Y = CH_3COO and Z = CH_3

II. Methyl (15*R*)-prostaglandin A_2 where X = H, Y = OH and Z = CH_3

III. (15*R*)-Prostaglandin A_2 where X = H, Y = OH and Z = H

IV. Prostaglandin A_2 where X = OH, Y = H and Z = H



V. (15*R*)-Prostaglandin E_2 where X = H, Y = OH and Z = H

VI. Methyl (15*R*)-prostaglandin E_2 where X = H, Y = OH and Z = CH_3

VII. Prostaglandin E_2 where X = OH, Y = H and Z = H

dins. This report describes a complex mixture of prostaglandins in chloroform-methanol extracts of the gorgonian *Plexaura homomalla*, including significant quantities of (15*R*)-prostaglandin E_2 (V), and the presence of small quantities of (15*S*)-prostaglandins.

MATERIAL AND METHODS

Prostaglandin Standards

Prostaglandin E_2 (VII) and prostaglandin $F_{2\alpha}$ were obtained from Dr John Pike (Upjohn Company, Kalamazoo, Michigan). (15*S*)-prostaglandin A_2 (IV) was prepared by dehydration of prostaglandin E_2 [4]. (15*R*)-prostaglandin E_2 (V) was prepared from prostaglandin E_2 by the method described by Pike *et al.* [5] for the epimerization of prostaglandin E_1. It was purified by preparative thin-layer chromatography (developing solvent, ethylacetate—benzene—formic acid, 75:25:1.25, v/v/v). Methyl esters were prepared by treatment of the acids with diazomethane in ether. *O*-Methyloxime and trimethylsilyl ether derivatives were prepared as described by Gréen [6]. Reduction of the 9-oxo group of prostaglandin E compounds to produce a mixture of prostaglandin F_α and prostaglandin F_β isomers was carried out by treatment with an excess of sodium borohydride in methanol [7].

After addition of acetone to destroy the excess borohydride, the solvent was evaporated, dilute HCl was added, and the product was isolated by ether extraction. Acetyl derivatives of prostaglandin F methyl esters were prepared by dissolving the ester (1—3 mg) in 0.5 ml pyridine and 0.5 ml acetic anhydride. The mixture was left at room temperature for 18—22 h, water was added, and the product was extracted three times with ether. The ether extracts were washed with 1 N HCl, water, saturated sodium bicarbonate, and saturated sodium chloride. The ether was dried by filtering over anhydrous sodium sulfate, and the solvent was evaporated under a stream of argon.

Chromatographic Techniques

Silicic acid column chromatography was carried out on Mallinckrodt Silicar CC-4, 100—200 mesh, which had been stored at 120 °C. Thin-layer chromatography was carried out either on glass plates prepared from silica gel G (E. Merck A.G., Darmstadt) or on silica gel pre-coated aluminium sheets (E. Merck A.G., Darmstadt).

Gas-liquid chromatographic analyses were performed on glass columns (2 m × 3 mm) in an F & M Biomedical instrument model-400 equipped with a flame ionization detector. Column packings were prepared by coating Gas Chrom Q (60/80 mesh) with the stationary phase as previously described [8] and conditioned prior to use.

Se-30, ultraphase and OV-17 were obtained from Pierce Chemical Co. (Rockford, Illinois, U.S.A.) QF-1 was obtained from F & M Scientific Corporation (Avondale, Pensylvania, U.S.A.) EGSSX was obtained from Applied Science Laboratories (State College, Pennsylvania, U.S.A.).

Normal fatty acid methyl esters were run as standards under the same conditions as the substances being analyzed. A plot of the logarithm of the retention time *versus* the number of carbon atoms in the fatty acid chain (*C*-value) produced a straight line which was used to convert retention times of other substances into equivalent "*C*-values" [9].

Mass Spectrometry

Mass spectra were obtained from the combined gas chromatograph—mass spectrometer, LKB 9000, equipped with either a 1% Se-30 or a 2.2% QF-1 column. The electron energy was 22.5 eV.

Extraction of Gorgonians

Samples of the gorgonian *Plexaura homomalla* were collected on two occasions off the coast of southeast Florida and stored frozen at −20 °C or −80 °C. Analyses of both collections were quite similar. For extraction of total material the frozen gorgonian tissue (10—20 g, cortex plus spiny skeleton) was cut into small pieces and blended with chloroform—methanol (2:1, v/v), 2 ml per gram wet weight, using an Ultra turrax model TP 18/2 blendor (Janke and Kunkel Kg, Staufen, W. Germany). After filtration the residue was stirred for 5 min with two additional portions (4 ml per gram wet weight each) of chloroform—methanol (2:1, v/v). The combined extracts were washed with 0.2 volumes of 1% formic acid and the solvent was removed *in vacuo*. The residue was dried by adding benzene and evaporating *in vacuo* and was then dissolved in benzene.

Oxidative Ozonolysis

The ozonolysis procedure was similar to that described earlier [10]. The derivative of the sample to be degraded by ozonolysis (0.5—3 mg) was dissolved in chloroform (3—6 ml), cooled to −10 °C, and ozone was passed through the solution for 2—3 min. After 15 min at room temperature the solvent was removed under a stream of argon or *in vacuo* and the residue was dissolved in 1 ml of glacial acetic acid and 0.2 ml of 30% hydrogen peroxide. The solution was kept in a stoppered tube at 50 °C for 16—19 h, and the solvent was distilled *in vacuo* almost to dryness, with care taken to avoid loss of the volatile 2-acetylheptanoic acid product. The residue was dissolved in methanol and aliquots were taken for analysis of the various cleavage fragments.

(−)Menthyloxycarbonyl Derivatives

The (−)menthyloxycarbonyl derivatives of hydroxy esters were formed as described by Hammarström for the preparation of 2-menthyloxycarbonyloxy fatty acid methyl esters [11], a micro-scale modification of the procedure of Westley and Halpern [12]. The hydroxy ester (100−500 μg) was dissolved in 60 μl benzene with 15 μl pyridine and 80 μl menthylchloroformate reagent was added. The reagent (about 1 μmol/μl) was prepared as described by Westley and Halpern [12] and kindly furnished by S. Hammarström. After 30 min at room temperature, 3 ml benzene and 1 ml water were added, mixed and the water layer was discarded. The benzene layer was washed a second time with water and the solvent removed under a stream of argon. At this stage the menthyloxycarbonyl derivatives of methyl 2-hydroxyheptanoate (XIV and XV were purified by preparative thin-layer chromatography (developing solvent, benzene−dioxane, 98:2,-v/v). The spots were made visible under ultraviolet light by spraying with dichlorofluorescein and the appropriate areas were eluted with ether. Occassionally impurities from the reagent and from the silica gel which interfered with the gas-liquid chromatographic analysis of XIV and XV would also be obtained from the preparative plate. These could be minimized by more narrowly circumscribing the area to be eluted and by placing the silica gel scrapped from the plate on top of a 0.5 g column of Silicar and eluting with 5% ether in hexane rather than ether.

The menthyloxycarbonyl derivatives of prostaglandin methyl esters were subjected to oxidative ozonolysis before further purification. After ozonolysis and oxidation as described above, the products were treated with diazomethane, and the resulting 2-menthyloxycarbonyl oxyheptanoic acid methyl esters were purified by preparative thin-layer chromatography.

Standard racemic 2-hydroxyheptanoic acid was prepared by bromination and hydrolysis of heptanoic acid [13]. Standard (2*S*)-hydroxyheptanoic acid was obtained by anodic coupling of valeric acid and monomethyl(2*S*)-acetoxysuccinate (M. Hamberg, unpublished results). Both were obtained from Dr M. Hamberg.

RESULTS AND DISCUSSION

The total lipid extracted from one gorgonian sample amounted to 4.5% of the wet tissue weight or 13% of the dried, lipid-free residue. The total lipid in the second sample was 3% of the wet weight and 9.5% of the extracted dry weight. Analysis of prostaglandin content by alkaline treatment to generate the prostaglandin B chromophore at 278 nm [14] indicated that 28% of the lipid in the first sample and 19% of the lipid in the second sample could be prostaglandins of the A or E type. Weinheimer and Spraggins [1] reported that the air-dried cortex of this organism contained 0.2%(15*R*)-prostaglandin A2 (III) and 1.3% methyl-acetyl-(15*R*)-prostaglandin A_2 (I), which is about equivalent to the prostaglandin content of our second sample. Analysis of the total lipids by silicic acid column and thin-layer chromatography showed these two compounds to be major components in both samples, but also revealed the presence of other prostaglandin constituents. The distribution of components in both samples was quite similar. Fig. 1 shows a thin-layer chromatographic analysis of fractions from the silicic acid chromatography of the total lipids. Fraction 1 (eluted with 3% ethyl acetate in benzene) gave no chromophore at 278 nm upon treatment with alkali and presumably was devoid of prostaglandins. The major spot in fraction 2, compound A, gave a *C*-value of 22.9 upon gas-liquid chromatography analysis on 1.5% Se-30, and is presumably the previously identified methyl-acetyl-(15*R*)-prostaglandin A_2 (I). Likewise the major spot in fraction 5, compound D, was presumed to be (15*R*)-prostaglandin A_2 (III). Its R_F on thin-layer chromatography was slightly greater than (15*S*)-prostaglandin A_2 (IV). Upon treatment with diazomethane followed by the silylation reagents, fraction 5 gave one major peak on gas-liquid-chromatography (1.5% Se-30) with a *C*-value of 22.5. The *C*-value for the methyl ester, trimethylsilyl ether of standard (15*S*)-prostaglandin A_2 is also 22.5. Compound B, the leading spot in fraction 4, was tentatively identified as the methyl ester of (15*R*)-prostaglandin A_2 (II). Silylation of this fraction produced a single major peak on gas-liquid chromatography with a *C*-value of 22.5, and the size of this peak was not increased by treatment with diazomethane. Furthermore, treatment of fraction 5 (predominantly compound D) with diazomethane yielded one major spot on a thin-layer chromatogram with an R_F identical to compound B.

Identification of (15R)-Prostaglandin E_2 (V) in the Gorgonian Lipid Extract

Of particular interest was the observation that the fractions eluted from a silic acid column with 60% ethyl acetate in benzene produced a chromophore at 278 nm upon treatment with alkali, which accounted for 7−8% of the chromophore in the total lipid sample. As can be seen in Fig. 1 B, fraction 8 contains a distinct spot (compound G) with an R_F identical to synthetic (15*R*)-prostaglandin E_2 (V). As prostaglandins of the E type have not previously been reported in the gorgonian, a more rigorous identification of compound G was undertaken.

Compound G was first purified from other components in fraction 8 by preparative thin-layer chro-

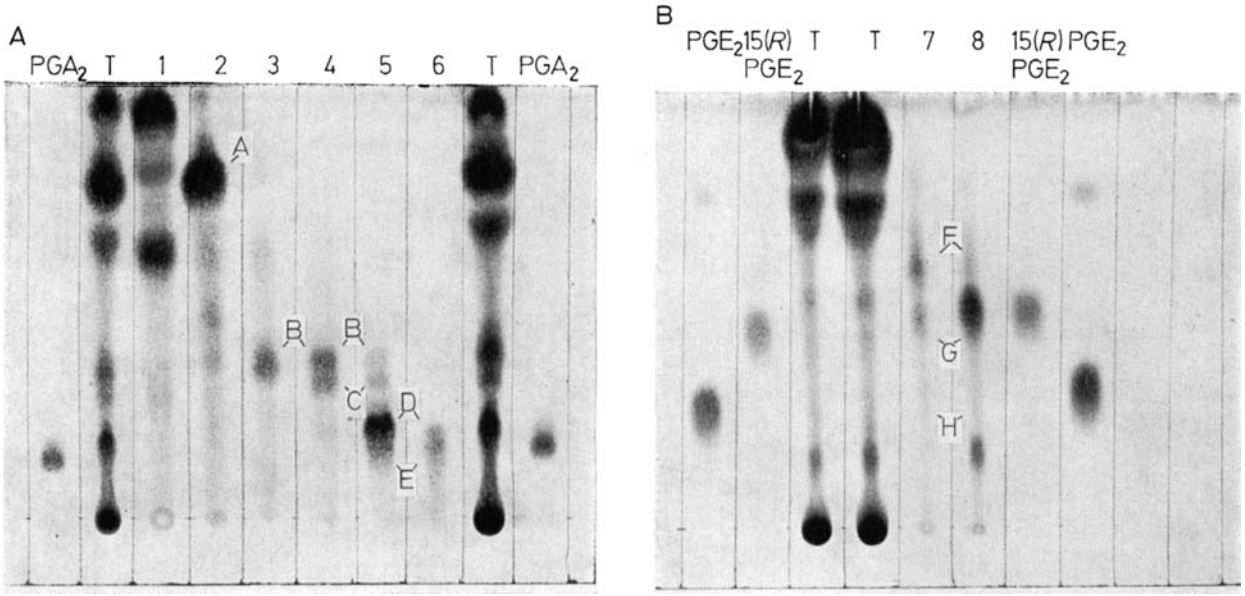


Fig. 1. *Thin-layer chromatography of silicic-acid-column fractions of gorgonian total-lipid extract.* A sample (310 mg) of total lipid in benzene was applied to a 20-g column of silicic acid, and the column was eluted with mixtures of ethyl acetate in benzene. Aliquots of the chromatographic fractions were analyzed by thin-layer chromatography as shown. Spots were made visible by spraying the plates with phosphomolybdate reagent and heating at 120 °C. (A) Developing solvent for the plate was ethylacetate—benzene—formic acid (25:75:1, v/v/v). Standard prostaglandin A_2 (PGA_2) and samples of the total lipid (T) were applied as standards. The numbered samples correspond to the following pooled column chromatographic fractions, expressed as volume of eluting solvent and percentage ethyl acetate in benzene: (1) 300 ml, 3%; (2) 400 ml, 12%; (3) 200 ml, 12% plus 50 ml, 30%; (4) 50 ml, 30%; (5), 200 ml, 30% ; and (6) 200 ml, 30%. (B) Developing solvent for the plate was ethylacetate—benzene—formic acid (80:20:1, v/v/v). Standard prostaglandin E_2 (PGE_2), (15*R*) · prostaglandin E_2, and samples of the total lipid (T) were applied as standards. Fractions 7 and 8 were 60% ethyl acetate in benzene elution obtained from a separate chromatographic experiment on a smaller silicic acid column, but correspond to consecutive fractions of 120 ml each on a 20-g column. The letters point out substances which are discussed in the text

matography. The adsorbent in this region of the plate was eluted with methanol, the methanol evaporated, and the residue was treated with 1% formic acid and extracted with ethyl acetate. Part of the material was converted to the methyl ester, *O*-methyloxime, trimethylsilyl ether derivative. Gas-liquid chromatography analysis of this derivative on 1.5% Se-30 showed two peaks with *C*-values of 23.8 and 24.3, presumably corresponding to the *syn*- and *anti*-isomers of the *O*-methyloxime group [6, 15]. The retention times of these two peaks were identical with those from the methyl ester, *O*-methyloxime, trimethylsilyl ether derivatives of both (15*S*)-prostaglandin E_2 (VII) [6] and synthetic (15*R*)-prostaglandin E_2 (V).

Fig. 2 shows the mass spectrum of the major isomer (*C*-value 24.3) from the methyl ester, *O*-methyloxime, trimethylsilylether derivative of compound G compared to the mass spectrum of the same derivative of synthetic (15*R*)-prostaglandin E_2. These spectra are identical and both are almost identical to the published spectrum for the same derivative of (15*S*)-prostaglandin E_2 [6], establishing the identity of the carbon skeleton of compound G with that of prostaglandin E_2.

Compound F, which was enriched in the early 60% ethyl acetate-benzene fraction (fraction 7), co-chromatographed with the methyl ester of (15*R*)-prostaglandin E_2 (VI). Furthermore the *O*-methyloxime, trimethylsilyl derivative of fraction 7 gave two peaks on gas-liquid chromatography with *C*-values of 23.8 and 24.3, indicating that this fraction contained (15*R*)-prostaglandin E_2, already in the methyl ester form.

The *O*-methyloxime of 8-iso prostaglandin E_2 shows only a single peak on gas-liquid chromatography [15], and so the R_F value on thin-layer chromatography and the retention time of a derivative on gas-liquid chromatography provide some evidence that the stereochemistry of compound G is also the same as (15*R*)-prostaglandin E_2. Since four asymmetric centers are involved, however, further evidence for the stereochemistry was obtained by analysis of oxydative ozonolysis fragments from compound G. To prevent dehydration and preserve the stereochemistry at position 11, compound G was

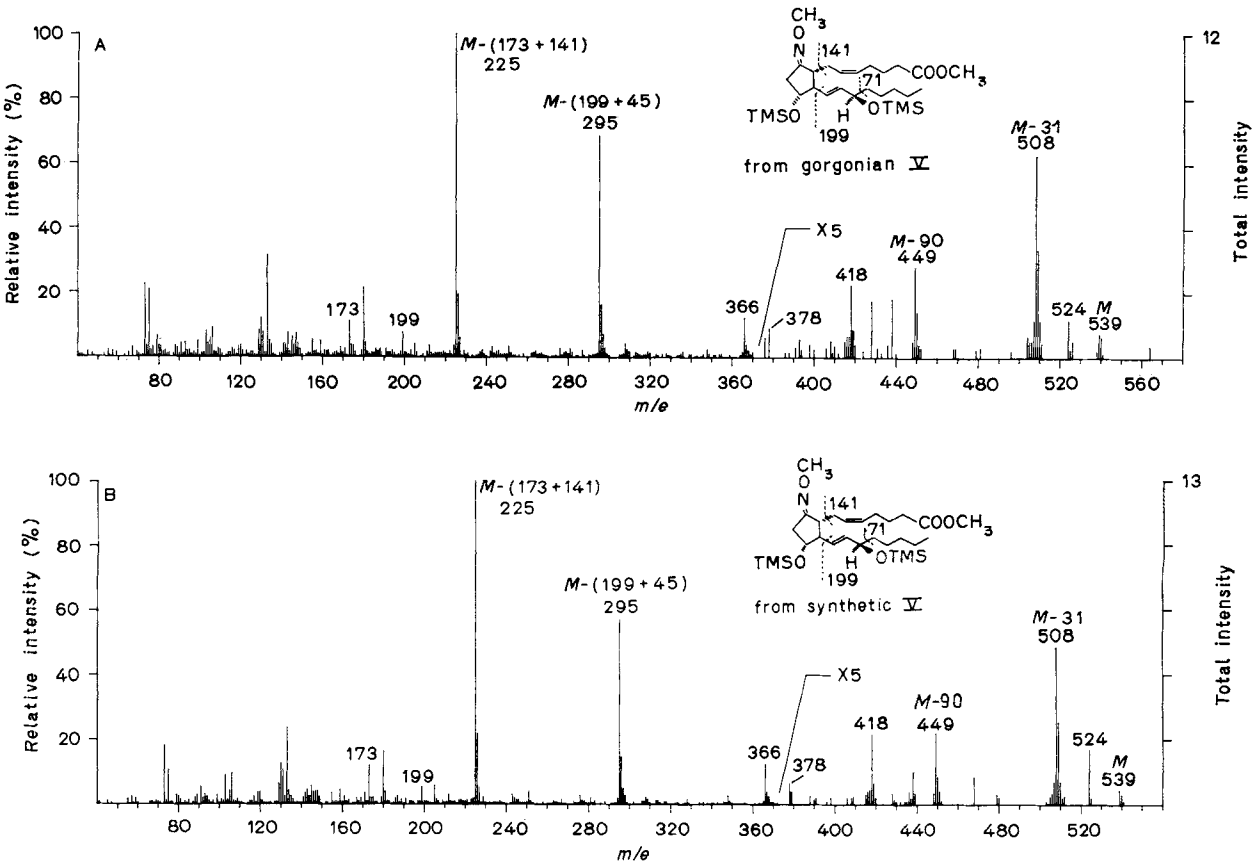
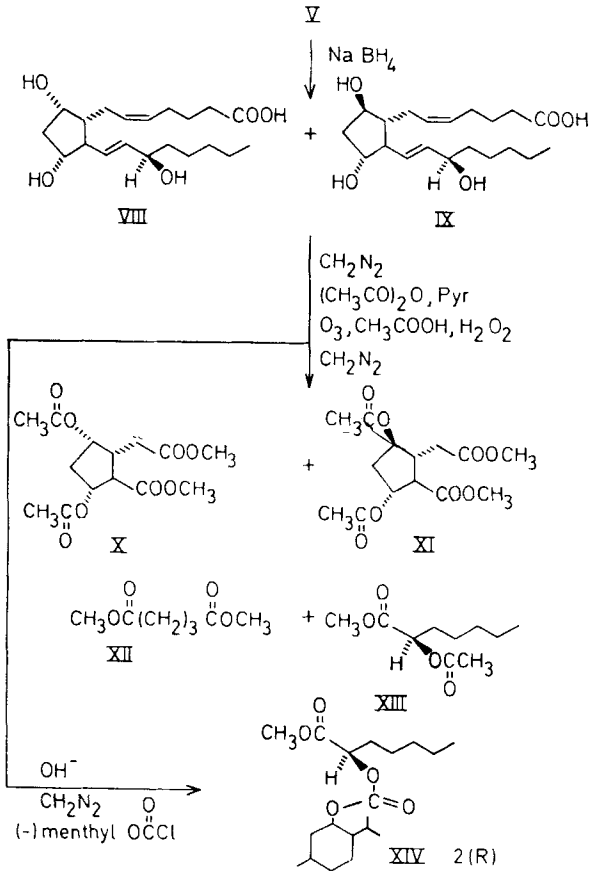


Fig. 2. *Mass spectrum of the methyl ester, O-methyloxime, trimethylsilyl ether derivative (A) of (15R)-prostaglandin E_2 isolated from the gorgonian lipids and (B) of synthetic (15R)-prostaglandin E_2.* Both spectra were obtained from the major isomer of the *O*-methyloxime derivative. Gas-liquid chromatography was performed on 1% Se-30 at 239 °C. TMS = trimethylsilyl

first reduced with sodium borohydride to a mixture of the 9α- and 9β-hydroxy isomers. Thin-layer chromatography of the reduction products (developing solvent, ethyl acetate-benzene, 100:1, v/v) showed two spots with R_F values slightly greater than prostaglandin $F_{2\alpha}$. The mixture (3 mg) was converted to the methyl ester, triacetyl derivative which was purified by preparative thin-layer chromatography (developing solvent, hexane—ether, 50:50, v/v) and subjected to oxidative ozonolysis. As a standard for the ozonolysis degradations 3 mg of (15*S*)-prostaglandin E_2 (VII) was treated in the same way to produce a mixture of prostaglandin $F_{2\alpha}$ and prostaglandin $F_{2\beta}$ methyl ester triacetyl derivative. A second standard ozonolysis was performed on 1.7 mg of methyl triacetyl prostaglandin $F_{2\alpha}$.

Identification of Cyclopentane-Ring Fragments X and XI

Scheme 1 summarizes the reactions involved in the degradation of compound G which establish its structure as (15*R*)-prostaglandin E_2 (V). An aliquot of the ozonolysis products was treated with diazomethane and purified by preparative thin-layer chromatography. Compounds X and XI gave an incompletely separated double spot at R_F 0.48 when the plate was developed in ether—hexane (20:80, v/v). The product from the standard degradation of reduced (15*S*)-prostaglandin E_2 gave a double spot at the same R_F, while the standard degradation of prostaglandin $F_{2\alpha}$ yielded a single spot for compound X. The purified fragments were compared by gas-liquid chromatography. Table 1 shows the retention times, expressed as *C*-values, for the fragments from the two standard degradations along with the fragments from the degradations of compound G. Isomers X and XI from compound G have the same retention times on four gas-liquid chromatography columns of differing polarity as the isomers produced from (15*S*)-prostaglandin E_2. Likewise, isomer X has identical retention times to the same product from prostaglandin $F_{2\alpha}$. Compounds X and XI differ by the configuration of one acetyl group on the cyclopentane ring. It is extremely unlikely that an inversion at one of the three remaining



Scheme 1

Table 1. *Retention times on gas-liquid chromatography of cyclopentane-ring fragments X and XI from oxidative ozonolysis of prostaglandins*

The retention times are expressed as *C*-values. The triacetyl methyl ester derivatives of the compounds listed were degraded

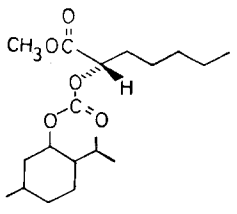
Compound degraded	Product	*C*-values on column of: 1% SE-30 148 °C	1% OV-17 166 °C	5% QF-1 200 °C	10% EGSSX 208 °C
Prostaglandin $F_{2\alpha}$	X	15.7	17.95	22.3	29.9
Reduced prostaglandin E_2	X	15.7	17.95	22.3	29.9
	XI	15.6	17.85	21.5	29.5
Reduced compound G	X	15.7	17.95	22.3	29.9
	XI	15.6	17.85	21.5	29.5

asymmetric centres on the cyclopentane ring would fail to show a retention time difference for either X or XI on at least one of the four gas-liquid chromatography columns. On this basis the relative stereochemistry at positions 8, 11, and 12 of compound G are established as being identical to that of (15*S*)-prostaglandin E_2 (and prostaglandin $F_{2\alpha}$). This data alone does not rule out the possibility that the absolute stereochemistry of these three positions is just the opposite of that found in (15*S*)-prostaglandin E_2, and hence that X and XI from compound G could be enantiomers of X and XI from (15*S*)-prostaglandin E_2. This possibility is ruled out below when the configuration of the 15-hydroxy group is established as 15*R*. If positions 8, 11, and 12 of compound G also had absolute configurations opposite those in prostaglandin E_2, then compound G would be an enantiomer of (15*S*)-prostaglandin E_2, and the two compounds would not separate on thin-layer chromatography.

The carbon skeletons of compound X and XI were confirmed by a comparison of their mass spectra with the standard cleavage products. The mass spectra of X and XI (separated on a 2.2% QF-1 column) were very similar, and they were identical with the spectra of the corresponding isomers from the standard cleavages.

Configuration of the 15-Hydroxyl Group

A second aliquot of the ozonolysis products was dissolved in 3 ml of 2 N methanolic sodium hydroxide and heated at 71 °C for 2.5 h. The mixture was cooled, acidified with 5 N HCl, and extracted four times with ethyl acetate. The combined extracts were washed with water, filtered over anhydrous sodium sulfate, and dried under a stream of argon. The residue was treated with etheral diazomethane and then converted to the (−)menthyloxycarbonyl derivative as described in Materials and Methods. The resulting methyl 2-menthyloxycarbonyloxyheptanoate (XIV) was analyzed by gas-liquid chromatography on a 2.2% QF-1 column. Fig. 3A shows that racemic methyl 2-hydroxyheptanoate produces diastereomeric menthyloxycarbonyl derivatives which can be separated on this column. The peak with the shorter retention times (*C*-value 19.3) corresponds to methyl (2*S*)-menthyloxycarbobyloxyheptanoate (XV) while the peak with the longer retention time (*C*-value 19.5) corresponds to methyl (2*R*)-menthyloxycarbonyloxyheptanoate (XIV). This is in line with the order of elution of diastereomers formed from long-chain 2-hydroxy fatty acid methyl esters [11], and from the methyl esters of lactic acid,



XV 2(S)

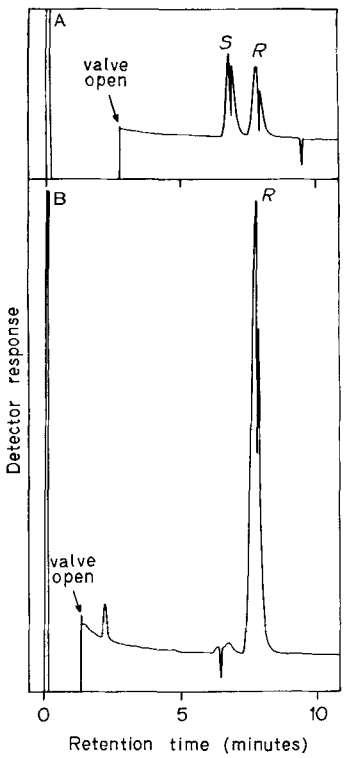


Fig. 3. *Gas-liquid chromatography of (A) the methyl (2S)- and (2R)-menthyloxycarbonyloxyheptanoate mixture obtained from racemic 2-hydroxyheptanoic acid and (B) the methyl (2R)-menthyloxycarbonyloxyheptanoate obtained from degradation of the gorgonian (15R)-prostaglandin E_2.* The compounds were analyzed at 161 °C on a 2.2% QF-1 column in the gas-liquid chromatography unit of the combined gas chromatograph-mass spectrometer, LKB 9000. Zero time is marked at the appearance of the solvent peak, and the recorder tracing began when the valve to the ion source was opened. The "detector response" records a fraction of the total ions produced in the mass spectrometer ion source, and spikes in the recorder tracing represent the recording of a mass spectrum on that portion of the sample. Background spectra obtained on column bleed were subtracted from the recorded spectra. These mass spectra are shown in Fig. 4 and 5

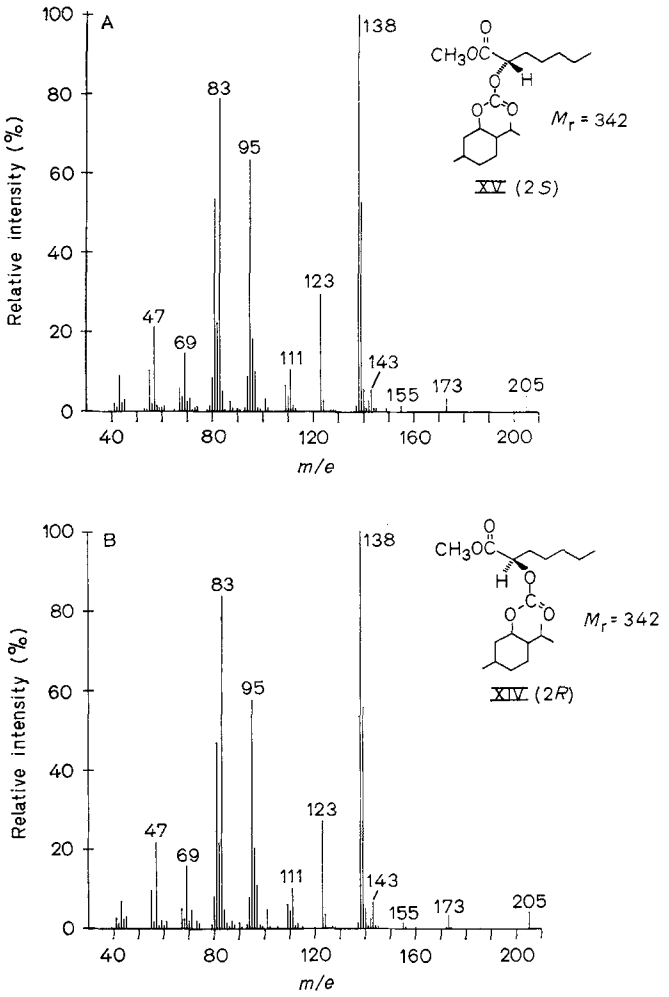


Fig. 4. *Mass spectra of standard samples of (A) methyl (2S)-menthyloxycarbonyloxyheptanoate and (B) methyl (2R)-menthyloxycarbonyloxyheptanoate.* The spectra were obtained on the gas-liquid chromatography samples shown in Fig. 3A

3-phenyllactic acid, 2-hydroxyisovaleric acid and 2-hydroxyisocaproic acid [12]. The identity of the peaks was confirmed with the menthyloxycarbonyl derivative of a standard resolved sample of (2*S*)-hydroxyheptanoate.

The standard ozonolysis of (15*S*)-prostaglandin E_2 yielded 2-hydroxyheptanoate whose menthyloxycarbonyl derivative gave a single peak on gas-liquid chromatography corresponding to the retention time of the *S* isomer showing that our degradation procedures did not lead to racemization at the original 15-hydroxy position. Fig. 3B shows that the 2-hydroxyheptanoate produced from cleavage of compound G has the *R* configuration at its hydroxyl group since its menthyloxycarbonyl derivative corresponds to the second peak in the racemic mixture. The correspondence of retention times was confirmed by injecting mixtures of samples. To make certain of the identity of the peak shown in Fig. 3B, its mass spectrum was compared with those of the standard *R* and *S* methyl 2-menthyloxycarbonyloxyheptanoates. Fig. 4A and 4B show the spectra of the standard isomers. There is very little difference between the spectra of the isomers. There is no molecular ion, and the base peak at *m/e* 138 is derived from the menthyl group. Ions at *m/e* 205, 173 and 143 appear characteristic of the fatty acid chains since they change in comparing compounds of a homologous series. (S. Hammarström, unpublished results). Fig. 5 shows the mass spectrum of the prod-

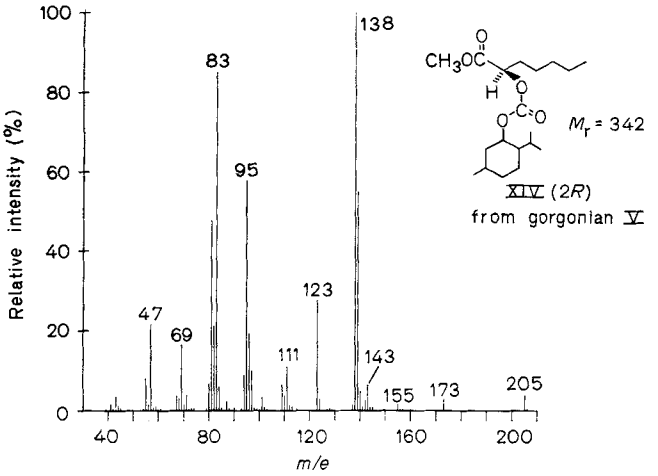


Fig. 5. *Mass spectrum of methyl (2S)-menthyloxycarbonyloxyheptanoate obtained from the degradation of gorgonian (15R)-prostaglandin E_2, compound G in the gorgonian lipid extract.* The spectrum was obtained on the gas-liquid chromatography sample in Fig. 3B

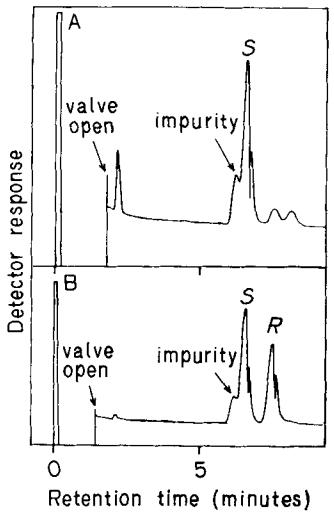


Fig. 6. *Gas-liquid chromatography of (A) the methyl (2S)-menthyloxycarbonyloxyheptanoate obtained upon degradation of compound H from the gorgonian lipid extract and (B) the methyl (2S) and (2R)-menthyloxycarbonyloxyheptanoate mixture obtained upon degradation of fraction 6 of the gorgonian lipid extract.* For experimental details see Fig. 3. Ions from the impurity showing as a shoulder on the (2*S*) peaks were almost completely absent in the spectra recorded at the positions indicated. Background spectra obtained on column bleed and on a separate sample of the impurity were subtracted from the recorded spectra. These mass spectra are shown in Fig. 7

uct derived from compound G, and it is identical with the spectra in Fig. 4. This degradation thus establishes the configuration of the 15-hydroxy group as *R*, and places the double bonds at positions 5 and 13.

Identification of (15 S)-Prostaglandins in the Gorgonian Lipid Extract

Fig. 3B also shows a small peak with the same retention time as methyl (2*S*)-menthyloxycarbonyloxyheptanoate. Furthermore several of the (15*R*) gorgonian prostaglandins were accompanied by slightly more polar materials that have the R_F on thin-layer chromatography of the corresponding (15*S*) derivatives. In Fig. 1A, compound C could be methyl (15*S*)-prostaglandin A_2 and compound E could be (15*S*)-prostaglandin A_2 (IV). In Fig. 1B, compound H (very faint in this chromatogram) could be (15*S*)-prostaglandin E_2 (VII). In order to obtain evidence for the occurrence of the (15*S*) configuration in the gorgonian prostaglandins, an oxidative ozonolysis was carried out on two samples. Fraction 6 (enriched in compound E) and compound H isolated from preparative thin-layer chromatography of the total 60% ethyl acetate-benzene fraction) were treated with diazomethane and converted to the menthyloxycarbonyl derivatives. The menthyloxycarbonyl derivative was subjected to oxidative ozonolysis, and the ozonolysis product was treated with diazomethane and purified on preparative thin-layer chromatography as described in the Material and Methods. Fig. 6 shows the gas-liquid chromatography analysis of methyl 2-menthyloxycarbonyloxyheptanoates from the degradations. It can be seen that degradation of compound H gives principally a peak corresponding to the (2*S*) derivative XV, while degradation of fraction 6 gives both the (2*S*) and (2*R*) derivatives XV and XIV.

Fig. 7 shows the mass spectra of the (2*S*) products. The spectra were taken on the trailing side of the (2*S*) peak in both cases to minimize the contribution of the silica gel impurity (*cf.* to the gas-liquid chromatography tracings in Fig. 6). The mass spectrum of the impurity showed a base peak at *m/e* 149. Only a small peak was seen at *m/e* 149 (3% of the base peak) in the spectra of Fig. 7A and 7B, and this peak was considered to come from the impurity and was subtracted from the two spectra. The spectra in Fig. 7 are seen to be identical to the standard spectra of the compounds shown in Fig. 4A. Hence the gorgonian lipid extract contains compounds which yield (2*S*)-hydroxyheptanoate upon oxidative ozonolysis, and these compounds have polarities on thin-layer chromatography identical with standard (15*S*)-prostaglandins.

In summary the present work shows that the gorgonian, *Plexaura homomalla*, contains, in addition to the previously identified (15*R*)-prostaglandin A_2 and its 15-acetate methyl ester, (15*R*)-prostaglandin E_2 and the methyl esters of (15*R*)-prostaglandin E_2 and (15*R*)-prostaglandin A_2. The data presented on

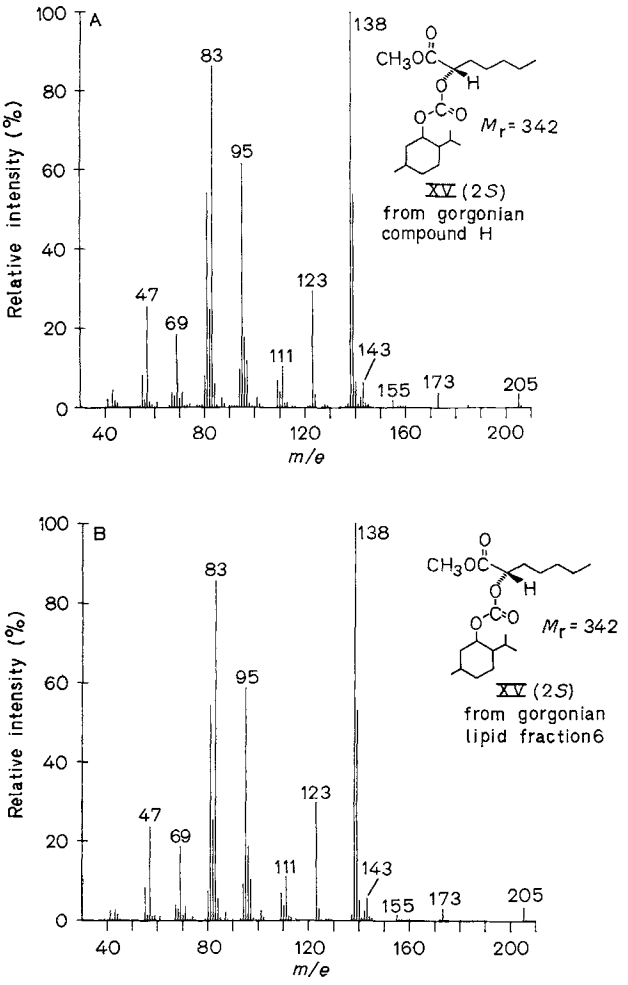


Fig. 7. *Mass spectra of methyl(2S)-menthyloxycarbonyloxyheptanoate obtained (A) upon degradation of compound H from the gorgonian lipid extract and (B) upon degradation of fraction 6.* The spectra were obtained on the gas-liquid chromatography samples shown in Fig. 6

the properties and degradation of the isolated (15*R*)-prostaglandin E_2 establish its carbon skeleton, the stereochemistry at positions 8, 11, 12 and 15 and the positions of the double bonds. The levels of (15*R*)-prostaglandin E_2 found in the gorgonian tissue amount to 0.1—0.2% of the dry weight of the organism. Furthermore, chemical degradation of material with chromatographic properties identical to prostaglandin E_2 and prostaglandin A_2 gave 2-hydroxy heptanoate with the *S* configuration at C-2, demonstrating the presence of (15*S*)-prostaglandins in the gorgonian.

Previous work on the biosynthesis of prostaglandins has shown that the prostaglandin synthetase consists of a membrane-bound enzyme system. This has been solubilized and fractionated; however, as with many particulate enzymes, conventional purification and characterization is difficult [16]. The isolation of (15*S*)-prostaglandin E_2 and (15*R*)-prostaglandin E_2 from the marine invertebrate, *Plexaura homomalla* indicates that the same primary prostaglandin is formed from arachidonic acid in this tissue as in mammalian tissues. Further studies are required to establish the usefulness of the gorgonian tissue for studies of the biosynthesis of prostaglandins at the enzyme level.

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