Isolation and Chemical Conversions of Prostaglandins from *Plexaura homomalla*: Preparation of Prostaglandin E_2, Prostaglandin $F_{2\alpha}$, and Their 5,6-Trans Isomers[1]

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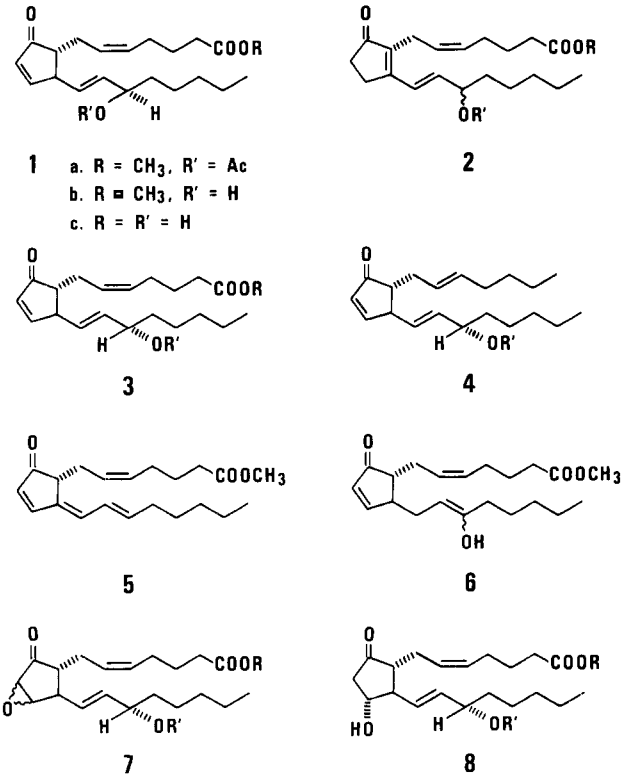
Abstract: The isolation and characterization of (15*R*)- and (15*S*)-prostaglandins A_2 and their esters from the sea whip, *Plexaura homomalla*, is described. Several routes are detailed for the conversion of these coral products to the primary, highly biologically active prostaglandins, PGE_2 and $PGF_{2\alpha}$. The presence of 5,6-*trans*-PGA_2 in this coral, its separation from PGA_2, and its conversion to 5,6-*trans*-PGE_2 and 5,6-*trans*-$PGF_{2\alpha}$ is also described.

In 1969, A. J. Weinheimer and R. L. Spraggins[2] reported the surprising discovery of the occurrence of relatively large amounts (1.3 and 0.2% of the dry weight, respectively) of two unusual prostaglandins **1a** and **1c** in air-dried specimens of a sea whip, *Plexaura homomalla*. These prostaglandins differed from the previously known prostaglandins isolated from mammalian sources in having the "unnatural" *R* configuration at carbon 15. These products, (15*R*)-prostaglandin A_2 and its acetate methyl ester, do not exhibit[3] the usual biological activities of the mammalian prostaglandins and their biological role in the sea whip is unknown. They do not occur in easily detectable amounts in any related corals or other marine animals investigated by Professor Weinheimer or by us.

We found that specimens of *P. homomalla* which were frozen in liquid nitrogen within minutes after collection in Florida waters gave almost exclusively the acetate methyl ester **1a** upon extraction. On allowing the coral to stand in contact with water for increasing times after collection, the acetate methyl ester underwent spontaneous enzymatic hydrolysis to the hydroxy methyl ester **1b** and finally after about 24 h to the hydroxy acid **1c**. Isolation of the prostaglandins from other predominately neutral products of the coral was facilitated by this hydrolysis to the acids.

The hydroxy acid **1c** could be reesterified to **1b** with diazomethane or on a larger scale by the method of Alvarez and Watt[4] using methyl iodide and sodium bicarbonate in dimethylacetamide, and finally to **1a** with acetic anhydride in pyridine without appreciable (<1%) isomerization of the labile enone double bond to the more stable (15*R*)-prostaglandin B_2 acetate methyl ester (**2a**). The (15*R*)-PGA_2 acetate methyl ester (**1a**), after purification by silica gel chromatography, amounted to about 50% of the weight of the crude organic extracts and approximately 1.5–2% of the wet weight of the collected coral.

Light and Samuelsson[5] have reported that prostaglandins from Florida *P. homomalla* contain minute amounts of the 15*S* isomers along with the predominate 15*R* compounds **1**. Previous to this report we had found, strangely, that one specimen of *P. homomalla* from a collection from Florida waters, when hydrolyzed and extracted, gave only (15*S*)-PGA_2 (**3c**) and (15*S*)-PGA_2 methyl ester (**3b**), rather than the 15*R* isomers previously encountered.[1a] The isomers **3b** and **3c** could be distinguished from **1b** and **1c** by thin layer chromatography, which served as a convenient method for screening specimens from experimental collections.[6] We were unable to find another specimen like this from Florida collections, but specimens from numerous other areas of the Caribbean such as the Cayman Islands consisted entirely of *P. homomalla* (Var. *S*), producing



the *S* prostaglandins **3**, while those collected from other areas varied, some containing the 15*S* isomers, some the 15*R* isomers, and on rare occasions, some single specimens containing both *R* and *S* isomers in approximately equal amounts.

The amounts of the (15*S*)-prostaglandins from *P. homomalla* Var. *S* were about the same as from the Var. *R* specimens from Florida. In addition to the PGA_2, a small amount (0.06%) of crystalline PGE_2 (**8c**) was isolated from Cayman *P. homomalla*, identical in physical and biological properties with that from mammalian sources.[7] An even smaller fraction from the column showed the same mobility and color reactions on TLC plates as does $PGF_{2\alpha}$ (**9c**), both as the acid and as the derived methyl ester **9b**, and also showed biological activity like that of $PGF_{2\alpha}$. It also gave a peak in the gas chromatogram (as the methyl ester, trimethylsilyl derivative) and a mass spectra like that of the corresponding derivative of $PGF_{2\alpha}$, but the amount was too small for further characterization. Another fraction gave a peak in the gas chromatogram after trimethylsilylation, having a mass spectral fragmentation compatible with that of a monoacetate of $PGF_{2\alpha}$ methyl ester.

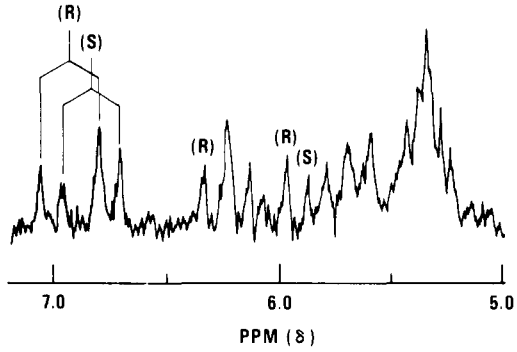
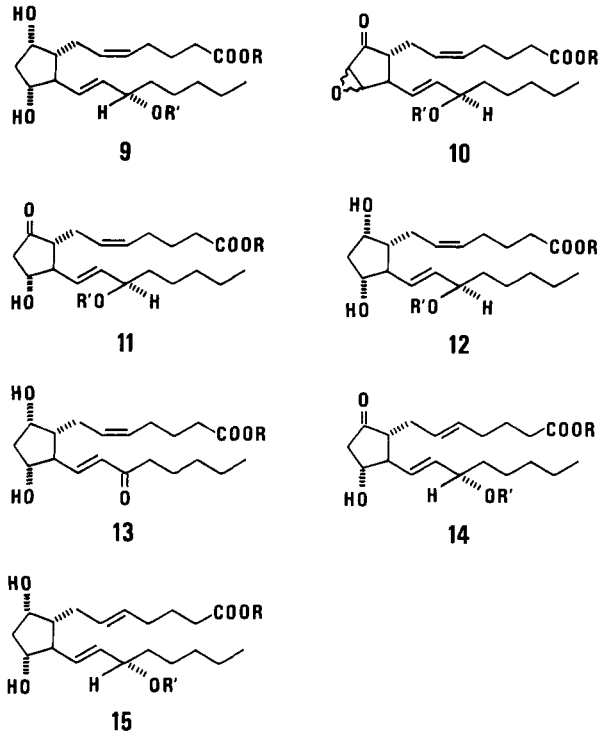


Figure 1. Portion of 60-MHz NMR spectrum of a mixture of 15*R* and 15*S* isomers (58:42) of 15-[(+)-α-methoxy-α-trifluoromethylphenyl]acetates of PGB_2 methyl ester.



While the TLC method is useful for screening coral specimens for (15*R*)- or (15*S*)-prostaglandin A_2 or its methyl ester, other methods were also developed for assaying mixtures of 15-epimers. Treatment of **1b** or **1c** or **3b** or **3c** with aqueous base gives the enantiomeric (15*R*)- or (15*S*)-PGB_2 (**2c**), and the magnitude and sign for the ORD or CD absorptions[8] of these derived materials can be used for determining the approximate stereochemical purity of the starting PGA_2. The accuracy of this method depends upon eliminating all impurities, both optically active and inactive ones, from the sample. A more useful method is based on the NMR method of Dale, Dull, and Mosher.[9] The 15-(+)-α-methoxy-α-trifluoromethylphenyl acetates of the derived PGB_2 methyl esters showed characteristic differences in chemical shifts for a number of protons, the doublet for the C(13) proton being particularly useful (Figure 1). It occurred at δ 6.79 (J = 16 Hz) for the (15*S*)-isomer and at δ 6.89 (J = 16 Hz) for the 15*R* isomer. Differences in chemical shifts of the diasteromeric methoxyl protons in these 15-esters were small and not well resolved at 60 MHz, particularly since they are quartets, split by the fluorines of the trifluoromethyl group. The quartets of the diastereomeric trifluoromethyl groups (split by OCH_3) were separated sufficiently in the fluorine NMR region at 94.1 MHz, so that comparison with computer-generated curves of mixtures of varying amounts of the diastereomers could be used

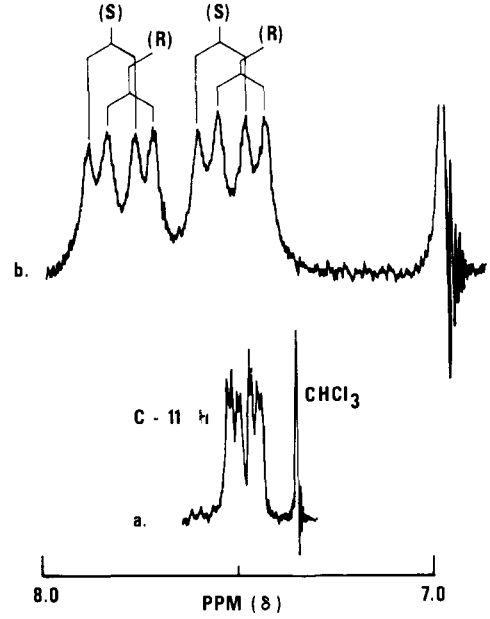


Figure 2. Mixture of about equal amounts of (15*R*)- and (15*S*)-PGA_2 acetate methyl ester, 100-MHz NMR in $CDCl_3$.

for estimation of the relative composition of mixtures.[10] The chiral shift reagent tris[3-(heptafluorobutyryl)-*d*-camphorato]europium(III)[11] also distinguishes (15*R*)- and (15*S*)-PGB_2 methyl esters. Both the C(13) and the methoxyl protons of the 15*R* isomer are shifted further downfield by about 5 Hz compared to those of the 15*S* isomer in a 2.06 M solution of the shift reagent in CCl_4 at 60 MHz. Other protons are also selectively shifted, but are not sharp enough to be diagnostically useful.

None of the above methods are useful for determining configuration at C(15) of the PGA_2 acetate methyl esters.[12] However, at 100 MHz, there is about a 1 Hz difference in chemical shifts for the C(11) proton in the two isomers **1a** and **3a** in $CDCl_3$, that of the 15*S* isomer being further downfield, which can be used at appropriate sweep width expansion to estimate the composition of mixtures[13] (Figure 2).

Both the (15*R*)- and (15*S*)-prostaglandin A_2 and the esters isolated by silica gel chromatography, while apparently homogeneous by the usual criteria, were found to contain a faster moving component when chromatographed on silver nitrate impregnated silica gel TLC plates.[1c] This component was isolated on a preparative scale by column chromatography of (15*S*)-PGA_2, using either silver nitrate impregnated silica gel, or better a macroreticular ion-exchange resin (Amberlyst 15) in the silver form, or by countercurrent distribution between organic solvents and aqueous silver nitrate solutions. It was found to account for 5–15% of the total, varying somewhat with different sources of *P. homomalla*. This material was very similar by analytical and spectral properties to PGA_2, except for increased intensity of absorption at 975 cm^{-1}, indicative of an additional trans double bond and changes in the vinylic proton region, 5.2–5.78 in the NMR spectrum. It was characterized as 5,6-*trans*-PGA_2 (**4c**) by its subsequent conversion to 5,6-*trans*-PGE_2 **14c** (see below).

Conversion of (15*R*)-PGA_2 or its esters **1** to the highly biologically active primary prostaglandins, PGE_2 and $PGF_{2\alpha}$, consists essentially of two parts: (1) the addition of the elements of water to the enone system in the ring, and (2) inversion of configuration at C(15). For the former, there are precedents in, for example, the steroid field,[14] involving epoxidation of the enone with alkaline hydrogen peroxide, followed by reductive opening of the oxirane ring with Cr(II) salts. One might anticipate difficulty with the use of alkaline solutions on the

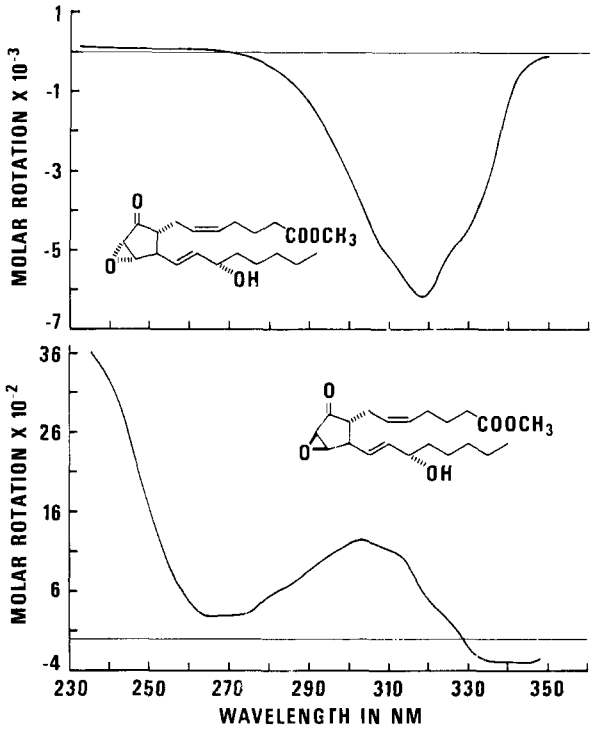


Figure 3. ORD curves, Carey 60, Model 6002 spectropolarimeter, in dioxane at ca. 1 mg/ml concentration.

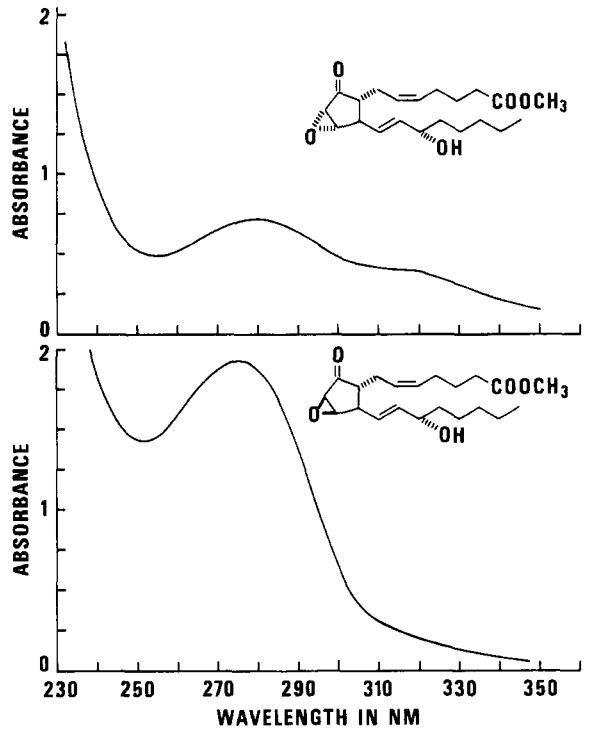


Figure 4. Absorbance curves, Carey 60, Model 6002 spectropolarimeter, in dioxane at ca. 1 mg/ml concentration.

PGA_2 compounds because of the known lability of the enone system with rapid formation of the alternative, more stable enone **2** and/or **5**. Inversion of the stereochemical configuration of allylic alcohols by displacement reactions are not usually high yielding reactions because of accompanying allylic rearrangements and eliminations. In fact, we previously had experience[15] with inversions at C(15) of PGE_1 and $PGF_1\alpha$ in formic acid, which gave about 25% yields of the C(15) epimers. Recently Spraggins[16] has reported the formic acid method to convert **1a** to **3b** and a number of other products, including the allylic rearrangement products **6**. We also had previously[15] inverted the configuration at C(15) of $PGF_2\alpha$ by the steps of selective oxidation of the allylic alcohol to the ketone followed by reduction. This leads to mixtures of 15*R* and 15*S* alcohols and 13,14-dihydro compounds, but reagents have recently been developed which favor reduction to 15*S* alcohols.[17] We describe below four routes to PGE_2 and/or $PGF_2\alpha$ based on the different starting materials, (15*R*)-PGA_2 methyl ester (**1b**), (15*R*)-PGA_2 acetate methyl ester (**1a**), (15*S*)-PGA_2 acetate methyl ester (**3a**), and (15*S*)-PGA_2 (**3c**), which illustrate successful and increasingly efficient methods for utilization of these coral prostaglandins.

Inversion of configuration of (15*R*)-PGA_2 methyl ester (**1b**) by solvolysis of its 15-mesylate in aqueous acetone or tetrahydrofuran gave about 25% of (15*S*)-PGA_2 methyl ester (**3b**) along with about the same amounts of starting **1b** and allylic rearrangement products **6**. This mixture is similar to that obtained by treating **1a** with formic acid and hydrolyzing the resulting mixture of formates.[16] The PGA_2 methyl ester produced in this way from "*R*" coral was identical with that obtained directly from "*S*" coral and to authentic samples. Epoxidation of the chromatographically separated PGA_2 methyl ester from either source at 0 °C in isopropyl alcohol with hydrogen peroxide was rapid upon dropwise addition of sodium hydroxide solution, giving about 90% of a mixture of 10,11-epoxides (**7b**) with very little base-catalyzed double bond migration to (15*S*)-PGB_2 methyl ester (**2b**). The latter is not epoxidized under these conditions. Although TLC showed essentially one spot slightly less polar than (15*S*)-PGA_2 methyl ester, the NMR spectrum indicated that a mixture of isomeric epoxides was formed. These could be separated by high pressure liquid chromatography, giving the 10,11α-epoxide (**7b**) as the first eluted from silica gel, followed by the β-epoxide in a ratio of about 1:4. The latter showed a 10-Hz downfield shift of the C(13),C(14) olefinic protons, an upfield shift of the C(12) proton, and smaller shifts of the C(10),C(11) protons, compared to the α-epoxide. The ORD curves (Figure 3) showed opposite signs for 300-nm bands and the α-epoxide showed considerably greater $n \rightarrow \pi^*$ absorbance than did the β-epoxide (Figure 4). Assignment of configuration was confirmed by reduction of the pure α-epoxide with aluminum amalgam[18] to prostaglandin E_2 methyl ester (**8b**) and a similar reduction of the β-epoxide to the less polar 11β isomer of **8b**. This isomer ratio was disappointing, but could be changed to nearly 1:1 by carrying out the epoxidation at −20 °C in methanol using potassium hydroxide as the base. Better ratios could be obtained (see below) by making derivatives of the C(15) hydroxyl group.

There is some rationalization for predominant β-epoxidation by applying the principle of overlap control of carbanionoid reactions as developed by Zimmerman[19] and particularly by Katsuhara.[20] If the ring carbons in the intermediate hydroperoxy anion are noncoplanar in the direction to allow the large side chains at C(8) and C(12) to have maximum equatorial character,[21] then maximum orbital overlap leading to elimination of OH and epoxide formation is developed in the case of the 11β-hydroperoxide. For maximum overlap with α-hydroperoxide, the ring must be noncoplanar in the direction which tends to make the side chains both axial. Thus any change in the steric requirements of the side chains which would tend to force them further apart could change the ring conformation toward the latter and increase the proportion of α-epoxide.[22] In the latter conformation, there is also increased steric hindrance to the β face of the ring in some conformations of the R_8 side chain, which would also favor α-epoxidation.

Ordinarily, separation of 10,11-epoxides was not attempted, but the mixture was directly reduced with Cr(II) salts[14] or better with aluminum amalgam, since the isomeric 11-alcohols

were more easily separable than the epoxides by silica gel chromatography. We were not able to find conditions for Cr(II) reductions of A_2 epoxides (**7b**) which did not also give 25–50% of the starting PGA_2 methyl ester **3b** in addition to the PGE isomers,[23] but aluminum amalgam reductions in ether–methanol–water were quite high yielding.[24] The 11β epimer of PGE_2 methyl ester showed characteristic differences in the NMR spectrum compared to that of PGE_2 methyl ester (**8b**). The C(13),C(14) olefinic protons were shifted downfield by about 9 Hz, and the C(11) and C(15) protons occurred at δ 4.15 and 4.4, where in PGE_2 methyl ester they occur together at about δ 4.15. Hydrolysis of the PGE_2 methyl ester (**8b**) with an acetone-insoluble esterase derived from *P. homomalla* gave PGE_2 in 43% yield, completing the synthesis from either (15*R*)- or (15*S*)-PGA_2 methyl esters.

Coral-derived (15*R*)-PGA_2 acetate methyl ester (**1a**) was converted to $PGF_{2\alpha}$ (**9c**) in the following way. Epoxidation of **1a** in methanol at −20 °C as above gave a mixture of epoxides **10a** in which the α-epoxide predominated, as judged by the NMR spectrum. After aluminum amalgam reduction, chromatographic separation gave (15*R*)-PGE_2 15-acetate methyl ester (**11a**) and its 11β isomer in yields of 49 and 11.4%, respectively, from **1a**.

The 9-keto group of **11a** was then reduced with sodium borohydride after temporarily converting the 11α-hydroxyl to its trimethylsilyl ether. By this means, a 9α/9β ratio of 68:32 was obtained and **12a** was isolated in 55% yield from **11a**. Basic hydrolysis of **12a** gave (15*R*)-$PGF_{2\alpha}$,[15] which was selectively oxidized with 2,3-dichloro-5,6-dicyanobenzoquinone to give **13c** in 40% yield. After protecting hydroxyl groups by trimethylsilylation the 15-ketone was reduced with zinc borohydride in dimethoxyethane[26] to give **9c** and **12c** in a ratio of 73:27, from which **9c** was isolated by chromatography in 57% yield. The $PGF_{2\alpha}$ was identical with authentic samples.

The conversion of (15*S*)-PGA_2 acetate methyl ester to PGE_2 and $PGF_{2\alpha}$ follows closely the procedures used for the 15*R* isomer. Alkaline hydrogen peroxide epoxidation gave epoxides **7a**, which were reduced by aluminum amalgam, and PGE_2 15-acetate methyl ester (**8a**) was separated by silica gel chromatography from the 11β epimer in a yield of 50% from **3a**. The 11β epimer could be dehydrated in high yield to **3a** by refluxing with Florisil in benzene or by Woelm alumina (activity I) in benzene at room temperature. Under these conditions essentially no PGB_2 acetate methyl ester or C(15) elimination products were produced.

Enzymatic hydrolysis of **8a** with the acetone-insoluble fraction from *P. homomalla* gave PGE_2 (**8c**), mp 66–67 °C after two recrystallizations. Sodium borohydride reduction of the 11-trimethylsilyl derivative of **8a**, followed by hydrolysis gave $PGF_{2\alpha}$ (**9c**) and $PGF_{2\beta}$ in a ratio of 62:38. Chromatographic separation afforded $PGF_{2\alpha}$ in 55% yield from **8a**.

During the development of the above processes, several other epoxidation procedures were investigated, including *tert*-butyl hydroperoxide and Triton B in benzene[27] and sodium hypochlorite in aqueous tetrahydrofuran, but these gave less favorable α/β ratios than the above method. Also, a number of other reducing agents, in addition to Cr(II) and aluminum amalgam, were tried for reductive opening of epoxides **7** or **10**. Electrolysis of **10a** in methanol–water containing potassium acetate (4 V, 0.5 A) gave satisfactory yields of the isomeric PGE's, while zinc in methanol in the presence of ammonium chloride gave a mixture of PGE_2 and PGA_2 acetate methyl esters. Two convenient methods were developed for in situ preparation of the very reactive Cr(II) acetate, which may be useful in other cases (see Experimental Section).

During some attempts to intercept an intermediate 11-hydroperoxide in the epoxidation step, PGA_2 methyl ester (**3b**) was treated with weak bases such as sodium bicarbonate and hydrogen peroxide or *tert*-butyl hydroperoxide and aliquots were added to potassium iodide–sodium thiosulfate solutions with the intention of reducing such intermediate peroxides to the 11-alcohols. Small amounts of PGE_2 and 11β-PGE_2 methyl esters were noted on TLC investigation of these aliquots. The PGE's did not arise, however, until some of the epoxides **7b** had been formed in the reaction, and it was found that preformed epoxide **7b** liberated iodine from potassium iodide.[28] This reducing system was then briefly investigated as an alternative to aluminum or Cr(II) reductions. Sodium thiosulfate was not a very satisfactory reducing agent for the liberated iodine, since it formed polar by-products evidently by reaction with the oxirane ring. Triphenylphosphine proved to be a very rapid and mildly basic reducing agent, converting liberated iodine to iodide and also neutralizing the hydrogen iodide formed. A better combination was a mixture of KI, triphenylphosphine, and aqueous sodium bicarbonate in tetrahydrofuran–methanol, which rapidly converted epoxides **7b** to a mixture of **8b**, its 11β epimer, and enone **3b**. Unfortunately, conditions were not found which gave only PGE's; usually at least an equal amount of **3b** was concurrently formed.

The above methods utilizing coral-derived PGA_2 esters require a hydrolysis step at the end of the synthesis to obtain PGE_2 or $PGF_{2\alpha}$, and in the case of PGE_2 an enzymatic step is required due to the lability of the β-hydroxy ketone system to usual chemical methods. Initial attempts to use PGA_2 directly in the epoxidation and aluminum amalgam reduction sequence (**3c** → **7c** → **8c**) pointed up two difficulties. First, the epoxidation of **3c** gave predominately the 10β,11β-epoxide and secondly, the aluminum amalgam reduction done as above was difficult to drive to completion and the resulting products included considerable amounts of regenerated **3c** and some $PGF_{2\alpha}$ (**9c**) and $PGF_{2\beta}$ from reduction of the 9-ketone. The first of these problems could be overcome by trimethylsilylating the PGA_2 before epoxidation. This temporary protecting group at C(15) changed the ratio of epoxides to about 7:1 α/β and was later easily removed by acidification during the workup of the aluminum amalgam reduction.[29]

It had been found previously that addition of acetic or citric acids to aluminum amalgam reductions of epoxides **7a** or **7b** had resulted in formation of PGA_2 esters in addition to the desired PGE esters. Presumably, **7c**, as the carboxylic acid, was also promoting the reversion of epoxide back to starting unsaturated ketone **3c** during aluminum amalgam reduction. It was found that if the crude epoxide mixture above in a solvent system of tetrahydrofuran, methanol, and water was first converted to the sodium salts by addition of sodium bicarbonate, then aluminum amalgam reduction was rapid and complete and the products contained little PGA_2 by TLC. Upon acidification and extraction a mixture was obtained, which upon silica gel chromatography gave overall yields (from **3a**) of about 70% PGE_2 and 10% 11β-PGE_2 as combined chromatographic fractions. Crystallization of the former gave 47.3% overall yield of PGE_2, mp 65–67.5 °C when purified (free from 5,6-trans) PGA_2 was used as starting material.

The 5,6-*trans*-PGA_2 (**4c**) separated as described above from coral-derived PGA_2 was also converted to 5,6-*trans*-PGE_2, mp 75–77 °C, by the process described immediately above. This was then converted to its trimethylsilyl derivative and reduced with sodium borohydride to give 5,6-*trans*-$PGF_{2\alpha}$, mp 94.8–95.8 °C, and 5,6-*trans*-$PGF_{2\beta}$, mp 77–79 °C. These products were identical with products obtained from the corresponding 5,6-cis isomers by irradiation in oxygen-free benzene–methanol with 3500-Å light in the presence of diphenyl sulfide, a process[30] known to isomerize cis to trans carbon–carbon double bonds without bond migration. 5,6-*trans*-PGE_2 has also been prepared by van Dorp and co-workers[31] by biosynthesis from 5-*trans*,8-*cis*,11-*cis*,14-cis-eicosatetraenoic acid.

The above studies show that coral-derived prostaglandins

of the PGA_2 type can serve as starting materials for efficient chemical synthesis of PGE_2 and $PGF_{2\alpha}$. Other studies indicate that *P. homomalla* can be harvested in limited amounts and under ecologically controlled conditions by careful hand pruning by trained personnel. When properly harvested, regrowth occurs at an appreciable rate. Long term studies of the effect of properly controlled harvests on the involved ecosystems are continuing.[32]

Experimental Section[33]

(15*R*)-PGA_2 Acetate Methyl Ester 1a from *P. homomalla* (Var. *R*). *P. homomalla* (Var. R) (25 g) collected off the Florida Keys[34] and frozen within minutes of collection in liquid nitrogen and maintained in liquid nitrogen until extraction was placed in a Waring Blender with 150 ml of 95% ethanol and blended, while maintaining the temperature below room temperature by addition of dry ice. After filtration, the solid residue was again blended with an additional 50 ml of 95% ethanol. The combined filtrates were concentrated in vacuo to an aqueous slurry, pH 6, which was extracted with ethyl acetate. The extracts were washed with 1 N HCl and saturated NaCl, dried with Na_2SO_4, and evaporated to a residue of 800 mg of dark oil. A thin layer chromatogram (AIX system[35]) of this showed an intense spot corresponding to authentic (15*R*)-PGA_2 acetate methyl ester[2] (**1a**) and a much fainter spot like the 15-acetate of (15*R*)-PGE_2 methyl ester (**11a**) (see below) as the only prostaglandin-like materials. The mixture was chromatographed on 50 g of silica gel, eluting with 600 ml of a gradient of 10–100% ethyl acetate–Skellysolve B,[36] giving 429 mg (1.7% of the wet weight of the coral) of (15*R*)-PGA_2 acetate methyl ester identical in IR and NMR spectral characteristics with an authentic sample.[2] The material corresponding in mobility to **11a** amounted to less than 30 mg.

The (15*R*)-PGA_2 acetate methyl ester fractions, on thin layer chromatography on silver nitrate impregnated plates (developed with 2% acetone in chloroform) showed the presence of small amounts of the faster moving 5,6-trans isomer.

(15*S*)-PGA_2 (3c), (15*S*)-PGA_2 Methyl Ester (3b), and PGE_2 (8c) from *P. homomalla* (Var. *S*). Approximately 700 g of *P. homomalla* (Var. S) from the Cayman Islands, which had been kept frozen in dry ice since collection, was cut into pieces and then ground in a Waring Blender with 1500 ml of water. This slurry was stirred for 20 h at room temperature, acidified with 25 ml of 12 N HCl, and then extracted four times with ethyl acetate. The extracts were filtered through a filter aid (Celite), washed with saturated NaCl, dried with sodium sulfate, and evaporated. The solids filtered from the above aqueous layer were stirred for 2 h with enough methanol to cover, filtered, and the filtrate concentrated in vacuo. This residue was extracted with ethyl acetate and the extracts washed and dried as above. The residue from this, 14 g, was combined with the first ethyl acetate extracted material, giving a total of 25 g of dark oil which was chromatographed on 1.5 kg of silica gel, eluting with a gradient of 16 l. of 25–100% ethyl acetate–Skellysolve B. Fractions of 500 ml each were evaporated. Fractions 8–12 contained 2.8 g of (15*S*)-PGA_2 methyl ester (**3b**), fractions 15–18, 9.54 g of (15*S*)-PGA_2 (**3c**), and, fractions 35–40, 414 mg of (15*S*)-PGE_2 (**8c**) contaminated by some other materials. This latter material, after decolorizing with charcoal in ether and concentrating, gave 190 mg of crystalline (15*S*)-PGE_2, mp 63–66.5 °C, identical in NMR and IR spectra and TLC mobility to authentic samples.

The (15*S*)-PGA_2 fraction above showed approximately 15% of a faster moving isomer on silver nitrate impregnated silica gel thin-layer plates and was further purified as follows.

Amberlyst 15 (3 kg) was washed with water to remove colored impurities, then packed into three 5.8-cm columns connected in series with tubing. The free space between columns was kept to a minimum. Total column length was 8–9 ft. The resin was converted to the silver form by eluting with 10% aqueous silver nitrate solution until the pH of the effluent rose to 3.5–4.0. A moderately slow flow rate was maintained. $AgNO_3$ (3–4 kg) was required. The column was washed with deionized water until the effluent was free of silver ion; then with two column volumes of 3A alcohol (about 5 l.).

A solution of 25 g of chromatographed (15*S*)-PGA_2 mixture (about 16% 5,6-*trans*-(15*S*)-PGA_2 by TLC assay) in 35 ml of 3A alcohol was carefully applied to the top of the column and rinsed on with another 10 ml of solvent. The column was eluted with 3A alcohol at a flow rate of approximately 1 l./h. The first 2.5 l. of eluent was the column holdup. The next 5 l. was collected in 500-ml fractions and individually assayed by TLC using $AgNO_3$ impregnated silica gel plates and A-IX[35] development. The trans isomer is slightly less polar. Elution with 3A alcohol was continued until all of the slower cis isomer was eluted (10–12 l.). As the last of the cis isomer was eluted a second 25-g portion of mixture was applied to the top of the column. The column was repeated as before. Two more 25-g portions were chromatographed. Like fractions from each column were combined in methylene chloride, washed with H_2O, dried, and evaporated to give 11 g of 5,6-*trans*-PGA_2 (**4c**), 11 g of mixed material, and 73 g of 5,6-*cis*-PGA_2 (**3c**). The products were deep yellow colored oils.

The *cis*-PGA_2 fraction was further purified by chromatographing over 400 g of acid-washed silica gel. The material was applied in benzene, and the column washed with Skellysolve B. The *cis*-PGA_2 was then rapidly eluted with 60% ethyl acetate in Skellysolve B. The solvent was evaporated, the residue taken up in ether, treated with decolorizing charcoal, filtered, and evaporated under house vacuum and then high vacuum, yield 68.6 g yellow oil. This material gave the following analytical data: melt solvate, 0% ether or ethyl acetate; $[\alpha]_D$ +145° ($CHCl_3$); UV λ_{max} 217 mμ (ε 10 300); IR (neat) λ_{max} 3400–3100, 2650 sh (OH/COOH), 1705 (C=O), 1585 (C=C), 1405, 1345 (CH/other), 1300, 1235, 1175, 1150 sh, 1060, 1015 (C-O/other), 970 (*trans*-CH=CH) cm^{-1}; NMR ($CDCl_3$) δ 7.57 (1 H, dd, C(10) vinylic), 6.93 (2 H, s, COOH + OH), 6.2 (1 H, dd, C(11) vinylic), 5.6 (2 H, m, C(13,14) vinylic), 5.4 (2 H, m, C(5,6) vinylic), 4.12 (1 H, m, C(12)H), 3.23 (1 H, m, carbinolic), 0.89 (3 H, t, CH_3).

A portion of the 5,6-*trans*-PGA_2 fraction was similarly rechromatographed over acid-washed silica gel. The purified material was combined in methylene chloride, washed with water, dried, treated with decolorizing charcoal, filtered, and evaporated to a pale yellow oil: $[\alpha]_D$ +137° ($CHCl_3$); IR (neat) λ_{max} 3380–3000, sh 2640 (OH/COOH), 1705 (C=O), 1585 (C=C), 1295, 1240, 1180, 1150 sh, 1045, 1015 (C-O/other), 970 (*trans*-CH=CH) cm^{-1}; UV λ_{max} 216 mμ (9250); mass spectrum as Me_4Si derivative, calcd, 478.2932, found, 478.2998.

(15*R*)-PGA_2 (1c) and (15*R*)-PGA_2 Methyl Ester (1b) from *P. homomalla* (Var. *R*). In the same manner as the preceding experiment, 500 g of frozen *P. homomalla* (Var. R) from off the Florida Keys was hydrolyzed in water for 24 h, extracted, and chromatographed. Fractions 15–17 contained (15*R*)-PGA_2 methyl ester, 1.37 g, and fractions 18–22 contained 6.57 g of (15*R*)-PGA_2.[2]

(15*S*)-PGA_2 Acetate Methyl Ester (4a) from *P. homomalla* (Var. *S*). Frozen Caribbean *P. homomalla* (1 kg) was extracted in a Waring Blender using a total of about 5 l. of methylene chloride. After filtration of the crude extracts through Celite, the filtrate was concentrated at reduced pressure (maximum temperature 30 °C), thereby affording 27.0 g of a dark green oil. Analysis by TLC (A-IX solvent system[35]) indicated about a 1:1:2 mixture of PGA_2 (**3c**), PGA_2 methyl ester (**3b**), and PGA_2 acetate methyl ester (**3a**).

The crude product was dissolved in 125 ml of reagent grade *N*,*N*-dimethylacetamide (DMA) and treated with 25 ml of methyl iodide and 6.0 g of solid sodium bicarbonate. The resulting suspension was stirred vigorously for 18 h, then poured into 1500 ml of 5% aqueous sodium chloride and extracted thoroughly with Skellysolve B. The combined extracts were washed with water, aqueous sodium bicarbonate, and brine, and dried over anhydrous sodium sulfate. Removal of the solvent at reduced pressure afforded 27.2 g of a dark green oil. Analysis by TLC indicated the absence of PGA_2.

The crude product was dissolved in 35 ml of pyridine and treated with 25 ml of acetic anhydride. After 2 h at room temperature, TLC indicated no 15-OH. The mixture was cooled in an ice bath and treated with ice chips at a rate which allowed the temperature of the reaction mixture to remain below 10 °C. Then 20 ml of cold water was added and the mixture was stirred 10 min longer. After dilution of the mixture with 300 ml of water the product was isolated by extraction with ethyl acetate. The organic layers were washed with 3 N hydrochloric acid until the washes were acidic, then with water, saturated aqueous sodium bicarbonate, and brine, and dried over anhydrous sodium sulfate. Removal of the solvent gave 27.4 g of a green oil, which was chromatographed on 1.7 kg of Brinkmann silica packed in Skellysolve B. Elution with 20% ethyl acetate–Skellysolve B gave fractions homogeneous by TLC which, after charcoal treatment and concentration, afforded 13.5 g of PGA_2 acetate methyl ester (**3a**) (50% yield from crude extracts).

This material could be further purified by argentation counter-

current distribution to remove small amounts of the 5,6-trans isomer as follows. The Craig countercurrent distribution apparatus used for this separation was a 200-tube machine, 10 ml per phase in each tube. The solvent system was hexane–methanol–water (10:9:1). The aqueous methanol was made to 0.3 N silver nitrate before equilibration with the hexane.

A partially purified coral extract (15 g) containing predominantly the 5,6-cis and 5,6-trans isomers of 15-acetyl PGA_2 methyl ester was loaded into the first 15 tubes of the Craig apparatus and the distribution began. After 200 transfers, the contents of tubes 0–15 were removed and discarded. These contained the bulk of the polar impurities and most of the color. The tubes were refilled with fresh solvent and the distribution continued (by recycling the upper phase) for an additional 1400 transfers. The location of the products was determined by removing 20 μl of lower phase from every sixth tube, treating it with 5 ml of 1 N potassium hydroxide in methanol, and measuring the absorption at 278 nm.

The contents of the tubes were pooled as follows: cis fraction, tubes 160–199, 0–10, 6.21 g; trans fraction, 29–75, 1.64 g.

The product was isolated by diluting each pool with an equal volume of water and separating the two phases. The aqueous (lower) phase was reextracted with 0.5 vol of hexane (based on the original pool volume) and the hexane extracts pooled. This was washed with brine to remove residual silver nitrate, dried over sodium sulfate, and evaporated to an oil.

Thin-layer chromatography on silver nitrate impregnated plates (developed with 2% acetone in chloroform) indicated complete separation of the cis and trans isomers. Combined material from the 5,6-cis fraction gave the following data: IR λ_{max} 1735, 1710, 1585, 1240, 1170, 1020, 970 cm^{-1}; NMR ($CDCl_3$) δ 7.52 (C(11)H, 4 line pattern, 1 H), 6.20 (C(10)H; 4 line pattern; 1 H), 5.8–5.0 (vinyl and C(15)H, complex m, 5 H), 3.67 (CO_2CH_3, s, 3 H), 3.4–3.1 (C(12)H, m, 1 H), 2.04 (CH_3CO_2, s, 3 H), 0.90 (C(20) methyl, unresolved t, 3 H); UV (EtOH) λ_{max} 217 mμ (ϵ 10 500); $[\alpha]D$ +117° ($CHCl_3$, 1.026 g/100 ml); mass spectrum *m/e* 390 (M^+), M^+ − (HOAc + OMe). Anal. Calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.41; H, 8.57.

15-[(+)-α-Methoxy-α-trifluoromethylphenyl]acetate of PGB_2 Methyl Ester (2). A solution of 40 mg of (15*S*)-PGA_2 methyl ester (**3b**) in 4 ml of methanol and 4 ml of 1 N NaOH was stirred overnight under nitrogen, acidified with 4.5 ml of 1 N HCl, extracted with ethyl acetate, and the extracts were dried and evaporated. The residue containing (15*S*)-PGB_2 (**2c**) was reesterified with excess ethereal diazomethane, evaporated, and the residue chromatographed on 10 g silica gel, eluting with 100 ml each of 20, 30, and 50% ethyl acetate in Skellysolve B. The material having TLC mobility like that of authentic PGB_2 methyl ester (30 mg) was treated for 1 h at 25 °C with 0.3 ml of pyridine and 7 drops of (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride.[9] Water was added and the product was extracted with ethyl acetate, which was washed, dried, and evaporated. Carbon tetrachloride was added and removed by evaporation and the residue was dissolved in deuteriochloroform for NMR studies. The 13-proton appeared as a doublet at δ 6.79 (J = 16 Hz). When the above procedure was repeated with (15*R*)-PGA_2 methyl ester (**1b**), the doublet occurred at δ 6.89 (J = 16 Hz). The above procedure has also been applied to ca. 100-mg samples of crude hydrolysis extracts from various samples of *P. homomalla* to determine approximate composition of the desired prostaglandins, see Figure 1.

(15*S*)-PGA_2 Methyl Ester (3b) from (15*R*)-PGA_2 Methyl Ester (1b). A solution of 1.8 g of **1b** in 145 ml of tetrahydrofuran was cooled to 0 °C under nitrogen. To this was added 11 ml of tri-*n*-butylamine and then slowly 7 ml of methanesulfonyl chloride. The mixture was stirred for 40 min at 0 °C and then diluted with 70 ml of ice and water. After stirring an additional 1 h, it was concentrated in vacuo, extracted with ethyl acetate, and the extracts were washed with sodium bicarbonate, dried, and evaporated. The residue was chromatographed on 200 g of silica gel, eluting with 5 l. of 20–70% ethyl acetate in Skellysolve B. Fractions containing material having the same TLC mobility and NMR and IR spectra as (15*S*)-PGA_2 methyl ester (**3b**) contained 380 mg of material. Earlier fractions contained 350 mg of material identical with starting **1b** and 220 mg of the allylic rearrangement product **6**[16] as a mixture of isomers. This latter material showed, in the NMR spectrum, two sets of doublets of doublets at δ 7.74 and 7.63 for the C(11) proton, a doublet of doublets at δ 6.20 (J = 2 and 6 Hz) for the C(10) proton and a one proton multiplet between δ 3.9 and 4.3 for the epimeric C(13) protons, and was identical with that of material obtained by the method of Spraggins.[16] The mass spectrum showed ions at 348 (M^+), 330, 299, 222, 190, 141, 127, 109, 96, 82, 57, 55, 43.

10α,11α- and 10β,11β-Epoxides of (15*S*)-PGA_2 Methyl Ester (7b). A solution of 1.0 g of (15*S*)-PGA_2 methyl ester (**3b**) in 20 ml of methanol was cooled to −20 °C and treated with 1.3 ml of 30% hydrogen peroxide and then slowly with 0.20 ml of 1 N aqueous KOH. After a further 1 h at −15 °C, no starting material was evident by TLC. After adding 0.3 ml of 1 N HCl, the methanol was removed at reduced pressure and the residue extracted with ethyl acetate. The extracts were washed, dried, and evaporated to give 1.04 g of a mixture of epoxides **7b**. These were of very similar mobility on TLC (A-IX system[35]), but were partially separated by chromatography on 300 g of silica gel, eluting with 8 l. of 20–70% ethyl acetate–Skellysolve B. Further purification of early and late fractions from this column on Porasil T silica gel 15–25 μ (Waters Associates, Framingham, Mass.), eluting with 50% ethyl acetate–Skellysolve B, gave pure samples of the faster moving 10α,11α-epoxide, 101 mg, and the slower moving 10β,11β-epoxide, 75 mg, characterized by differences in their CD and UV spectra (Figures 3 and 4). A 10-mg sample of the former, stirred with amalgamated aluminum[18] in 1 ml of 100:10:1 mixture of ether–methanol–water for 2 h gave a TLC spot of the same mobility as authentic PGE_2 methyl ester. The latter under the same conditions gave a spot with the mobility of 11β-PGE_2 methyl ester (see below).

PGE_2 Methyl Ester (8b) and 11β-PGE_2 Methyl Ester. A solution of 136 mg of the mixture of α- and β-epoxides of PGA_2 methyl ester (**7b**) prepared as above, in 3 ml of acetic acid and 1 ml of H_2O, was cooled to 0 °C under argon and 450 mg of chromous acetate, prepared as in ref 38, was added. The mixture was stirred at 5 °C overnight, ice was added, and the products extracted with ethyl acetate. The extracts were washed with water, 1 N HCl, and sodium bicarbonate, dried, and evaporated. The residue was chromatographed on 20 g of silica gel, eluting with 600 ml of 20–100% ethyl acetate–Skellysolve B. Early portions contained 65 mg of PGA_2 methyl ester, followed by 47 mg of the 11β-isomer of PGE_2 methyl ester and 13 mg of PGE_2 methyl ester (**8b**). The latter was identical in spectral properties with authentic samples, the former differed in the NMR spectrum from PGE_2 methyl ester in having the C(13),C(14) olefinic protons shifted downfield by 9 Hz and the C(11) and C(15) protons occurred at δ 4.15 and 4.4, whereas in PGE_2 methyl ester, these occur together at δ 4.1. Mass spectra were essentially identical for the two isomers.

Conversion of (15*R*)-PGA_2 Acetate Methyl Ester (1a) to $PGF_{2\alpha}$ (9c). A. Epoxidation to 10a. To a stirred solution of 265 g (0.68 mol) of (15*R*)-PGA_2 acetate methyl ester in 5000 ml of methanol maintained at −20 °C under a nitrogen atmosphere was added 350 ml of 30% hydrogen peroxide in one portion. To this well-stirred solution was added dropwise over 1 h 50 ml of 1 M aqueous potassium hydroxide. After an additional 2 h at −20 °C, thin-layer chromatographic assay of an aliquot indicated that no starting material remained (TLC solvent: 3% acetone in methylene chloride; starting material, R_f 0.27; epoxide mixture **10a**, R_f 0.37). The reaction mixture was then treated with 80 ml of 1 M aqueous hydrochloric acid and concentrated in vacuo (bath temperature 35 °C). The residue was diluted with 3 l. of ethyl acetate and washed with three 500-ml portions of water. The aqueous washes were extracted with 300 ml of ethyl acetate and the combined extract was washed with 500 ml of brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product (275 g, 100% of theory) was homogeneous by TLC and was reduced directly without further purification.

B. (15*R*)-PGE_2 15-Acetate Methyl Ester 11a. Preparation of aluminum amalgam. Granular aluminum (50 g, 20 mesh) was washed with two portions of ether, then two portions of methanol. The washed aluminum was then added to a solution of 50 g of mercuric chloride in 2000 ml of water, and the resulting suspension was swirled for about 25–40 (until hydrogen evolution from the metal surface becomes appreciable). The aqueous solution was removed by rapid filtration through a coarse porosity sintered-glass funnel and the aluminum amalgam was washed rapidly with two 200-ml portions of methanol and two 200-ml portions of anhydrous ether. The aluminum amalgam was then stored under ether.

A stirred solution of 275 g (0.68 mol) of (15*R*)-PGA_2 acetate methyl ester, 10,11-epoxide **10a** in 2500 ml of ether, 250 ml of methanol, and 25 ml of water was cooled to about 10 °C and treated with the aluminum amalgam from 50 g of aluminum. The reaction mixture (exothermic) was stirred in a cool water bath to maintain an internal temperature of 15–25 °C. After 1 h, an additional 50 g of

aluminum amalgam was added and after 2 h total reaction time a further 50 g of aluminum amalgam and 25 ml more water were added. After a total reaction time of 3 h, TLC analysis (ethyl acetate–cyclohexane–acetic and 40:60:2) indicated that the reaction was complete (starting epoxide mixture, R_f 0.64; products R_f 0.20 (11α), 0.25 (11β)). Following the addition of 100 g of magnesium chloride, the reaction mixture was filtered through a pad of Celite and the solids were washed thoroughly with methylene chloride. The combined filtrate was concentrated in vacuo, thereby affording a viscous oil weighing 247 g.

A 210-g portion of this C(11) epimeric mixture was chromatographed on a column containing 30 kg of E. Merck silica gel. The column was eluted (1-gal fractions) with 60-l. portions of 25, 30, 35, 40, 45, 50, 55, and 60% ethyl acetate–hexane. Fractions 29–38 afforded 14.3 g of (15*R*)-PGA_2 acetate methyl ester (**1a**). Fractions 71–76 contained 27 g of 11β-(15*R*)-PGE_2 15-acetate methyl ester. Fractions 77–80 upon combination yielded 25 g of a mixture of C(11) epimers and fractions 81–110 afforded 116 g of pure (15*R*)-PGE_2 15-acetate methyl ester. (The recovery of 116 g of pure **11a** from 210 g of crude product corresponds to 136 g from the total crude product; this represents an isolated yield of **11a** of 49% from **1a**. The yields can be improved some by rechromatographing the mixed fractions.)

C. Reduction of (15*R*)-PGE_2 15-Acetate Methyl Ester (11a). A stirred solution of 63 g of (15*R*)-PGE_2 15-acetate methyl ester (155 mmol) in 400 ml of tetrahydrofuran was treated under a nitrogen atmosphere with 100 g of hexamethyldisilazane followed by 20 g of chlorotrimethylsilane. After 2 h at 25 °C, TLC analysis of an aliquot indicated that silylation at C(11) was complete (30% ethyl acetate–cyclohexane; starting material **11a**, R_f 0.09; silyl derivative, 0.44). The reaction mixture was concentrated in vacuo with the aid of a vacuum pump (maximum bath temperature 30 °C) and the residue was taken up in 150 ml of toluene. Following filtration through Celite to remove the ammonium chloride, the filtrate was concentrated in vacuo. An additional 150-ml portion of toluene was added and evaporated to ensure removal of excess hexamethyldisilazane. The crude product (75 g, 100% yield) was homogeneous by TLC and was reduced without further purification.

A solution of 30.7 g (64 mmol) of the C(11) trimethylsilyl derivative of (15*R*)-PGE_2 15-acetate methyl ester in 500 ml of absolute ethanol was cooled to 0 °C and treated with 1.42 g of sodium borohydride, added in one portion. After 3.5 h at 0 °C, TLC analysis of an aliquot indicated that the reduction was complete [30% ethyl acetate–cyclohexane; starting material, R_f 0.44; products, R_f 0.30 (9α), 0.19 (9β)]. The reaction mixture was then treated dropwise, still at 0 °C, with 10 ml of acetic acid followed by 100 ml of water. The mixture was allowed to stir overnight at 25 °C to ensure silyl group removal. Most of the ethanol was then removed in vacuo and the residue was taken up in 400 ml of brine and extracted with three portions of ethyl acetate (400, 250, 150 ml). The combined organic extract was washed with two 100-ml portions of water, one 100-ml portion of aqueous sodium bicarbonate, and two 100-ml portions of brine. Removal of the solvent in vacuo after drying (sodium sulfate) afforded 24.5 g of the crude product (94% yield). Analysis of the product by TLC showed only the two C(9) epimers (A-IX solvent system;[35] R_f 0.42 (9α), 0.33 (9β)).

D. (15*R*)-$PGF_{2\alpha}$ (12c) and (15*R*)-$PGF_{2\beta}$. A solution of 48 g (117 mmol) of the above mixture of 9-epimers in 350 ml of methanol was cooled to 0 °C and treated with 275 ml of 10% aqueous sodium hydroxide. The resulting pale yellow solution was allowed to warm to room temperature and was stirred for 3 h at 25 °C. Following removal of the methanol in vacuo, the residue was extracted with 100 ml of 1:1 ether–methylene chloride to remove any neutral material. The aqueous layer was acidified with 260 ml of 3 M hydrochloric acid, saturated with sodium chloride, and extracted with ethyl acetate (400, 250, 150 ml). The combined extract was washed with water and brine, dried over sodium sulfate, and concentrated, thereby affording 42 g (100%) of crude (15*R*)-PGF_{2a} and (15*R*)-$PGF_{2\beta}$ (R_f 0.21 and 0.13, respectively, in the A-IX solvent system[35]). Since no other products were evident by TLC, the crude product was oxidized directly without further purification.

E. 15-Keto-$PGF_{2\alpha}$ (13c) and 15-Keto-$PGF_{2\beta}$. A solution of 42 g of crude product from part D and 40 g of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in 950 ml of anhydrous dioxane was stirred at 50 °C under a nitrogen atmosphere. The reaction mixture was then cooled to 25 °C and filtered through a medium porosity sintered-glass funnel to remove the hydroquinone. The solids were washed several times with methylene chloride and the combined filtrate was evaporated in vacuo. The crude product (66 g, contains some DDQ and some hydroquinone) was divided into two equal portions, each of which was chromatographed on a column containing 3 kg of silica gel. The column was eluted (650-ml fractions) with 10 l. of 60% ethyl acetate–hexane, 10 l. of 70% ethyl acetate–hexane, 10 l. of 80% ethyl acetate–hexane, 20 l. of 90% ethyl acetate–hexane, and 15 l. of pure ethyl acetate.

Fractions 42–53 were homogeneous by TLC and upon combination afforded 8.3 g of 15-keto-$PGF_{2\alpha}$ (**13c**). Fractions 54–63 contained 4.4 g of a mixture of 9α/9β isomers, while fractions 64–95 yielded 3.9 g of 15-keto-$PGF_{2\beta}$. (TLC, A-IX solvent:[35] 15-keto-$PGF_{2\alpha}$, R_f 0.25; 15-keto-$PGF_{2\beta}$, R_f 0.20.) Chromatographic purification of the other half of the above crude product also gave an additional 8.3 g, thus making the total yields of 15-keto-$PGF_{2\alpha}$ (**13c**) 16.6 g (40% of theory, this step; 38% of theory based on (15*R*)-PGE_2 15-acetate methyl ester (**11a**). These yields can be improved by rechromatographing mixed fractions.

F. $PGF_{2\alpha}$ (9c). A solution of 3.0 g (8.54 mmol) of 15-keto-$PGF_{2\alpha}$ (**13c**) in 350 ml of anhydrous tetrahydrofuran was treated with 70 ml of hexamethyldisilazane and 14 ml of chlorotrimethylsilane, and the resulting homogeneous mixture was stirred under nitrogen at 25 °C for 18 h. The 1,9,11-tris(trimethylsilyl derivative) of 15-keto-$PGF_{2\alpha}$ was isolated in exactly the same manner as in part C above.

A zinc borohydride solution was prepared as follows. To a suspension of 680 mg of sodium borohydride in 65 ml of 1,2-dimethoxyethane at 0 °C was added under nitrogen 1.23 g of anhydrous zinc chloride and the resulting mixture was stirred at 0 °C for 30 min. To the stirred 0 °C zinc borohydride solution was added dropwise over 10 min a solution of the crude 15-keto-$PGF_{2\alpha}$, tris(trimethylsilyl derivative) from above in 20 ml of 1,2-dimthoxyethane. The reaction mixture was then warmed to 25 °C, stirred for 4 h longer, then treated cautiously with 30 ml of water and 8 ml of acetic acid to destroy excess zinc borohydride and to hydrolyze the trimethylsilyl groups. After stirring 18 h at 25 °C, the reaction mixture was poured into 100 ml of 0.5 M hydrochloric acid with ice, saturated with sodium chloride, and extracted thoroughly with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product, 3.2 g, exhibited two spots of nearly equal intensity on TLC [A-IX solvent:[35] (15*R*), R_f 0.21, (15*S*), R_f 0.13; 10:10:80 methanol–acetic acid–chloroform solvent: (15*R*), R_f 0.54, (15*S*), R_f 0.43]. The crude product was chromatographed on 600 g of Mallinckrodt CC-4 acid-washed silica gel. The column was eluted (550-ml fractions) with 5 l. of 75% ethyl acetate–hexane, 5 l. of 90% ethyl acetate–hexane, and a gradient system consisting of 5 l. of 90% ethyl acetate–hexane and 5 l. of 10% methanol–ethyl acetate.

Fractions 21–26 afforded 653 mg of (15*R*)-$PGF_{2\alpha}$ (**12c**) (22% of theory), while fractions 28–36 yielded 1.73 g of pure $PGF_{2\alpha}$ (**9c**) (57% of theory). This material exhibited IR, NMR, and mass spectra identical with those of authentic $PGF_{2\alpha}$.[37]

Epoxidation of (15*R*)-PGA_2 Acetate Methyl Ester (1a). A solution of 250 mg of (15*R*)-PGA_2 acetate methyl ester (**1a**) in 15 ml of tetrahydrofuran was cooled to −15 °C and treated dropwise with stirring with 0.91 ml of aqueous sodium hypochlorite (Clorox). After 2 h at 25 °C, TLC analysis of the reaction indicated that about 25% starting material remained. An additional 0.5 ml of Clorox was added and the reaction was allowed to stir 4 h longer. TLC analysis then indicated about 10% of a material with some R_f as starting material, about 75% of the desired epoxide mixture, and 15% of a product less polar than the epoxides. The reaction mixture was diluted with brine and extracted with ethyl acetate. The combined extract was washed with aqueous sodium bicarbonate and brine, dried over sodium sulfate, and evaporated in vacuo.

A 10-mg portion of this epoxide mixture was reduced with aluminum amalgam as described in a previous experiment to determine the ratio of α/β epoxides. By TLC, the ratio of (15*R*)-PGE_2 15-acetate methyl ester (**11a**) to the corresponding 11β isomer appeared to be 1:1.

(15*R*)-PGE_2 15-Acetate Methyl Ester (11a) and its 11 Epimer. The Use of in Situ Prepared Chromous Acetate. To a solution of mixed α- and β-epoxides of (15*R*)-PGA_2 acetate methyl ester (**11a**) (3.8 g) in 50 ml of acetic acid was added 8 g of sodium acetate and 4 g of zinc dust. The mixture was cooled in an ice bath and with stirring under nitrogen, 4 ml of chromium chloride (1.8 g of $CrCl_3 \cdot 6H_2O$ in 5 ml of water) solution was added. After 1 h another 4 g of zinc dust was

added. After 4 h in the ice bath and 2.5 h at room temperature, 200 ml of ethyl acetate was added and the solution was washed several times with water, 1 N HCl, saturated $NaHCO_3$, and saturated salt, and dried over sodium sulfate. The residue after evaporation was chromatographed on 400 g of silica gel, eluting with 8 l. of 20–75% ethyl acetate–Skellysolve B gradient. Fractions 4–5 (300 ml each) contained 719 mg of a mixture of starting epoxide and (15*R*)-PGA_2 acetate methyl ester. Fraction 14 had 204 mg of the 11-epi-(15*R*)-PGE_2 15-acetate methyl ester. Fractions 15–16, 562 mg, were mixtures of this and (15*R*)-PGE_2 15-acetate methyl ester. Fractions 17–20, 683 mg, consisted of (15*R*)-PGE_2 15-acetate methyl ester (**11a**).

Essentially the same results were obtained when the epoxide and sodium acetate in acetic acid were stirred under argon and chromium(II) solution is added dropwise from a Jones reductor mounted on the flask. See ref 39 for details for preparing chromous solutions in this fashion.

(15*R*)-PGE_2 15-Acetate Methyl Ester (11a) by Electrolysis. A mixture of 520 mg of (15*R*)-PGA_2 acetate methyl ester 10,11-epoxide (**10a**) and 6 g of potassium acetate in 70 ml of methanol and 10 ml of water was electrolyzed (Pt electrodes, reaction stirred in a 150-ml beaker) at 4 V, 0.5 A for 2 h. The reaction mixture was then treated with 1 ml of acetic acid and concentrated in vacuo to remove most of the methanol. The residue was partitioned between brine and ethyl acetate and the organic layer was washed with aqueous sodium bicarbonate and brine, dried over sodium sulfate, and evaporated. By TLC, the crude product (490 mg) consisted of 10% starting (15*R*)-PGA_2 acetate methyl ester (**10a**) and 45% each of (15R)-PGE_2 15-acetate methyl ester (**11a**) and the corresponding 11β isomer. No other products were evident by TLC.

PGE_2 15-Acetate Methyl Ester (8a) and 11-*epi*-PGE_2 15-Acetate Methyl Ester. A solution of 6.0 g (15.4 mmol) of PGA_2 acetate methyl ester (**3a**) in 120 ml of methanol was cooled to −20 °C in a dry ice–isopropyl alcohol bath and treated with 7.8 ml of 30% aqueous hydrogen peroxide added in one portion. With good stirring, at −20°C, 1.2 ml of 1 M aqueous potassium hydroxide was added dropwise over a 1-h period. By the time the mixture had stirred 1 h longer at −15 °C, no starting material was evident by TLC (3% acetone–methylene chloride) and, after adding 1.8 ml of aqueous 1 M hydrochloric acid, the methanol was removed at reduced pressure (30 °C maximum temperature). The residue was diluted with water and extracted thoroughly with ethyl acetate. The combined extracts were washed with water, aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, and concentrated at reduced pressure. The resulting epoxide mixture, 6.25 g, was approximately 90–95% one spot on TLC.

The crude epoxidation product (6.25 g) from above was dissolved in a mixture of 60 ml of ether, 6 ml of methanol, and 0.6 ml of water, cooled to approximately 5–10 °C and treated with aluminum amalgam[18] made from 3.48 g of aluminum powder and 3.48 g of mercuric chloride dissolved in 232 ml of water. The temperature of the reaction rose slightly on addition of the amalgam. After 3 h at ambient temperatures, TLC (ethyl acetate–cyclohexane–acetic acid 40:60:2) indicated approximately 15–20% starting epoxide remained. Another approximately 3 g of aluminum amalgam was added and 30 min later no epoxide was left. The mixture was filtered through Celite and the filter cake was washed thoroughly with ether and methylene chloride. Upon concentration of the filtrate, a 6.3 g C(11) epimeric mixture of PGE_2 diesters was obtained, which was chromatographed on 1.8 kg of Brinkmann silica gel packed in 25% ethyl acetate–Skellysolve B. Elution proceeded as follows: 8 l. of 35% ethyl acetate–Skellysolve B—discard; 10 l. of 50% ethyl acetate–Skellysolve B—fractions 1–28; 8 l. of 55% ethyl acetate–Skellysolve B—fractions 29–44.

Fractions were combined on the basis of their TLC mobility (ethyl acetate–cyclohexane–acetic acid 40:60:2). Fractions 17–20 yielded 1.26 g of 11-*epi*-PGE_2 15-acetate methyl ester: IR λ_{max} 3460, 1735, 1240, 1160, 1075, 1040, 1020, 975 cm^{-1}; NMR ($CDCl_3$) δ 6.0–5.1 (vinyl and C(15) H, m, 5 H), 4.5–4.3 (*CH*OH, br symmetrical peak, 1 H), 3.66 (CO_2CH_3, s, 3 H), 2.85 (*OH*, s, 1 H), 2.04 ($OCOCH_3$, s, 3 H), 0.90 (terminal CH_3, t, J = 5.5 Hz, 3 H); UV (basic) λ_{max} 238 (ϵ 9150), 326 mμ (ϵ 17 850); $[\alpha]_D$ −47° (EtOH, 0.5496 g/100 ml); mass spectrum *m/e* 408 (M^+), M − HOAc, M − (HOAc + H_2O), M − OCH_3, M − (OCH_3 + HOAc), M − (HOAc + C_5H_{11}). Anal. Calcd for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.54; H, 9.42.

Fractions 21–24 contained 732 mg of a mixture epimeric at C(11). Finally fractions 25–36 afforded 3.23 g of PGE_2 15-acetate methyl ester (**8a**): IR λ_{max} 3460, 1735, 1240, 1160, 1075, 1020, 970 cm^{-1}; NMR ($CDCl_3$) δ 5.8–5.0 (vinyl and C(15), m, 5 H), 4.4–3.9 (*CH*OH, four line pattern, 6 Hz between peaks, 1 H), 3.69 (CO_2CH_3, s, 3 H), 3.15 (*OH*, br s, 1 H), 2.05 ($OCOCH_3$, s, 3 H), 0.90 (terminal CH_3, t, J = 5.5 Hz, 3 H); UV (basic EtOH) λ_{max} 240 (ϵ 8900), 327 mμ (ϵ 17 900); $[\alpha]_D$ −86° (EtOH, 0.5002 g/100 ml); mass spectrum no M^+, peaks present at M − HOAc, M − (HOAc + H_2O), M − (HOAc + OCH_3), M − (HOAc + C_5H_{11}). Anal. Calcd for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.03; H, 11.26.

Dehydration of 11-*epi*-PGE_2 15-Acetate Methyl Ester to PGA_2 Acetate Methyl Ester. A. A solution of 120 mg of 11-*epi*-PGE_2 15-acetate methyl ester in 5 ml of benzene was treated with 600 mg of Florisil and the resulting suspension was stirred vigorously and refluxed for 2 h. The reaction mixture was then cooled to 25 °C, filtered to remove the Florisil, and concentrated in vacuo. The NMR spectrum of the crude product (100 mg) was superimposable with that of authentic PGA_2 acetate methyl ester (**3a**). UV analysis of the crude product showed the absence of any PGB_2 methyl ester (i.e., no 278-nm peak) and the presence of 3% of 9-oxo-prosta-(5*Z*,10*Z*,12*E*,14*E*)-tetraenoic acid methyl ester (**5**) (326 nm) as the only UV absorbing impurity. The absence of any starting 11-*epi*-PGE_2 15-acetate methyl ester was readily demonstrated by TLC (40% ethyl acetate–hexane).

B. A solution of 100 mg of 11-*epi*-PGE_2 15-acetate methyl ester in 5 ml of benzene was treated with 500 mg of Woelm acidic aluminum oxide, and the resulting suspension was stirred vigorously at 25 °C for 12 h. The reaction mixture was filtered, the alumina washed with ethyl acetate, and the combined filtrate was evaporated in vacuo. The crude product (90 mg) was homogeneous by TLC (40% ethyl acetate–hexane) and showed no UV absorbing impurity (i.e., no 278 or 326 nm).

Preparation of Crude Coral Esterase. Frozen, ground *P. homomalla* (10 kg) was thawed and allowed to autolyze at room temperature for 21 h. Acetone (30 l.) was added and the suspension stirred for 1 h. The dark brown insoluble residue was recovered by filtration and washed on the Buchner funnel with 1–2 l. of acetone. The residue was then air dried and stored at −20 °C.

To reduce the level of endogenous lipid contamination in the enzyme preparation, the powder was stirred with 10 vol (v/w) of water for 24 h at room temperature. Acetone (3 vol) was added with constant stirring. After filtering, the residue was washed with a small volume of acetone and air dried.

Enzymatic Hydrolysis of PGE_2 15-Acetate Methyl Ester (8a). Prepared as above, 300 g of *S* coral-derived, washed enzyme powder, prepared as above, was suspended in 2500 ml of water. The pH was adjusted to 6.5 (narrow-range pH paper) by the addition of a small amount of phosphoric acid. A solution of 47 g of PGE_2 15-acetate methyl ester (**8a**) in 75 ml of 95% ethanol was added and the resulting mixture was stirred vigorously for 20 h at ambient temperature. The mixture was then diluted with 6 l. of acetone, stirred at ambient temperature for 45 min, and filtered through Celite. After removal of the acetone at reduced pressure (30 °C), the aqueous residue was acidified to pH 3.5 with 2 M citric acid and extracted twice with 1 l. of methylene chloride. After removal of the methylene chloride at reduced pressure, the residue was dissolved in 1.5 l. of a buffer solution (0.2 M Na_2HPO_4, 3A alcohol, 4:1—pH adjusted to 8 with H_3PO_4) and extracted with toluene. The buffer was then acidified to pH 3.5 with 2 M citric acid and extracted three times with 1.5 l. of methylene chloride. The organic layer was washed with water (containing a trace of H_3PO_4) and brine, stirred 30 min with 50 g of Darco G-60, filtered through Celite and concentrated. The crude product was a light yellow viscous oil weighing 36.5 g (90% theor). By TLC (A-IX solvent[35]), the product contained <5% PGA_2, about 15% 5,6-*trans*-PGE_2 (**14c**), and a trace of 8-*iso*-PGE_2.

A 10-g aliquot of this product was crystallized from an ether–hexane mixture, yielding 4.7 g of PGE_2, mp 64–65 °C, which by TLC still contained 8% 5,6-*trans*-PGE_2. Two recrystallizations from ether–hexane afforded 3.2 g of PGE_2, mp 66–67 °C, containing <2% of the corresponding trans isomer and identical in spectral properties with authentic samples.

Enzymatic Hydrolysis of PGE_2 Methyl Ester (8b). In the same manner as the preceding experiment, **8b** was converted to PGE_2 (**8c**), mp 64–66 °C, in 43% yield.

Reduction of PGE_2 15-Acetate Methyl Ester (8a), Hydrolysis to $PGF_{2\alpha}$ (9c). A solution of 4.45 g (10.9 mmol) of PGE_2 15-acetate methyl ester (**8a**), 13 ml of hexamethyldisilazane, and 2.3 ml of tri-

methylchlorosilane in 40 ml of tetrahydrofuran was stirred under nitrogen for 18 h at ambient temperature. The excess reactants and solvent were removed at reduced pressure (30 °C maximum temperature, vacuum pump) and the residue was diluted with reagent grade xylene (150 ml). After filtration through Celite to remove ammonium chloride, the xylene was removed at reduced pressure. The crude product, 5.31 g (>100% contains xylene), was a light yellow mobile oil, homogeneous by TLC (40% ethyl acetate in cyclohexane).

The total crude trimethylsilyl derivative was dissolved in 80 ml of absolute ethanol, cooled to 0 °C, and treated with 500 mg of sodium borohydride. After stirring at 0 °C for 1 h, excess borohydride was decomposed by the cautious addition of dilute aqueous acetic acid (4 ml glacial acetic acid in 60 ml water). The resulting homogeneous, colorless solution was stirred at ambient temperature for 18 h under nitrogen to ensure removal of the silyl group. After removal of the solvents at reduced pressure, the residue was taken up in ethyl acetate–water and extracted thoroughly with ethyl acetate. The combined extracts were washed with aqueous sodium bicarbonate solution, water, and brine, and dried over anhydrous sodium sulfate. Removal of the solvent afforded 4.6 g of a colorless viscous oil—only two spots on TLC. This mixture could be easily separated by chromatography at this point, but in this instance was hydrolyzed first.

The mixture of $PGF_{2}\alpha$ and $PGF_{2}\beta$ diesters from above (4.6 g) was dissolved in 50 ml of methanol and the solution was purged with nitrogen via a gas dispersion tube. To this solution, well stirred at −10 °C, was added 50 ml of 1 M aqueous potassium hydroxide solution over 15 min. The mixture was allowed to warm gradually to room temperature and stirred 18 h. After removal of the methanol at reduced pressure, the aqueous residue was extracted with methylene chloride to remove any neutral material. The aqueous layer was acidified using 2 M potassium bisulfate, saturated with sodium chloride, and extracted thoroughly with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated, thereby affording 4.0 g of a mixture of $PGF_{2}\alpha$ (**9c**) and $PGF_{2}\beta$. The two isomers accounted for >99% of material visible by TLC on a vanillin-sprayed plate. Separation was achieved using a 400-g column of CC-4 silica packed in 50% ethyl acetate–Skellysolve B. Fractions of 400 ml were collected as follows: 2.5 l. of 50% EtOAc–Skellysolve B, fractions 1–4; 5 l. of 75% EtOAc–Skellysolve B, fractions 5–20; 4 l. of 75% → 4 l. of 100% EtOAc, fractions 21–38; and 5 l. of 5% MeOH–EtOAc, fractions 39–51.

Fractions 15–42 contained 2.14 g (56% from PGE_{2} diester) of $PGF_{2}\alpha$ (contaminated only by the 5,6-trans isomer). Removal of the *trans*-$PGF_{2}\alpha$ and characterization of it and pure $PGF_{2}\alpha$ is described below. Fraction 43 was a mixture of isomers and weighed 20 mg. Fractions 44–50 were combined and yielded 1.30 g (34%) of crystalline $PGF_{2}\beta$, mp 92–94 °C.

Argentation Chromatographic Isolation and Purification of 5,6-*trans*-$PGF_{2}\alpha$ (15c) and $PGF_{2}\alpha$ (9c). Amberlyst 15 resin (500 g) was washed with hot water, packed in a 3.5 × 80 cm column, and converted to the silver form by washing with 6 l. of 10% aqueous silver nitrate. The column was then washed with water until the eluate was free of silver ion, and then washed with 2 l. of ethyl alcohol. A 5-g sample of $PGF_{2}\alpha$, containing 8–10% 5,6-trans isomer, was dissolved in 15 ml of the same solvent, and put on the column, which was then eluted with ethyl alcohol at the rate of 150 ml/h, collecting 50-ml fractions. Fractions 22–69, on evaporation, gave 180 mg of **15c**, greater than 90% pure by gas chromatography (4-ft. 1% SE52, 185 °C). Fractions 70–110 contained 1.7 g of a mixture of **15c** and $PGF_{2}\alpha$, while fractions 111–147 contained 2.8 g of $PGF_{2}\alpha$ (**9c**).

The 5,6-*trans*-$PGF_{2}\alpha$ (**15c**) from the above column was further purified on 25 g acid-washed silica gel, eluting with 75–100% ethyl acetate–Skellysolve B. Fractions homogeneous by TLC were crystallized from diethyl ether, giving 105 mg of **15c**, mp 92–94 °C, 98% pure by GLC. Further crystallization gave mp 94.8–95.8 °C: IR ν_{max} 3470, 3280, 3180 sh, 2730, 2620, 2560, 1700, 1665 w, 1345, 1290, 1230, 1215, 1090, 1025, 990, 975, 965, 805 cm^{-1}; NMR (acetone-d_6) δ 5.7–5.4 (vinyl H, m, 4 H), 4.9–4.35 (OH, COOH, br, exchangeable), 4.35–3.55 (C*H*OH, m, 3 H), 0.92 (C(20) methyl, t); $[\alpha]_D$ +9° (EtOH, 43.27 mg/5 ml); equiv wt 345 (theor 354); mass spectrum M^+ 354, 336, 318, 264, 247. Anal. Calcd for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67. Found: C, 67.99; H, 9.64.

The $PGF_{2}\alpha$ fractions from the silver column above were further purified on acid-washed silica gel as described for **15c** and the resulting $PGF_{2}\alpha$ was crystallized at −15 °C from anhydrous ethyl ether. The crystalline residue was recrystallized at −15 °C from ethyl acetate, filtered at low temperature, giving material melting at about room temperature, which was dried in vacuo at 40 °C. The resulting colorless oil retained solvent tenaciously, amounting to 4.9% of ethyl acetate by a melt solvate analysis. The C, H, and equivalent weight analyses below are corrected for solvent content: $[\alpha]_D$ +26° (EtOH); equiv wt 359 (theor 354); IR ν_{max} 3300, 1720, 1220, 1075, 1050, 1025, 970 cm^{-1}; NMR (acetone-d_6) δ 5.4 (vinyl H, m, 4 H), 5.12 (OH, COOH, br, exchangeable), 4.0 (C*H*OH, m, 3 H), 0.9 (CH_3, t, 3 H); GLC analysis, 4 ft 3.8% UC-W98 on Diatoport-S 80/100 column at 215 °C, 99.6% (as tetra Me_4Si derivative), retention time 30 min. Anal. Calcd for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67. Found: C, 67.45; H, 10.13.

Prostaglandin PGE_{2} (8c) from PGA_{2} (3c). A solution of 6.8 g of pure, trans-free PGA_{2} (**3c**) in 40 ml of tetrahydrofuran under nitrogen was treated with 9.5 ml of hexamethyldisilazane and 0.5 ml of trimethylchlorosilane with stirring for 2 h at room temperature. The solvent was removed in vacuo, benzene (50 ml) was added, and removed in vacuo. The residue was dissolved in 150 ml of isopropyl alcohol, immediately cooled to −40 °C under N_2 in a dry ice–acetone bath, and with stirring, 12 ml of 30% hydrogen peroxide was added, followed dropwise by 17 ml of 3 N LiOH in water. The first 15 ml were added over about 30 min at −30 to −40 °C, and after about 3 h TLC showed incomplete epoxidation, so the additional 2 ml of LiOH was added and after an additional 0.5–1 h at about −30 °C the reaction was judged to be done. Then 56 ml of 1 N HCl was added and the mixture was concentrated in vacuo to remove most of the isopropyl alcohol. The residue was extracted with ethyl acetate, washed with 1 N HCl, saturated salt, dried with sodium sulfate, and evaporated, leaving a residue of about 10 g of yellow oil. TLC (A-IX system[35]) showed a major spot, gray with vanillin–H_3PO_4 spray, moving slightly faster than PGA_{2}. This was stored in the refrigerator overnight, then dissolved in 200 ml of tetrahydrofuran and 20 ml of methanol, and 40 ml of saturated aqueous sodium bicarbonate was added while stirring with an efficient motor stirrer under nitrogen. This was cooled in a cold water bath (15 °C) and amalgamated aluminum (from 10 g of granular aluminum, 20 mesh and finer) was added. After stirring 0.75 h, TLC showed no epoxide remaining, and a strong PGE_{2} spot was formed. No appreciable PGA_{2} was seen. The gray suspension was decanted from the unreacted aluminum and was concentrated to a small volume in vacuo. This was diluted with 150 ml of ethyl acetate, and with stirring, a total of 200 ml of 1 N HCl was added. The organic layer was separated, the aqueous was extracted twice with ethyl acetate, and the extracts were washed with 1 N HCl and saturated salt, dried with sodium sulfate, and evaporated in vacuo to leave 8.9 g of a yellow oil. A little 50% ethyl acetate–cyclohexane was added, cooled in the refrigerator, and seeded with PGE_{2} to produce a crystalline precipitate. After refrigerating overnight, the solvents were removed by pipet, the crystals washed twice with a little more of the same solvent mixture to leave 4.15 g of pale yellow crystals. These were dissolved in 35 ml of anhydrous ether, filtered to remove some cloudy materials, diluted with 10 ml of Skellysolve B, and cooled in ice to produce a crystalline precipitate. After refrigerating, 7 ml more Skellysolve B was added, and the flask was put in the freezer overnight (−12 °C), filtered, washed with 2:1 Skellysolve B–ether obtaining 3.08 g, mp 63.5–66 °C, of PGE_{2} (**8c**).

A TLC of this on 2 × 8 in. silver nitrate–silica gel plate run in A-IX system[35] showed only one spot corresponding to PGE_{2}, and no 11-*epi*-PGE_{2} was seen on an ordinary silica gel TLC plate.

This corresponds to 43.5% overall yield from PGA_{2} by direct crystallization. A sample, 1.00 g, was recrystallized from ether–Skellysolve B, wt 929 mg, mp 65–67.5 °C (40.2% overall yield), one spot on $AgNO_3$ and regular silica gel TLC plates.

The mother liquor from the above 4.15 g of crude crystals was chromatographed on acid-washed silica gel and eluted with 50–100% ethyl acetate–Skellysolve B to obtain 520 mg (7.5% overall yield) of 11-*epi*-PGE_{2}, 337 mg of fixed fractions, and 1.37 g of combined PGE_{2} fractions, which crystallized on seeding with PGE_{2}. This was recrystallized from ethyl acetate–cyclohexane to give 510 mg, mp 59–66.5 °C, or 7.1% additional yield of PGE_{2}.

5,6-*trans*-PGE_{2} (14c) from 5,6-*trans*-PGA_{2} (4c). In the same manner as in the preceding experiment, 1.3 g of **4c** was silylated, epoxidized, and the resulting mixture of epoxides reduced with aluminum amalgam. The residue from the ethyl acetate extracts was

chromatographed on 100 g of acid-washed silica gel. Elution with ethyl acetate and 5% methanol in ethyl acetate gave 5,6-*trans*-PGE_2 (**14c**), which after crystallization from ether–Skellysolve B melted at 75–76 °C, 0.40 g. It was identical in TLC mobility and spectral properties with material prepared by isomerization of PGE_2 (**8c**), see below, and gave no melting point depression on admixture.

Use of KI, Triphenylphosphine, and Bicarbonate for Reduction of Epoxides (7c). A solution of 680 mg of PGA_2 in 4 ml of tetrahydrofuran was treated with 1 ml of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride overnight under nitrogen. The mixture was concentrated in vacuo, some benzene was added, and again concentrated in vacuo, and was then cooled to −25 °C and dissolved in 14 ml of methanol. To this was added 1.2 ml of 30% H_2O_2, followed dropwise by 3.0 ml of 1 N NaOH. TLC showed then complete conversion to epoxide. The mixture was acidified with 3.5 ml of 1 N HCl, concentrated in vacuo, and extracted with ethyl acetate, which was washed with water and salt, dried, and evaporated.

The crude epoxide was dissolved in 20 ml of tetrahydrofuran and to it was added 830 mg of triphenylphosphine, 2 ml of methanol, and 1 ml of saturated $NaHCO_3$ in which 300 mg of powdered potassium iodide had been dissolved. The reaction mixture was stirred and followed by removing aliquots for TLC. A mixture of PGE_2, 11-*epi*-PGE_2, and PGA_2 was formed in which the latter seemed about equal in amount to the two PGE's.

5,6-*trans*-PGE_2 via Irradiation of PGE_2. A solution of 0.50 g of prostaglandin E_2 and 0.50 g of diphenyl sulfide in 100 ml of benzene and 10 ml of methanol was placed in a quartz tube, purged of oxygen by bubbling nitrogen slowly beneath the surface of the solution, and irradiated with 3500 Å UV light for 24 h in a Rayonet photochemical reactor. The pale yellow solution was evaporated at reduced pressure, and the oily residue chromatographed over 20 g of acid-washed silica gel. Elution with 40 and 60% ethyl acetate in cyclohexane separated the diphenyl sulfide and approximately 100 mg of less polar by-product. Continued elution with 100% ethyl acetate afforded 412 mg of a crude mixture of unchanged 5,6-*cis*-PGE_2 and 5,6-*trans*-PGE_2. The latter was slightly less polar upon silica gel TLC plates impregnated with 25% silver nitrate (chloroform–acetic acid–methanol 80:10:10), visualization by spraying with 50% sulfuric acid and charring.

The 412-mg fraction was chromatographed over a column prepared as follows from 25 g of Amberlyst-15 ion-exchange resin. The resin was washed with warm water several times, then packed into a 100-ml burette as an aqueous slurry. The resin was converted to the silver form by washing the column with a 10% aqueous solution of silver nitrate until the pH of the effluent was about 3.5. The column was then washed free of excess silver ion with water, and the water replaced by washing with 3–4 vol of 3A alcohol. The PGE_2 mixture was dissolved in 2–3 ml of 3A alcohol and applied to the column. Elution was continued with 75 ml of 3A alcohol and then with 25 ml of 3A alcohol containing 10% redistilled cyclohexene at a flow rate of about 1 ml in 2 min. The eluate was assayed by TLC on plates coated with silica gel impregnated with 25% silver nitrate using the 10:10:80 system. 5,6-*trans*-PGE_2 exhibited an Rf of about 0.45 and 5,6-*cis*-PGE_2 an R_f of about 0.36. The first 30 ml of eluate contained 206 mg of partially crystalline 5,6-*trans*-PGE_2. The remaining 3A alcohol contained 175 mg of mixture and the cyclohexene fraction 60 mg of 5,6-*cis*-PGE_2. The mixed fractions were rechromatographed on the same column to afford an additional 60 mg of the trans isomer.

The total trans product (0.26 g) was again chromatographed over 20 g of acid-washed silica gel to remove color and silver residues. Elution with 125 ml each of 20, 40, and 60% ethyl acetate in cyclohexane gave 52 mg of miscellaneous less polar impurities, while 80 and 100% ethyl acetate eluted 166 mg of product which, after crystallization from ether–Skellysolve B, was obtained as colorless plates, mp 75–77 °C, yield 108 mg (22%). The material appeared as one spot on both ordinary silica gel (A-9 system—ethyl acetate–acetic acid–2,2,4-trimethylpentane–water 90:20:50:100) and on silver nitrate–silica gel plates. The R_f on regular silica gel was the same as *cis*-PGE_2. The material was recrystallized for analysis from the same solvents, mp 76–77 °C. Anal. Calcd for $C_{20}H_{32}O_5$: C, 68.14; H, 9.15. Found: C, 68.52; H, 9.23.

The mass spectrum did not show an appreciable molecular ion at 352, but did exhibit strong peaks at 334 (M − 18), 316 (M − 36), and 190. Principal peaks were 3340, 3160 (OH and acid), 1730, 1710 sh (C=O), 1250, 1170, 1080, 1075, 960 (trans CH=CH). $[\alpha]_D$ −95° (c 0.903 in $CHCl_3$); UV (after treatment with base) 278 mμ (24 550).

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Conformations of Proline

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Abstract: The present study concerns the energies of the conformations of proline. We present results of an improved molecular mechanics calculation for ring conformations of Ac-Pro-OCH_3 and for the *s*-cis and *s*-trans conformations. Internal coordinates including all torsions have been calculated from crystal coordinates for more than 40 x-ray determinations to give a consistent set of data which define proline ring geometries. Results from the present work and from the many previous studies on proline derivatives by other workers permit the following definitive statements: (1) Although four parameters are theoretically necessary to define the conformational state of a five-membered ring having fixed bond lengths, in practice two parameters ordinarily suffice for the proline ring. (2) There are two broad energy minima (Figures 1 and 2) with a barrier high enough to rule out pseudorotation, but affording such flexibility of structure as to preclude the meaningful designation as *envelope* and *chair* or *exo* and *endo*. The most common conformational state approximates a range of C^{β}–C^{γ} half chairs.[1] (3) There is as yet no really good method to get experimental measures of the conformational state of the proline ring in solution, although useful limits can often be established by treating NMR coupling constants by the Karplus relationships. (4) ^{13}C NMR is especially useful for studying *s*-cis–*s*-trans equilibria of *N*-acylproline derivatives. Ratios range from about 15 to 40% *s*-cis for open chain derivatives. (5) The difference of the torsions $\chi_5 - \phi$ is not a constant but ranges from 54 to 80°. This lack of constancy must be taken into account if proper conclusions are to be drawn from energy maps of proline-containing peptides.

The importance of proline and of hydroxyproline in structural proteins, in enzymes, and in hormones is too well known to require comment. Structural features have been extensively investigated by x-ray crystallography, by ^{1}H and ^{13}C NMR, by various theoretical calculations, and by other methods such as IR and CD.

One purpose of the present study is to bring together this extensive and scattered body of information so as to provide a definitive picture of the conformational properties of proline, particularly the energies of ring conformations and of *s*-cis and *s*-trans acyl groups. We have also performed new and sophisticated molecular mechanics calculations which lead to the energy profiles shown in Figures 1 and 2. Since previous calculations by other groups based on simplified force fields have given generally similar results, we conclude that the energy profiles are not very sensitive to the details of the force field and are therefore reasonably well defined. Although x-ray data are available for some three dozen proline rings, it is almost impossible to make a rational comparison of the x-ray data because some of it is incomplete and because every author uses a different numbering system. We have therefore recalculated all the internal coordinates of the proline skeletal atoms from crystal coordinates and present a unified and accurate tabular summary of the x-ray results. We have made a complete survey of NMR studies on proline derivatives and show in what respect these are relatable to the energy profiles.

How to Describe Conformations of Five-Membered Rings. The biggest problem we faced in this study was to devise a convenient method for describing the conformational state of a five-membered ring. Even assuming that all bond lengths remain constant, there remain four angles and torsions to be defined. Is it necessary to treat these independently, or is there some sound reason for supposing that, say, two would suffice? We were eventually able to show by molecular mechanics calculations that two parameters give an adequate definition for conformations having energies up to a few kilocalories above the minimum, but that more parameters are necessary to define conformations of high energy. We present our recommendations below.

The starting point for defining conformations of five-membered rings is cyclopentane, and the recommended conformational equations may be represented by the equation[2-7]

$$\chi_i = a_0 \cos [t + (i - 1)4\pi/5] \qquad i = 1, 2, 3, 4, 5 \quad (1)$$

In the earliest paper χ_i represented the vertical displacement of a given atom from the average plane.[2,7] But χ_i can also represent a torsion; or it can represent certain other geometrical properties such as bond angles.[4] Whatever the meaning of χ, t is the same quantity throughout, a phase angle that defines the distribution of puckering. The constant a_0 is a puckering amplitude which defines the maximum value assumed by χ and will necessarily depend on what quantity is being represented by χ. For $t = 0°, 36°, 72°, \ldots$, the ring conformation is of C_2 symmetry ("half chair"). For $t = 18, 54°, \ldots$, the conformation is of C_s symmetry ("envelope"). For cyclopentane the conformational energy is independent of t, and in consequence the two normal ring vibrations are degenerate. The result is denoted as pseudorotation.[2,3,7,8] As rings are made less flexible by substitution, the energy becomes dependent on t and