the reaction rate constant at 560 °C is $1.2 \times 10^6\ M^{-1}\ s^{-1}$. We would expect the rate constant with 1,2-diphenylethyl in place of methyl to be smaller. The closeness to the limit suggests that we may find reactions in which line broadening occurs, permitting the measurement of rate constants.

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# Synthesis of Prostaglandin $H_2$†

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**Abstract:** Prostaglandin $H_2$ ($PGH_2$) has been prepared by chemical synthesis from $PGF_{2\alpha}$. The synthesis proceeds from the intermediate 9β,11β-dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ to the endoperoxide $PGH_2$ in 17–24% yield upon reaction of the dibromide with silver trifluoroacetate and hydrogen peroxide in diethyl ether. Synthetic $PGH_2$ was purified by high-pressure liquid chromatography and was characterized by comparison with a biological sample and by conversion to $PGF_{2\alpha}$ methyl ester, $PGE_2$, and $PGH_2$ methyl ester under appropriate reaction conditions. Synthetic $PGH_2$ aggregates human platelet rich plasma that has been preincubated with indomethacin.

Since the two prostaglandin endoperoxides $PGG_2$ and $PGH_2$, **1** (Figure 1), were first isolated from incubations of the microsomal fraction of sheep vesicular gland with arachidonic acid,[2] considerable attention has been directed toward developing an understanding of the biochemical mode of action of these unstable species. $PGH_2$, in particular, lies at a crucial biochemical branch point in the oxidation of arachidonic acid by human platelets. This endoperoxide is converted by platelets to thromboxane $A_2$, which acts as a potent aggregator of platelets and as a stimulant to vascular smooth muscle.[3] $PGH_2$, on the other hand, is converted by an enzyme present in vessel walls to $PGI_2$, an inhibitor of platelet aggregation and a smooth muscle relaxant.[4] The diametrically opposed action of these two intermediates prompted Vane et al. to suggest that they are responsible for the homeostasis of the circulatory system.

Although $PGH_2$ can be prepared in milligram quantities biologically,[5] preparation of an active cyclooxygenase enzyme is tedious. Further, the enzyme will apparently tolerate very little variation of substrate structure so that the biological preparation of a broad spectrum of analogues has not been possible. Analogues of $PGH_2$ in which the peroxide linkage (-O-O-) has been replaced by -CH=CH-,[6] -N=N-,[7] $-CH_2O-$,[8] and -S-S-[9] have been chemically synthesized[10,11] and the pharmacology of these analogues has been extensively investigated. Although two syntheses of the methyl ester of $PGH_2$ have recently been reported[12,13] along with several successful syntheses of simple bicyclic peroxide analogues,[14,15] the parent peroxide, $PGH_2$, has remained elusive to chemical synthesis. $PGH_2$ is intrinsically a highly unstable substance ($t_{1/2}$ about 5 min in aqueous solution at 37 °C and pH 7.4) and approaches to its synthesis must utilize mild reaction conditions. In the present paper, we report a chemical synthesis of $PGH_2$ that proceeds from $PGF_{2\alpha}$ as the starting material. Synthetic $PGH_2$, purified by high-pressure liquid chromatography, has the full activity of the biological material in platelet aggregation assays.

## Experimental Section

Solvents for LC were purified by distillation before use (ethyl acetate from $P_2O_5$, hexane from sodium). Silver trifluoroacetate was prepared by the method of Janssen and Wilson (see ref 13). $H_2O_2$ (90%) was obtained as a gift from FMC. LC was carried out on a Waters ALC 202 instrument with a refractive index detector. TLC was carried out on 5 cm × 20 cm × 0.25 mm silica gel plates that were either Merck 60F-254 or Whatman LK5D. $^1H$ NMR spectra were obtained on a Bruker HFX-90 by FT or CW mode at 0 °C. $^{13}C$ spectra were obtained in $CD_2Cl_2$ solvent by the use of a JEOL PS-100 spec-

† Abbreviations: PG, prostaglandin; LC, high-pressure liquid chromatography; PRP, platelet rich plasma; TLC, thin layer chromatography; TX, thromboxane; HHT, hydroxyheptadienoic acid.

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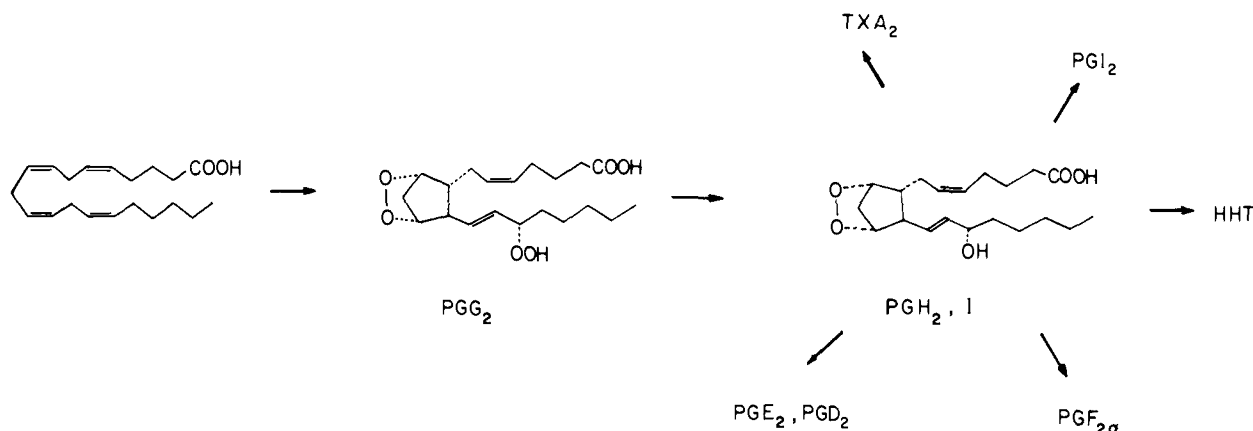



**Figure 1.** Prostaglandin cyclooxygenase products from arachidonic acid.

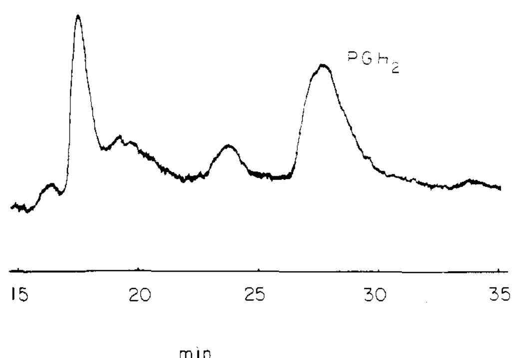



**Figure 2.** LC trace for $PGH_2$ purification (see Experimental Section for conditions).

trometer operating at 0 °C; 2500 accumulations gave the spectrum shown in Figure 3.

**Prostaglandin $F_{2\alpha}$ 9,11-Bis(benzenesulfonate) Methyl Ester (2).** A solution of prostaglandin $F_{2\alpha}$ 15-(dimethyl *tert*-butylsilyl ether) methyl ester (**12**, 0.640 g) in pyridine (8.15 mL) was treated with benzenesulfonyl chloride (2.709 g) for 30 h at room temperature. The solution was poured into cold 10% HCl and extracted four times with equal volumes of diethyl ether. The ether extracts were combined and washed with saturated brine and then bicarbonate solution followed by drying over $MgSO_4$ and solvent removal in vacuo. Chromatography on silica gel with ethyl acetate-hexane (ethyl acetate gradient from 0 to 45%) provided pure prostaglandin $F_{2\alpha}$ 9,11-bis(benzenesulfonate) 15-*tert*-butyl dimethylsilyl ether, $R_f$ 0.46 (ethyl acetate-hexane, 50/50, v/v). A solution of the 15-*tert*-butyldimethylsilyl 9,11-bis-(benzenesulfonate) compound (0.8075 g) was prepared in 8.4 mL of acetic acid-water-THF (3/1/1, v/v). After stirring for 24 h, 50 mL of water was added and the aqueous layer was extracted four times with ethyl ether. After washing with 10% sodium carbonate and brine, the solution was dried over magnesium sulfate and filtered, and the ether was removed in vacuo, leaving **2,** 89% yield, $R_f$ 0.23 (ethyl acetate-hexane, 50/50, v/v).

**9β,11β-Dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ Methyl Ester (3).** Lithium bromide (1.051 g) was added to dry dimethylformamide (DMF, 5.45 mL) followed by addition of the bis(benzenesulfonate) **2** (0.3927 g) in 12 mL of DMF. The solution was heated for 30 min at 65 °C under nitrogen. The cooled reaction mixture was poured into 100-150 mL of cold saturated sodium chloride solution and extracted four times with equal volumes of ether. The ether extracts were washed with saturated sodium chloride solution and dried over $MgSO_4$, and the solvent was removed. TLC (acetone-hexane, 20/80, v/v) showed products with $R_f$ 0.23, 9β,11α-dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ methyl ester; 0.18, 9α,11α-dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ methyl ester; 0.16, **3;** and 0.11, 9β-bromo,11α-benzenesulfonyl-prostaglandin $F_{2\alpha}$ methyl ester (**4**). Chromatography on silica gel at −11 °C with acetone-hexane (acetone gradient from 0 to 14%) provided pure **3** and **4.** Recovered **4** was resubjected to the lithium bromide-DMF reaction yielding more **3.** The total yield of **3** based on starting **2** was 20%. **3** prepared in this manner was identical with **3** prepared by Johnson et al.[10] in every respect.

**9β,11β-Dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ (5).** Powdered type II crude (Steapsin) porcine pancrease lipase (E.C. 3.1.1.3) (Sigma) (100 mg) was added to 100 mL of Tris-HCl pH 8 buffer and the solution allowed to equilibrate at 37 °C for 10 min. To this mixture was added 0.028 g of **3** in 2.5 mL of acetone and the solution was stirred for 3 h at 37 °C. The reaction was stopped by acidification to pH 2 with 10% HCl. The aqueous layer was extracted with ether, and the ether extracts were washed with saturated sodium chloride solution and then dried over magnesium sulfate. Removal of solvent and chromatography on silica gel with acetone-hexane (acetone gradient from 5 to 50%) provided pure **5,** 0.025 g, 92%. **5** was converted back to **3** upon treatment with diazomethane, $R_f$ for **5** 0.30 (acetone-hexane, 40/60, v/v).

**$PGH_2$ (1).** The dibromide **5** (0.011 g) was dissolved in anhydrous diethyl ether (1.55 mL) and to this solution was added 208 μL of 90% $H_2O_2$. Silver trifluoroacetate (0.182 g) was added as a solid and the mixture was stirred at room temperature for 20 min. At this time, the reaction mixture was diluted to 20 mL with ether and washed with a cold KCl-HCl buffer solution (pH 1.68, 25 mL of 0.2 M KCl and 6.5 mL of 2 M HCl). The aqueous layer was extracted four times with equal volumes of ethyl ether. The combined ether extracts were cooled to 0 °C, washed with saturated sodium chloride solution, and dried over magnesium sulfate at 0 °C, and the solvent was removed in vacuo at 0 °C. The residue was immediately taken up in 200 μL of ethyl acetate-hexane (60/40, v/v) and this solution was chromatographed at room temperature on a Whatman Partisil Magnum 9 preparative LC column (9.4 mm i.d., 12.7 m o.d. × 50 cm) with ethyl acetate-hexane (60/40, v/v) solvent (Figure 2). The retention time of **1** was 27.5 min with a solvent flow rate of 3 mL/min. Solvent was removed from the fraction collected from LC containing **1** by a rotary evaporator operating at <1 mmHg and at 0 °C. Final traces of solvent were removed by evacuation at 0.7 mmHg and 0 °C for 45 min. Yield of **1** was 1.4 mg (17%), homogeneous by TLC and LC.

The synthetic $PGH_2$ (LC pure) from four 10-38-mg reactions of dibromide **5** (105.7 mg total **5**) was pooled giving 18.9 mg of **1** (24% yield). The $^1H$ NMR of synthetic **1** was identical with that reported[5] in every respect. The $^{13}C$ NMR of **1** (Figure 3) showed absorptions at δ (downfield from $Me_4Si$, $CD_2Cl_2$ solvent) 178.5 (C-1), 133.5, 130.4, 130.0, 128.4, 82.1 and 79.6 (C-9 and C-11), 72.7 (C-15), 50.4, 48.2, 42.4, 37.0, 33.1, 31.7, 26.9, 26.3, 25.1, 24.5, 22.7, 14.0.

**Authentic $PGH_2$.** $PGH_2$ was biosynthesized by a modification of the method of Gorman et al.[5] Acetone powder of beef seminal vesicles (Miles Laboratories) was suspended in cold 0.06 M Tris buffer (pH 8.5) containing 1 mM phenol at a final concentration of 20 mg powder/mL. This suspension was homogenized in a glass-glass homogenizer for 1 min at 0 °C and allowed to stand at 0 °C for 30 min. The suspension was warmed to 37 °C, and *p*-hydroxymercuribenzoate in 0.1 M Tris buffer (pH 8.5) was added to a final concentration of 0.003 mM. The mixture was then incubated at 37 °C for 5 min. [1-$^{14}C$]-Arachidonic acid in ethanol, as the fatty acid substrate, was added to a final concentration of 0.40 μmol/mL (49.5 nCi/μmol). The reaction mixture was shaken by hand for 1 min, then diluted immediately with ten volumes of 0.2 M citrate buffer to a final pH of 2.8. The dilution mixture was extracted three times with double volumes of cold

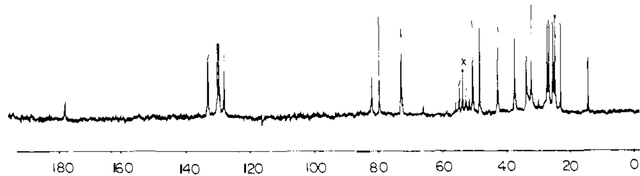


**Figure 3.** $^{13}C$ NMR spectrum of synthetic **1**. X indicates $CD_2Cl_2$ solvent signal.

hexane-diethyl ether (1:4). The extracts were dried over sodium sulfate and evaporated to dryness under reduced pressure at < 0 °C. The residue was dissolved in cold diethyl ether, transferred to a smaller tube, dried under nitrogen at 0 °C, and redissolved in 50 $\mu$L of cold acetone. A 5-$\mu$L aliquot was counted in a Beckman LS-100C to determine the amount of radioactivity recovered from the extractions, and the remainder was spotted on Quanta plates and developed in one of three solvent systems.

**Chromatography.** TLC was performed on Quanta plates (Quanta/gram, Whatman) in three solvent systems. Solvent system A consisted of ethyl acetate-hexane-acetic acid (50/50/0.5 v/v); solvent system B, ethyl acetate-isooctane-acetic acid-$H_2O$ (90/50/20/100, organic layer); solvent system C, ethyl ether-hexane-acetic acid (85/15/0.5, v/v). The dried chromatograms were sprayed with 10% phosphomolybdate in absolute ethanol and heated to 100 °C to stain the lipid products.

$R_f$ values follow: system A, arachidonic acid (0.44), $PGH_2$ (0.22); system B, arachidonic acid (0.67), $PGH_2$, (0.32); system C, $PGH_2$ (0.49).

**Platelet Aggregation.** Blood obtained from healthy donors, who had not taken aspirin for 1 week, was collected from the antecubital vein in 0.13 volume of 3.2% trisodium citrate solution. Platelet-rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 425 g for 8 min at room temperature. The PRP was removed, maintained at room temperature, and used within 3 h.

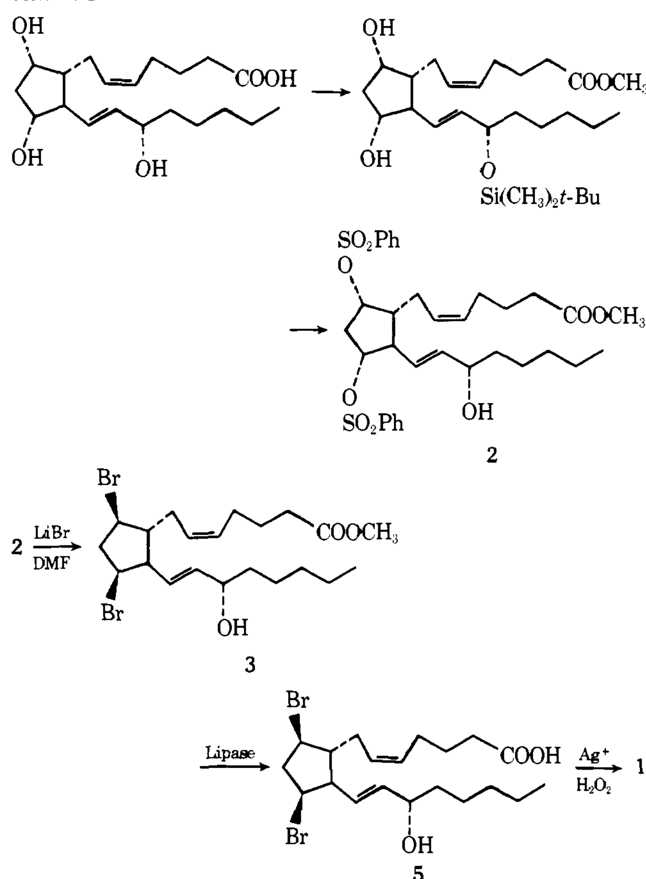
Platelet aggregation was measured photometrically with a Chronolog 330 aggregometer (Chrono-Log Corp., Broomall, Pa.) at 37 °C with stirring at 1200 rpm. Complete aggregation was initiated by the addition of the following compounds to the indicated final concentrations in 0.4 mL of PRP: 822 $\mu$M arachidonic acid; 14.5 $\mu$M epinephrine; 1.9 $\mu$M ADP; 0.78 $\mu$M synthetic $PGH_2$.

Indomethacin, as a complete inhibitor of arachidonic acid, was present in a final concentration of 14 $\mu$M in 0.4 mL of PRP. Platelets were preincubated with indomethacin for 1 min prior to initiation of aggregation.

## Results and Discussion

$PGH_2$ was prepared in seven steps from $PGF_{2\alpha}$ as outlined in Scheme I in an overall yield of 2.3%. Thus, $PGF_{2\alpha}$ was converted to 15-*tert*-butyldimethylsilyl prostaglandin $F_{2\alpha}$ methyl ester by published procedures[12] in 98% yield. This 15-OH protected compound was converted to the 9,11-bis(benzenesulfonate) and the 15-*tert*-butyldimethylsilyl group then removed to give **2**, prostaglandin $F_{2\alpha}$ 9,11-bis(benzenesulfonate) methyl ester. Treatment of **2** with LiBr in DMF led to three isomeric 9,11-dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ methyl esters. It had previously been reported that the 9,11-bis(tosylate) of prostaglandin $F_{2\alpha}$ methyl ester was converted to three 9,11-dibromo isomers (the $9\beta,11\alpha$, $9\alpha,11\alpha$, and $9\beta,11\beta$) of $PGF_{2\alpha}$ methyl ester. The $9\alpha,11\alpha$-dibromo-9,11-dideoxyprostaglandin $F_{2\alpha}$ methyl ester was the major product formed from the ditosylate and the desired $9\beta,11\beta$ isomer, **3**, was formed in only 9% yield. Johnson et al.[12] reasoned that **3** was the kinetic reaction product and that the other dibromide isomers resulted from subsequent bromide ion exchange with **3**. By using leaving groups at 9,11 better than tosylate (benzenesulfonate and *p*-bromobenzenesulfonate) and reducing reaction times, **3**, the kinetic product, could be prepared in an isolated yield of 20% from **2**. Thus, the simple expedient of changing the leaving group from tosylate to benzenesulfonate improves the yield of the desired isomer by over twofold. It seems likely that further improvement in the yield of dibromide

**Scheme I**




product is possible and other methods for conversion of $PGF_{2\alpha}$ methyl ester to **3** are under study.

Several methods were explored for converting **3** to **5**. Basic hydrolysis of **3** (KOH, $CH_3OH$-$H_2O$) was successful. However, some elimination accompanied hydrolysis, yields were only 80-85%, and reaction times were long. Hog pancrease lipase is known to be a rather unselective catalyst for lipid ester hydrolysis[16] and, in fact, lipase hydrolysis of **3** proceeded smoothly in 3 h and the free acid, **5**, could be isolated in 92% yield. Esterase catalysts were used in attempted hydrolyses of **3** without success. Thus, commercial lipase would appear to be the reagent of choice for mild ester hydrolysis of sensitive PG derivatives. Hog pancrease lipase is readily available from several commercial sources and it is convenient and easy to use.

The conversion of dibromide **5** to $PGH_2$, **1**, is best carried out with a large excess of silver trifluoroacetate and hydrogen peroxide and with reaction times that are less than 0.5 h. Longer reaction times and less silver-$H_2O_2$ reagent lead to significant decomposition of **1** during its synthesis. In addition to $PGH_2$, a byproduct is observed from this reaction by TLC that is slightly less polar than **1**. Careful column chromatography on silica gel led to no purification of **1** from the byproduct and high-pressure liquid chromatography (LC) of the reaction mixture was required for purification of $PGH_2$. LC could be carried out at room temperature on a micro silica gel column with detection by refractive index. Reinjection of recovered $PGH_2$ onto the LC showed it to be homogeneous with only approximately 20% material loss during one LC cycle. Room temperature LC on silica seems ideally suited for $PGH_2$ purification and experiments are in progress to determine if $PGG_2$ can also be purified by LC. Although chromatography of **1** may be carried out at room temperature, purified material was immediately cooled to 0 °C for solvent removal and the product was stored in acetone or ether at −78 °C.

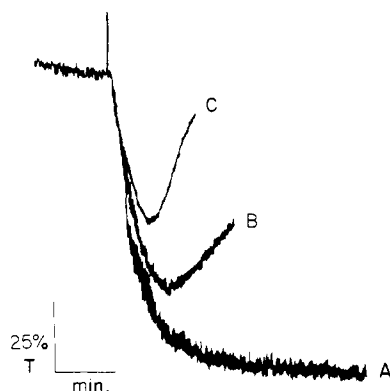



**Figure 4.** Aggregation of human platelets in plasma by the addition of (A) 0.78 $\mu$M; (B) 0.55 $\mu$M; or (C) 0.23 $\mu$M synthetic $PGH_2$ as described in the text.

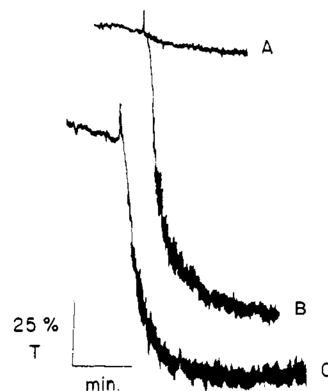
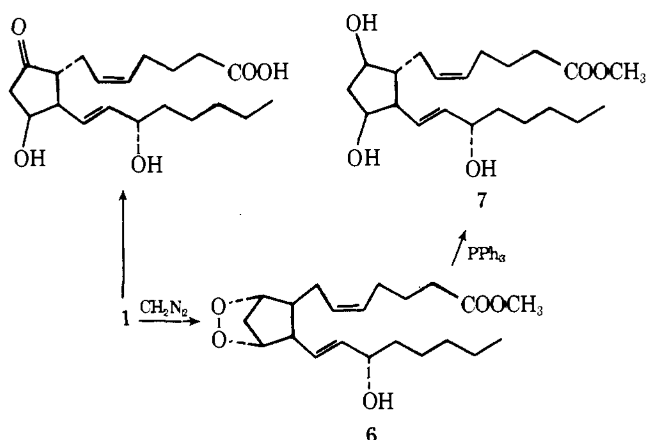



**Figure 5.** Aggregation of human platelets upon inhibition by indomethacin. (A) Platelets were preincubated with 14 $\mu$M indomethacin for 1 min and then treated with 822 $\mu$M arachidonic acid. (B) Platelets not treated with indomethacin were completely aggregated by addition of 822 $\mu$M arachidonic acid. (C) Indomethacin incubated platelets treated with 0.78 $\mu$M synthetic $PGH_2$.

**Scheme II**




**1** had the same $R_f$ on thin layer as the biological material in three distinctly different solvents. It was peroxide positive to ferrous thiocyanate spray and it converted, with diazomethane, to material that was identical by thin layer chromatography with $PGH_2$ methyl ester **6**. The methyl ester **6**, thus prepared, was reduced with triphenylphosphine to $PGF_{2\alpha}$ methyl ester, **7**. The tris(trimethylsilyl) derivative of **7** was shown to be identical with authentic material by GC-MS. Major mass spectrum fragments were observed at $m/e$ 585 (parent ion), 570, 513, 494, 423, 307, 74 (base). The $^1$H NMR of **1** is identical with the reported spectrum[5] and its $^{13}$C spectrum is totally consistent with the proposed structure (Figure 3).

When **1** was let stand at room temperature on silica gel, the major product observed upon subsequent chromatography was $PGE_2$, with minor amounts of $PGD_2$ also being formed. This is in accord with previous observations[2] that $PGH_2$ decomposes thermally to give $PGE_2$, $PGD_2$, and hydroxyheptatrienoic acid (HHT).

Freshly purified **1** was tested as an initiator of human platelet aggregation. Synthetic $PGH_2$ induced aggregation of platelets (six separate experiments and two donors) at the same concentrations (50-300 ng/mL) reported for aggregation induced by the biologically prepared endoperoxide.[17] The extent as well as the reversibility of aggregation was dependent on the concentration of **1** added (Figure 4). Further, synthetic **1** induced aggregation of platelets that had been previously incubated with 14 $\mu$M indomethacin (Figure 5). In addition, imidazole reduced the proaggregatory effects of synthetic $PGH_2$.[18]

The demonstration here that the F series of prostaglandins may serve as synthetic precursors to the H series opens the way for the preparation of prostaglandin endoperoxides that have been previously difficult or impossible to obtain biologically and calls for further efforts to develop and expand this approach. The procedures involved are straightforward and some improvement in overall yield may be possible.

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