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Concerning the Biosynthesis of Prostaglandin I_2[1]

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Two diastereoisomers, 5*R*,6*R*-5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid (**7**) and 5*S*,6*S*-5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid (**10**) were synthesized for evaluation as possible biosynthetic intermediates in the enzymatic transformation of PGH_2 or PGG_2 into PGI_2. The synthetic sequence entails the stereospecific reduction of the 9-keto function in PGE_2 methyl ester after protecting the C-11 and C-15 hydroxyls as t-butyldimethylsilyl ethers. The resulting $PGF_{2\alpha}$ derivative was epoxidized exclusively at the C-5 (6) double bond to yield a mixture of epoxides, which underwent facile rearrangement with SiO_2 to yield the 5*S*,6*S* and 5*R*,6*R*-5-hydroxy-6(9α)-oxido cyclic ethers. It was found that dog aortic microsomes were unable to transform radioactive 9β-5*S*,6*S*[^{3}H] or 9β-5*R*,6*R*[^{3}H]-5-hydroxy-6(9α)-oxido cyclic ethers into PGI_2. Also, when either diastereoisomer was included in the incubation mixture, neither isomer diluted the conversion of [1-^{14}C]arachidonic acid into [1-^{14}C]PGI_2.

INTRODUCTION

The prostaglandin endoperoxides, PGG_2, and PGH_2, are key intermediates in the enzymatic transformations of arachidonic acid. They are known to be precursors of prostaglandins (*1*, *2*), thromboxane A_2 (TXA_2) (*3*), a potent inhibitor of platelet aggregation, 12L-hydroxy-5,8,10-heptatnenoic acid (*4*) (HHT), and 6-keto-$PGF_{1\alpha}$ (*5*).

Recently, Vane and his colleagues (*6–9*) reported the presence of an enzyme system in microsomes of arterial walls that transforms PG endoperoxides to an unstable product (PGI_2) which relaxes vascular smooth muscle and potently inhibits platelet aggregation. They postulated a biochemical interaction between platelets and vessel wall by which platelets feed the vessel wall with PG endoperoxides for PGI_2 formation to prevent the accumulation of platelets in the vessel wall. Since microsomes from rat stomach fundus also form a substance with the same biological activity as PGI_2, they suggested that PGI_2 could be an unstable intermediate in the formation of 6(9α)-oxido-11α,15*S*-dihydroxy-prosta-7,13-dienoic acid (*10*, *11*) or 6-keto-$PGF_{1\alpha}$ (*5*) from PG-endoperoxides. Subsequent to their reports, several other groups have reported the detection of substances possessing PGI_2 activity (*12–14*) and the isolation of 6-keto-$PGF_{1\alpha}$ from other tissues (*15*, *16*).

The chemical structure of PGI_2 was elegantly established by Johnson et al. (*17*) to be 6(9α)-oxido-11α,15*S*-dihydroxyprosta-5,13-dienoic acid and shown to be rapidly

[1] We dedicate this paper to Professor S. Morris Kupchan, a long-time colleague at the University of Wisconsin.

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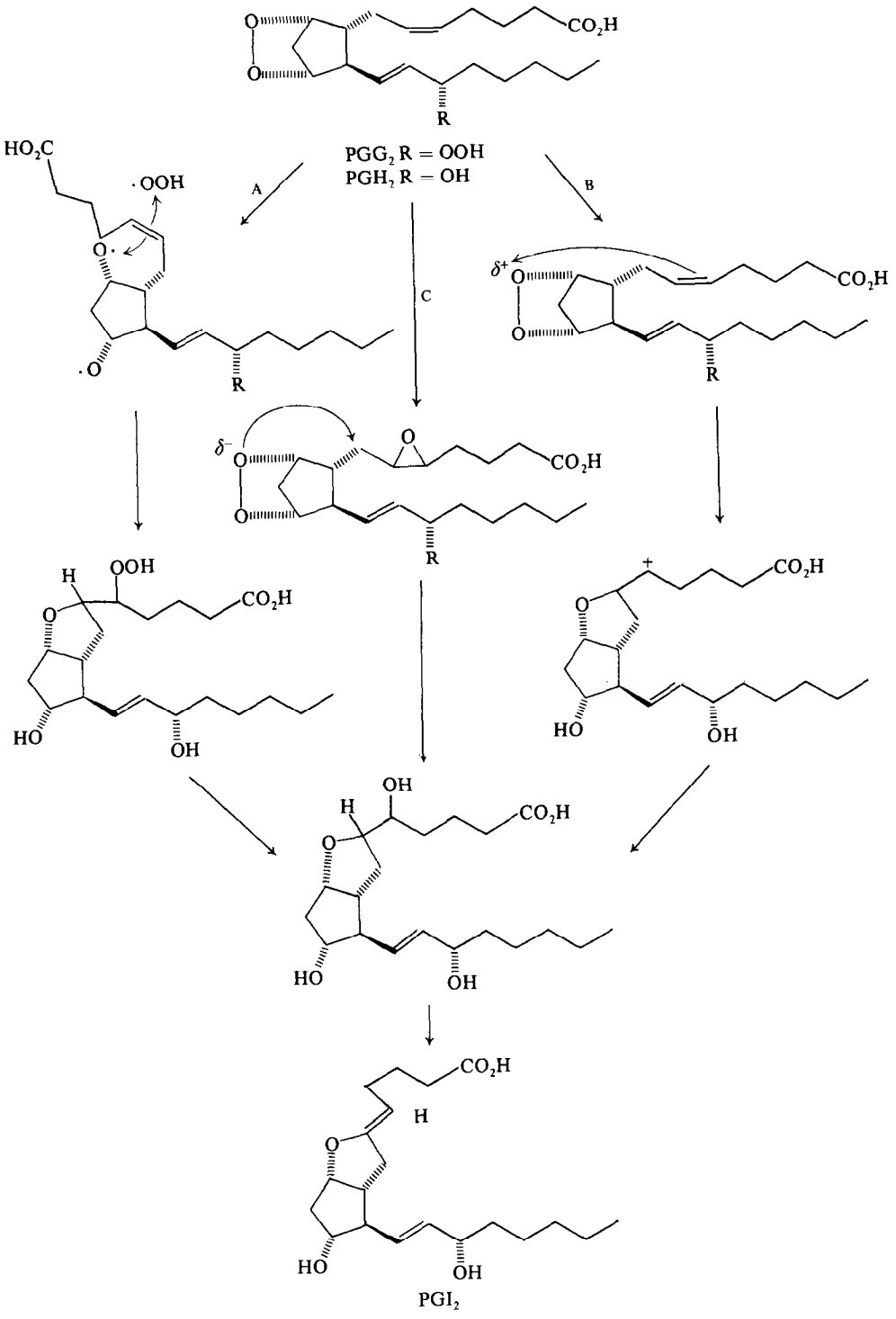


SCHEME 1. Three possible pathways for PGI_2 formation.

decomposed nonenzymatically into 6-keto-$PGF_{1\alpha}$ in biological fluids. Although 6(9α)-oxido-11α,15*S*-dihydroxyprosta-5,13-dienoic acid (PGI_2) had been identified as a minor component during the biosynthetic transformation of arachidonic acid by rat stomach homogenate (*10*, *11*), the stereochemistry of the C-5(6) double bond in PGI_2 was defined only recently and was confirmed by chemical syntheses (*18–20*).

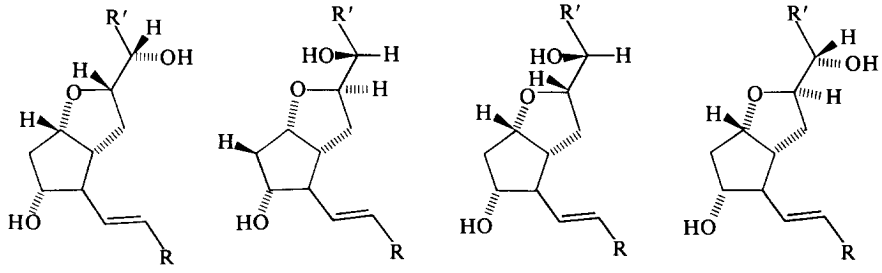
Several different pathways for the formation of PGI_2 from PGH_2 are possible, as shown in Scheme 1. Pathway A involves homolytic cleavage of the cyclic endoperoxide, followed by attack of the oxygen radical at C-9 on the C-6 position of the Δ^5 double bond with hydroperoxidation at C-5. Subsequent elimination of the hydroperoxy or hydroxyl group gives PGI_2 (*10*). Another possible pathway, B, involves charge separation to produce an incipient oxonium ion at C-9, which, upon addition to the *cis* olefin, results in the formation of a carbonium ion at C-5. This transient carbonium ion intermediate may be satisfied by the loss of the C-6 proton to generate the enol ether (*17*, *21*), or it may be intercepted by a hydroxide ion to yield 5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprosta-13-enoic acid, which gives PGI_2 upon enzymatic elimination. A third plausible pathway, C, entails the epoxidation of the Δ^5 double bond followed by a concerted attack of the endoperoxide 9-oxygen to C-6 with concomitant opening of the epoxide to generate the 5-hydroxy-6(9α)-oxido cyclic ether.

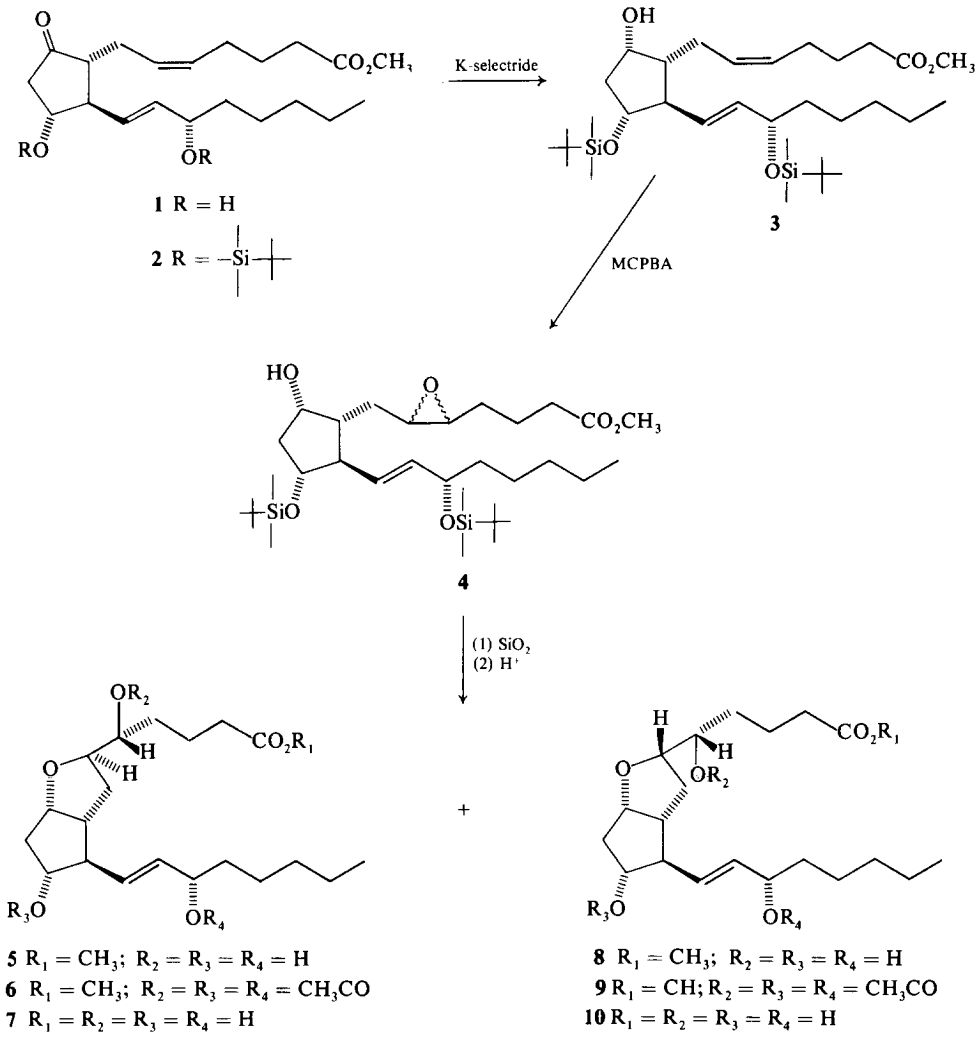
As all these mechanisms involve the participation of 5-hydroxy intermediates in the transformation of PGG_2 or PGH_2 into PGI_2, we herein report the chemical syntheses of 5*R*,6*R*-5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid (**7**) and 5*S*,6*S*-5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid (**10**), as well as the results of our incubation studies of these compounds in a PGI_2-forming microsomal system.

RESULTS AND DISCUSSION

The are four possible diastereoisomers of 5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid, and one of these could possibly serve as the biosynthetic intermediate in the enzymatic transformation of PGH_2 or PGG_2 into PGI_2. However, an analysis of the mechanisms outlined in Scheme 1 reveals that the orientation of the C-9 oxygen should be trans-periplanar to the C-5 hydroxyl in the initial addition product. Thus, we decided to synthesize the 5*S*,6*S* and 5*R*,6*R* isomers, since they appear more likely to operate as biosynthetic intermediates.

We envisaged a synthetic route (Scheme 2) for the preparation of the 5*S*,6*S* and 5*R*,6*R* isomers paralleling the reaction sequence of pathway C (Scheme 1). As we intend to





1 R = H
2 R = –Si–

3

4

5 $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$
6 $R_1 = CH_3$; $R_2 = R_3 = R_4 = CH_3CO$
7 $R_1 = R_2 = R_3 = R_4 = H$

8 $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$
9 $R_1 = CH$; $R_2 = R_3 = R_4 = CH_3CO$
10 $R_1 = R_2 = R_3 = R_4 = H$

SCHEME 2. Synthetic route for the preparation of **7** and **10**.

introduce eventually a radioactive label into these compounds, we elected to use PGE_2 methyl ester (**1**) as the starting material. To shield the *trans* C-13(14) double bond from subsequent oxidation, we protected the C-11 and C-15 hydroxyl groups in PGE_2 methyl ester as the t-butyldimethylsilyl ethers (**2**) (*22*). After the 9-ketone function was stereospecifically reduced with potassium selectride to the 9α-hydroxyl derivative (**3**), the resulting $PGF_{2\alpha}$ derivative was epoxidized exclusively at the C-5(6) *cis* double bond with *m*-chloroperbenzoic acid to afford a mixture of α- and β-epoxides (**4**). As these epoxides (**4**) were found to undergo facile intramolecular rearrangement on tlc plates, they were stirred with tlc silica gel in chloroform to effect cyclic ether formation. After acidic cleavage of the t-butyldimethylsilyl groups, two isomeric 5-hydroxy-6(9)oxido derivatives were noted on tlc plates with R_f values of 0.13 (**5**) and 0.08 (**8**) in system B.

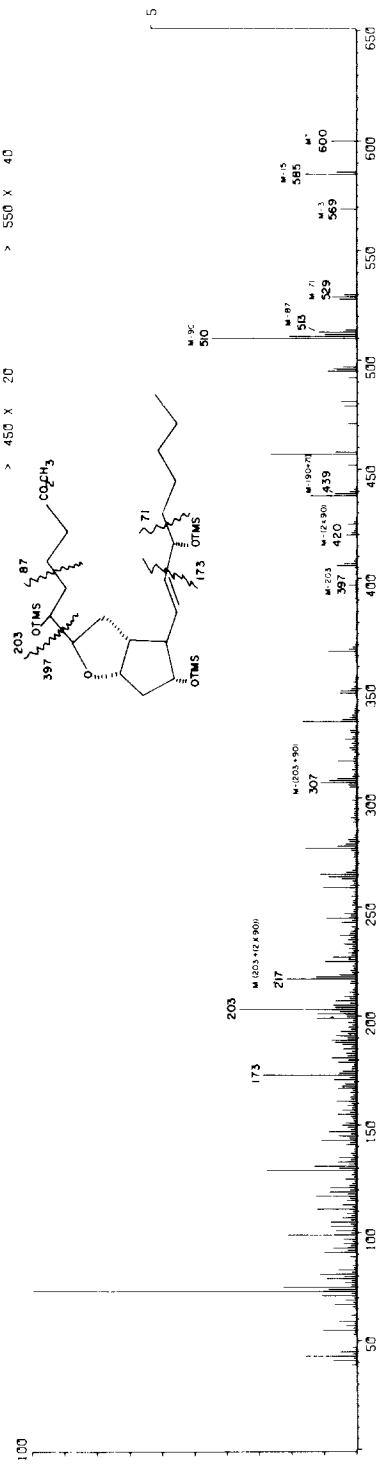


FIG. 1. The mass spectrum of the trimethylsilyl ethers of **5**.

The mass spectrum of the trimethylsilyl derivatives of **5** (Fig. 1) was found to be identical to that of **8**, and the nmr spectra of **5** and **8** exhibited no discernible features relative to the stereochemistry at the C-5, C-6 positions.

Recently, Posner and co-workers (*23*) reported the facile opening of epoxides on dehydrated alumina impregnated with alcohols, thiols, and acetic acids. As all of these

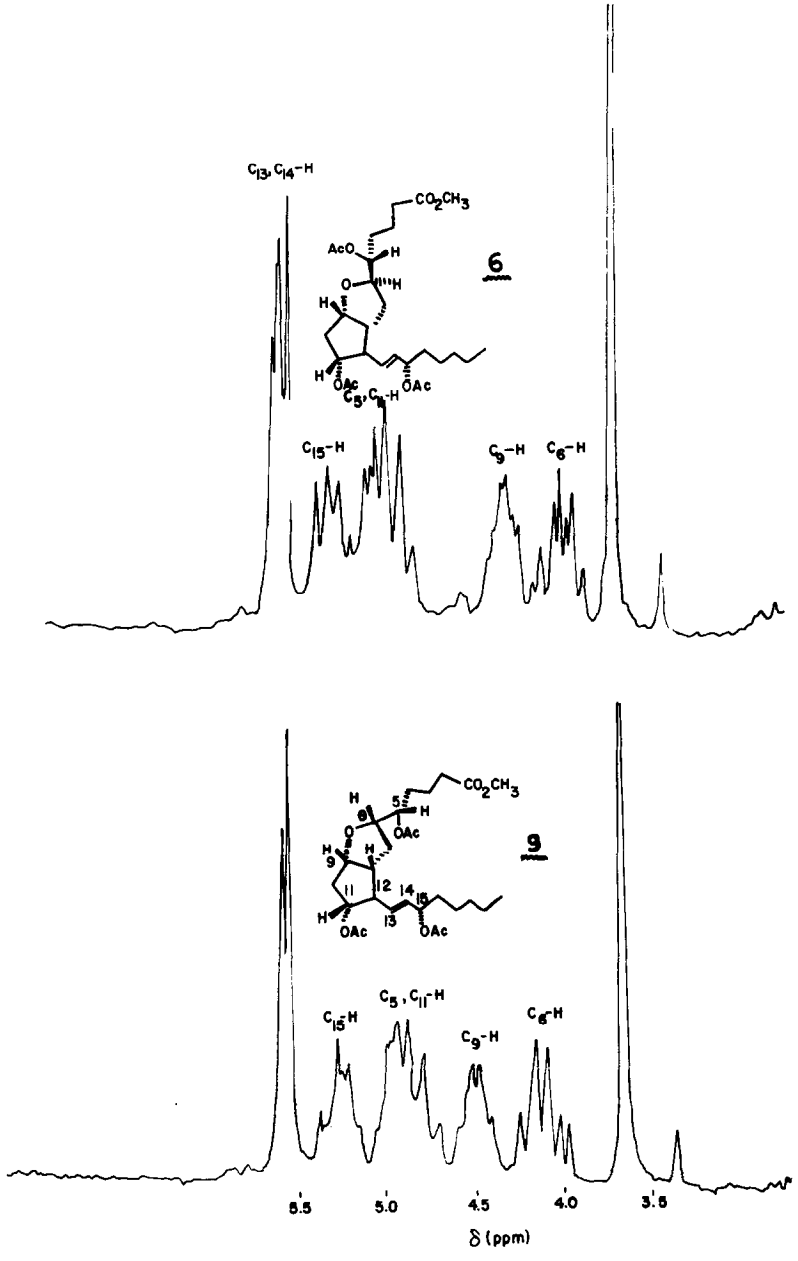


FIG. 2. Pertinent regions of the NMR spectra of **6** and **9**.

reactions were shown to proceed via S_N2 attack resulting in *trans* opening of the epoxides, it is reasonable to expect that the two diastereoisomers obtained in the SiO_2 rearrangement should possess the 5*R*,6*R* and 5*S*,6*S* configurations. To confirm and distinguish the stereochemistry of these two isomers, **5** and **8** were converted to their corresponding acetates **6** and **9**, respectively. The α-protons of the acetates were now shifted downfield, readily distinguishable from the methine protons of the tetrahydrofuran ring (Fig. 2).

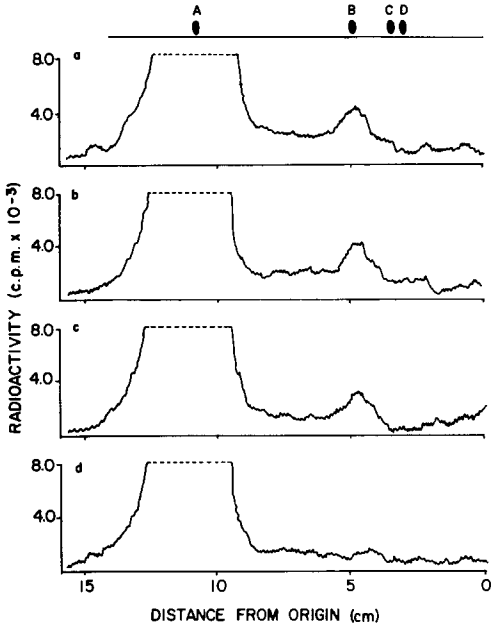


FIG. 3. Enzymatic transformations of [1-^{14}C]arachidonic acid in absence and presence of 5*R*, 6*R* (**7**) or 5*S*,6*S*(**10**)-5-hydroxy-6(9α)-oxido cyclic ethers. (a) [1-^{14}C]Arachidonic acid + microsomes; (b) [1-^{14}C]arachidonic acid + microsomes + 0.06 μmol of **7**; (c) [1-^{14}C]arachidonic acid + microsomes + 0.06 μmol of **10**; (d) [1-^{14}C]arachidonic acid + boiled microsomes. A, arachidonic acid methyl ester; B, 6-keto-$PGF_{1\alpha}$ methyl ester; C, (5*R*,6*R*)-5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid methyl ester (**5**); D, (5*S*,6*S*)-5-hydroxy-6(9α)-oxido-11α,15*S*-dihydroxyprost-13-enoic acid methyl ester (**8**).

The acetate of the more polar isomer, **9**, exhibited signals at δ4.47 and 4.14, whereas these corresponding signals appeared at δ4.25 and 3.94 in **6**. From spin-decoupling experiments between C-5 and C-6 methine protons of each isomer, the lower field signals (δ4.47 and 4.25) were assigned to the C-9 protons and the higher field signals (δ 4.14 and 3.94) to the C-6 protons of the two isomers. There was only a small change in the chemical shift of the C-9 protons in the acetate, **6** (δ4.47), from its parent alcohol, **5** (δ4.43). On the other hand, the C-9 proton in the acetate, **9**, (δ4.25) exhibited an upfield shift of 0.13 ppm with respect to its alcohol, **8** (δ4.38). This diamagnetic shift was probably due to a long-range shielding effect of the C-5 acetoxy function with an endo-orientation of the C-6 hydrogen. On the basis of this observation, we have *tentatively* assigned the 5*S*,6*S* configuration to the more polar isomer **9** and the 5*R*,6*R* configuration to the less polar isomer **6**. After hydrolyzing the methyl esters in **5** and **8** with 1 *N* ethanolic KOH at room temperature, the resulting free acids, **7** and **10**, were examined

as possible intermediates in the biosynthesis of PGI_2 from arachidonic acid. Figure 3a clearly shows that dog aortic microsomes in the presence of the cofactors tryptophan and methemoglobin converted radioactive arachidonic acid into a radioactive product with a mobility corresponding to 6-keto-$PGF_{1\alpha}$ (under the acidic workup, PGI_2 decomposes into 6-keto-$PGF_{1\alpha}$) methyl ester after diazomethane treatment of the extract. When either **7** (Fig. 3b) or **10** (Fig. 3c) was included in the reaction mixture, no significant new radioactive peaks corresponding in mobility to **5** and **8** were noted. Also, there was no significant decrease in the amount of 6-keto-$PGF_{1\alpha}$ methyl ester formed.

Using a similar synthetic sequence, except that NaB^3H_4 was substituted for K-selectride as the reducing agent, 9β-^{3}H-5*R*,6*R* and 9β-5*S*,6*S*-5-hydroxy-6(9α)-oxido isomers were prepared. When each of the 9-tritiated **7** and **10** was exposed to the dog aortic microsomal system, no radioactive 6-keto-$PGF_{1\alpha}$ was detected. On the basis of these observations, one can only conclude that **7** and **10** appear to be unlikely free intermediates in the biosynthesis of PGI_2.

EXPERIMENTAL

Materials

[1-^{14}C]Arachidonic acid (specific activity 54 mCi/mmol) and NaB^3H_4 (specific activity 244 mCi/mmol) were obtained from New England Nuclear. PGE_2 was generously donated by Dr. R. Pappo of G. D. Searle and Co.; 6-keto-$PGF_{1\alpha}$ methyl ester was a gift from Dr. H. Kluender of Miles Laboratories. Dog aortic microsomes (*12*) were kindly supplied by Dr. P. Ho of Eli Lilly and Co. Silica gel (MN-Kiesel gel, 70–270 mesh ASTM) was used for column chromatography.

Methods

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were determined on a Perkin–Elmer Model 257 recording spectrophotometer. Nuclear magnetic resonance spectra were determined in $CDCl_3$ on Varian EM390 or Bruker 90 spectrometers using tetramethylsilane as internal standard. Specific rotations were determined on a Perkin–Elmer polarimeter (Model 241). Evaporations were carried out at temperatures less than 40°C under reduced pressure. Thin-layer chromatography (tlc) was carried out on 0.25 mm-silica gel F254 precoated plates (EM-Brinkmann), and chromatograms were visualized by spraying with a 3% $Ce(SO_4)_2$–3 *N* H_2SO_4 solution followed by heating. Solvent systems used were: A, CH_2Cl_2:acetone (98:2); B, $CHCl_3$:acetone (1:1); C, $CHCl_3$:MeOH:HOAc:H_2O (90:9:1:0.65). Radioactivity was assayed in a Packard Model 2002 scintillation spectrometer after addition of 10 ml of Bray's solution. Radiochromatogram was assayed on a Vanguard Model 930 autoscanner. Mass spectra were obtained with a Varian MAT 112 mass spectrometer equipped with a SS100C data system using direct probe introduction with an ion source temperature of 220°C and electron potential of 80 eV.

General procedure for the enzymatic transformation of [1-^{14}C]arachidonic acid. Lyophilized dog aortic microsomes (*12*) (1.5 mg) were incubated with 0.01 μmol of [1-^{14}C]arachidonic acid, 1.0 μmol of L-tryptophan, and 0.0004 μmol of methemoglobin in

a total volume of 0.2 ml of 0.5 *M* Tris–HCl buffer, pH 8.0. Where indicated, 0.06 μmol of 5*R*,6*R* (**7**) or 5*S*,6*S* (**10**) was included. After incubation at 37°C for 7 min, the reaction mixture was acidified at pH 3 with HCl and extracted with 1 ml of ethyl acetate (three times). The combined extracts were evaporated to dryness, and the residue was treated with an excess of diazomethane. The residue was dissolved in methylene chloride, and an aliquot containing 100,000 cpm was spotted on a tlc plate and developed in system C. Results of the radiochromatograms are illustrated in Fig. 3.

Incubation of (5,R,6R)-9β5-[^{3}H]hydroxy-6(9α)-oxido-$PGF_{1\alpha}$ (**7**) *or (5S,6S)-9β-5[^{3}H]hydroxy-6(9α)-oxido-$PGF_{1\alpha}$* (**10**) *with dog microsomes.* Lyophilized dog aortic microsomes (1.5 mg) were incubated with 0.09 μmol of either (5*R*,6*R*)-9β-^{3}H or (5*S*,6*S*)-9β-5-[^{3}H]hydroxy-6(9α)-oxido-$PGF_{1\alpha}$ (39.7 mCi/mmol), 1.0 μmol of L-tryptophan, and 0.0004 μmol of methemoglobin in a total volume of 0.1 ml of Tris–HCl buffer, pH 8.0. After incubation for 7 min at 37°C, the reaction mixture was worked up, and the extract was chromatographed exactly as described above for the enzymatic transformation of [1-^{14}C]arachidonic acid.

$PGF_{2\alpha}$11,15-bis(t-butyldimethylsilyl ether) methyl ester (**3**). To a stirred solution of 135 mg (0.227 mmol) of PGE_2, 11,15-bis(t-butyldimethylsilyl ether) methyl ester (**2**), in 2 ml of dry tetrahydrofuran at −74°C under nitrogen was added 0.8 ml (0.4 mmol) of K-selectride (Aldrich) in tetrahydrofuran over a period of 3 min. The reaction mixture was stirred at −74°C for 1 hr and then quenched by the dropwise addition of 2 ml of water. The resulting solution was saturated with sodium chloride and then extracted with ethyl acetate (3 × 6 ml). The combined organic extracts were washed with 0.5 *N* HCl, saturated sodium bicarbonate solution, and brine, dried (sodium sulfate), and evaporated to give 112 mg of oil. Thin-layer chromatography (System A) showed mainly one spot (R_f 0.4) with traces of a more polar compound.

The crude product was chromatographed over 15 g of silica gel packed in methylene chloride. Elution was effected with 300 ml of 1% acetone in methylene chloride, and 3-ml fractions were collected. Fractions 32–60 were combined to give 90 mg of the $PGF_{2\alpha}$ derivative (**3**); nmr ($CDCl_3$) δ 0.07 (s, 12H), 0.9 (s, 18H), 0.8–2.9 (m, 23H), 3.68 (s, 3H), 4.08 (m, 3H), and 5.42 ppm (m, 4H). The mass spectrum showed ions at *m/e* 596 (M^+), 578 ($M-H_2O$), 539 ($M-C_4H_9$), 446 ($M-H_2O-C_6H_{15}SiOH$), 407 ($M-C_6H_{15}SiOH-C_4H_9$), and 314.

Epoxidation of $PGF_{2\alpha}$ 11,15-bis(t-butyldimethylsilyl ether) methyl ester (**3**). To a well-stirred solution of $PGF_{2\alpha}$ derivative (**3**), 172.6 mg (0.29 mmol) in 6 ml of chloroform and 6 ml of 0.2 *M* phosphate buffer (pH 7.2), was added dropwise 62.2 mg (0.36 mmol, 85% pure) of *m*-chloroperbenzoic acid in 8 ml of chloroform over a period of 10 min. After stirring at room temperature for an additional 3 hr, the reaction mixture was extracted with 50 ml of ethyl acetate. The organic solution was washed successively with water, saturated $NaHCO_3$ solution, and brine, dried over sodium sulfate, and evaporated to give 165 mg of an oily product. The nmr spectrum of the crude compound showed signals at δ 0.03 (m, 12H), 0.75–3.15 (m, 43H), 3.68 (s, 3H), 3.85–4.15 (m, 3H), and 5.45 ppm (m, 2H). The epoxide was used without further purification.

(5R,6R)-5-Hydroxy-6(9α)-oxido-$PGF_{1\alpha}$ methyl ester (**5**) *and (5S,6S)-5-hydroxy 6(9α)-oxido-$PGF_{1\alpha}$ methyl ester* (**8**). The mixture of epoxides (121 mg) obtained from above and 3.2 g of silica gel (EM tlc plates, Brinkmann) in 15 ml of chloroform was

stirred at room temperature for 18 hr. The reaction mixture was then extracted with methanol–methylene dichloride (1:1) (5 × 10 ml). After filtration, the organic solvent was evaporated and the residue was extracted again with methylene chloride (5 × 5 ml) and evaporated to dryness to afford 110 mg of crude residue. The residue was dissolved in 6 ml of acetic acid–water–THF (3:1:1) and stirred at room temperature for 20 hr. To this mixture was added 5 ml of water, and then the mixture was freeze-dried. The residue (70 mg) was dissolved in 2 ml of dioxane and treated with 1 ml of 2 *N* ethanolic KOH at room temperature for 20 min. The mixture was diluted with 2 ml of water, acidified to pH 2.5, and extracted with ethyl acetate (3 × 5 ml). After drying the organic layer over anhydrous Na_2SO_4, it was evaporated to dryness. The residue was reacted with an excess of an ethereal solution of diazomethane to give 60 mg of crude **5** and **8**. This mixture was chromatographed over 35 g of silica gel. The column was eluted with a gradient system composed of 250 ml of acetone–methylene chloride (1:9) in the mixing flask and 250 ml of acetone–methylene chloride (6:4) in the reservoir flask followed by another 250 ml of acetone–methylene chloride (3:7) and 250 ml of acetone–methylene chloride (7:3), respectively, and 5 ml fractions were collected. After combining fractions 120–128, 6 mg of **5** was obtained as an oil; $[\alpha]_D^{25} = +12.7°$ (*c*0.7, $CHCl_3$); δ 0.7–2.9 (m, 23H), 3.63 (s, 3H), 3.3–4.53 (m, 5H), and 5.5 ppm (m, 2H); ir 1735, 1460, 1440, 1240, 1075, and 975 cm^{-1}. The mass spectrum gave ions at *m/e* 366 ($M-H_2O$), 348 ($M-2H_2O$), and 322, 316, 290, 276, and 253.

Fractions 135–145 contained 13 mg of **8**; mp 94–95°C; $[\alpha]_D^{25} = +30°$ (*c*, 0.5, $CHCl_3$); δ 0.7–2.9 (m, 23H), 3.63 (s, 3H), 3.3–4.53 (m, 5H), and 5.52 ppm (m, 2H); ir 1735, 1455, 1440, 1380, 1365, 1265, 1235, 1070, 1020, 975, and 910 cm^{-1}. Its mass spectrum showed ions at *m/e* 366 ($M-H_2O$), 348 ($M-2H_2O$), and 322, 316, 290, 276, and 253.

(5R,6R)-5,11R,15S-Triacetoxy-6(9)oxido-prost-13-enoic acid methyl ester (**6**). To 10 mg of **5**, dissolved in 0.5 ml of acetic anhydride, was added 2 drops of pyridine, and the reaction mixture was allowed to stand at room temperature for 18 hr. After evaporation of the solvent, the residue gave the following spectral data: nmr δ 0.8–2.9 (m, 23H), 2.02 (s, 6H), 2.1 (s, 3H), 3.67 (s, 3H), 3.94 (m, 1H), 4.25 (m, 1H),4.8–5.3 (m, 3H), and 5.55 ppm (m, 2H). The mass spectrum showed ions at *m/e* 479 ($M-OCH_3$), 450 ($M-CH_3CO_2H$), 408 ($M-OCH_3-C_5H_{11}$), 390, 348, and 330.

(5S,6S)-5,11R,15S-Triacetoxy-6(9)-oxido-prost-13-enoic acid methyl ester (**9**). The isomer **9** was prepared by the above procedure and gave the following: nmr δ 0.8–3.0 (m, 23H), 1.99 (s, 3H), 2.02 (s, 3H), 2.08 (s, 3H), 3.67 (s, 3H), 4.14 (m, 1H), 4.48 (m, 1H), 4.85 (m, 2H), 5.2 (m, 1H), and 5.53 ppm (m, 2H); its mass spectrum showed ions at *m/e* 479 ($M-OCH_3$), 450 ($M-CH_3CO_2H$), 408 ($M-OCH_3-C_5H_{11}$), 390, 348, and 330.

(5R,6R)-9β-[^3H]-5-Hydroxy-6(9α)-oxy-$PGF_{1\alpha}$(**7**) *and (5S,6S)-9β-[^3H]-5-hydroxy-6(9α)-6(9α)-oxy-$PGF_{1\alpha}$*(**10**). A mixture of 11,15-bis(t-butyldimethylsilyl ether) PGE_2 methyl ester (**2**) and sodium boro[^{3}H]hydride (1.8 mg, specific activity 244 mCi/mmol) in 1 ml of methanol was stirred at −15°C for 1 hr. The reaction was terminated by the addition of 0.5 ml of acetic acid. After evaporation of the solvent, the residue was extracted with ethyl acetate (3 × 5 ml), dried over sodium sulfate, and evaporated to dryness. The residue containing the reaction products (9α and 9β isomers) was purified by tlc (system A). The compound with a mobility corresponding to authentic $PGF_{2\alpha}$

derivative (**3**), 3 mg (220 μCi, specific activity 39.7 mCi/mmol) was isolated. This compound was treated with 1.2 mg of *m*-chloroperbenzoic acid in 0.5 ml of chloroform and 0.5 ml of 0.2 *M* phosphate buffer (pH 7.2) and stirred for 3 hr at room temperature. The reaction mixture was then extracted with 5 ml of ethyl acetate, and the organic layer was washed successively with water and saturated $NaHCO_3$ solution, dried over sodium sulfate, and evaporated to yield 3 mg of an oily residue. To this residue in 1 ml of chloroform was added 100 mg of silica gel (EM tlc plates, Brinkmann), and the mixture was stirred for 18 hr at room temperature. The reaction mixture was then extracted with methanol–methylene dichloride (1 : 1) (5 × 2 ml). After filtration, the organic solvent was evaporated, and the residue was again extracted with methylene dichloride (2 × 2 ml) and evaporated to dryness. The residue was dissolved in 1 ml of acetic acid–water–THF (3 : 1 : 1) and stirred at room temperature for 20 hr. After removal of the solvent, the residue was dissolved in 0.5 ml of dioxane and treated with 20 μl of 1 *N* ethanolic KOH at room temperature for 10 min. The mixture was diluted with 1 μl of water, acidified to pH 2.5, and extracted with ethyl acetate (3 × 2 ml). After the organic layer was dried over sodium sulfate and evaporated, ethereal diazomethane was added to the residue. After reaction, the ether was evaporated. The products were separated by tlc (system B). The bands corresponding to **5** and **8** were eluted to give 25.9 μCi of (5*R*,6*R*)-9β-[^{3}H]-5-hydroxy-6(9α)-oxy-$PGF_{1\alpha}$ methyl ester (**5**) and 37.5 μCi of (5*S*,6*S*)-9β-[^{3}H]-5-hydroxy-6(9α)-oxy-$PGF_{1\alpha}$ methyl ester (**8**) with a specific activity of 39.7 mCi/mmol. The free acids were obtained by hydrolysis of the methyl esters in 1 *N* ethanolic KOH.

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REFERENCES

1. M. Hamberg and B. Samuelsson, *Proc. Nat. Acad. Sci. USA* **70**, 899 (1973).
2. D. H. Nugteren and E. Hazelhof, *Biochem. Biophys. Acta* **326**, 448 (1973).
3. M. Hamberg, J. Svensson, and B. Samuelsson, *Proc. Nat. Acad. Sci. USA* **72**, 2994 (1975).
4. M. Hamberg and B. Samuelsson, *Proc. Nat. Acad. Sci. USA* **71**, 3400 (1974).
5. C. Pace-Asciak, *J. Amer. Chem. Soc.* **98**, 2348 (1976).
6. S. Moncada, R. Gryglewski, S. Bunting, and J. R. Vane, *Nature (London)* **263**, 663 (1976).
7. R. Gryglewski, S. Bunting, S. Mondaca, R. J. Flower, and J. R. Vane, *Prostaglandins* **12**, 685 (1976).
8. S. Moncada, R. Gryglewski, S. Bunting, and J. R. Vane, *Prostaglandins* **12**, 715 (1976).
9. S. Bunting, R. Gryglewski, S. Moncada, and J. R. Vane, *Prostaglandins* **12**, 897 (1976).
10. C. Pace-Asciak and L. S. Wolfe, *Biochemistry* **10**, 3657 (1971).
11. C. Pace-Asciak, M. Nashat, and N. K. Merion, *Biochem. Biophys. Acta* **424**, 323 (1976).
12. P. P. K. Ho, R. G. Herrmann, R. D. Towner, and C. P. Walters, *Biochem. Biophys. Res. Commun.* **74**, 514 (1977).
13. A. Raz, P. C. Isakson, M. S. Minkes, and P. Needleman, *J. Biol. Chem.* **252**, 1123 (1977).
14. P. C. Isakson, A. Raz, S. E. Denny, E. Pure, and P. Needleman, *Proc. Nat. Acad. Sci. USA* **74**, 101 (1977).

15. W. C. CHANG AND S. I. MUROTA, *Biochim. Biophys. Acta* **486**, 136 (1977).
16. W. DAWSON, J. R. BOOT, A. F. COCKERILL, D. N. B. MALLEN, AND D. J. OSBORNE, *Nature (London)* **262**, 699 (1976).
17. R. A. JOHNSON, D. R. MORTON, J. H. KINNER, R. R. GORMAN, J. C. MCGUIRE, F. F. SUN, N. WHITTAKER, S. BUNTING, J. SALMON, S. MONCADA, AND J. R. VANE, *Prostaglandins* **12**, 915 (1976).
18. R. A. JOHNSON, F. H. LINCOLN, J. L. THOMPSON, E. G. NIDY, S. A. MIZAK, AND U. AXEN, *J. Amer. Chem. Soc.* **99**, 4182 (1977).
19. J. FRIED, Intra-Science Symposium "New Biochemical Aspects of Prostaglandins and Thromboxanes," December 1976.
20. E. J. COREY, G. E. KECK, AND I. SZEKELY, *J. Amer. Chem. Soc.* **99**, 2006 (1977).
21. J. FRIED AND G. JUST, *Prostaglandins* **11**, 433 (1976).
22. E. J. COREY AND A. VENKATESWARLU, *J. Amer. Chem. Soc.* **94**, 6190 (1972).
23. G. H. POSNER, D. Z. ROGERS, C. M. KINZIG, AND G. M. GURRIA, *Tetrahedron Lett.*, 3597 (1975).