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Synthesis and Characterization of Prostacyclin, 6-Ketoprostaglandin $F_1\alpha$, Prostaglandin I_1, and Prostaglandin I_3

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Abstract: The key intermediates, (5*S*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**7**) and (5*R*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**8**), have been prepared from the reaction of prostaglandin $F_2\alpha$ methyl ester (**6**) with iodine. The diastereomers, (5*R*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**17**) and (5*S*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**18**), have been prepared from the reaction of 5-*trans*-prostaglandin $F_2\alpha$ methyl ester (**16**) with iodine. Reductive removal of iodine from either **7** and **8** or from **17** and **18** gave (6*R*)-prostaglandin I_1 methyl ester (**15**) and (6*S*)-prostaglandin I_1 methyl ester (**12**), respectively. Compounds **15** and **12** have also been prepared from the reaction of **6** with mercuric acetate followed by reduction with sodium borohydride or from the reaction of 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**) with excess sodium cyanoborohydride. Catalytic reduction of **12** gave (6*S*)-13,14-dihydroprostaglandin I_1 methyl ester (**13**) and (6*S*)-13,14-dihydro-15-deoxyprostaglandin I_1 methyl ester (**14**). The parent PGI_1 structures, (6*R*)-PGI_1 (**23**, mp 97–99 °C) and (6*S*)-PGI_1 (**24**, mp 79–81 °C), have been prepared either by reaction of $PGF_2\alpha$ (**25**) with mercuric acetate followed by sodium borohydride or by saponification of the methyl esters **15** and **12**, respectively. Prostacyclin sodium salt (**32**, PGI_2 sodium salt) has been prepared from iodo ethers **7** or **8**. The reaction of **7** or **8** with 1,5-diazabicyclo[4.3.0]non-5-ene or with potassium superoxide gave PGI_2 methyl ester (**26**) together with traces of (4*E*,6*S*)-Δ^4-prostaglandin I_1 methyl ester (**27**) and (4*E*,6*R*)-Δ^4-prostaglandin I_1 methyl ester (**28**). Treatment of **26** with sodium hydroxide or sodium carbonate gave the desired sodium salt of PGI_2 (**32**). The isomeric (5*E*)-PGI_2 methyl ester (**31**) has been prepared from **18** by reaction with potassium superoxide and, upon saponification, gave (5*E*)-PGI_2 sodium salt (**34**). Δ^6-Prostaglandin I_1 methyl ester diacetate (**43**) together with PGI_2 methyl ester diacetate (**41**) and (5*E*)-PGI_2 methyl ester diacetate (**42**) have been prepared by dehydration of 6-ketoprostaglandin $F_1\alpha$ methyl 11,15-diacetate (**39**). Δ^6-PGI_1 sodium salt (**38**) has been prepared by hydrolysis and saponification of **43**. A radiolabeled sample of enzymatically produced PGI_2 did not contain significant amounts of either (5*E*)-PGI_2 or Δ^6-PGI_1. 6-Ketoprostaglandin $F_1\alpha$ methyl ester (**22**) has been prepared either from **8** via silver ion assisted elimination and hydrolysis or more directly from hydrolysis of **26**. 6-Ketoprostaglandin $F_1\alpha$ (**33**) has been prepared either by hydrolysis of **32** or by saponification of **22**. A convenient assay for the purity of prostacyclin samples has been developed. For this assay, prostacyclin is converted to the *p*-phenylphenacyl ester and analyzed by thin layer chromatography. This derivative of prostacyclin is stable to the chromatography conditions and is cleanly separated from impurities. (5*S*,6*S*)-5-Iodo-*cis*-Δ^{17}-prostaglandin I_1 methyl ester (**55**) and (5*R*,6*R*)-5-iodo-*cis*-Δ^{17}-prostaglandin I_1 methyl ester (**56**) have been prepared from the reaction of prostaglandin $F_3\alpha$ methyl ester (**54**) with iodine. From the major iodo ether **56**, prostaglandin I_3 methyl ester (**57**) was prepared and was converted to prostaglandin I_3 sodium salt (**52**). Hydrolysis of **52** and **57** gave respectively 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ (**53**) and 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ methyl ester (**58**).

The discovery of prostacyclin (originally called prostaglandin X) by Moncada, Gryglewski, Bunting, and Vane[1] has added an exciting new dimension to the role of arachidonic acid metabolism in biology.[2-7] Prostacyclin (**1**), acting via cyclic nucleotide mediation,[8,9] is the most potent inhibitor of platelet aggregation yet discovered as well as being a powerful vasodilator.[1-4] Prostacyclin is derived biosynthetically from arachidonic acid (**2**) by way of the intermediate prostaglandin endoperoxides, PGG_2 and PGH_2 (**3** and **4**).[1] The endoperoxides[10] also are precursors to thromboxane A_2 (**5**), a molecule having biological properties opposite those of prostacyclin.[11] Thromboxane A_2 is a potent inducer of platelet aggregation as well as being a powerful vasoconstrictor. The biosynthesis of prostacyclin in the circulatory system is concentrated within the vascular walls,[2] whereas biosynthesis of thromboxane A_2 is concentrated in the platelets.[11] The chemical properties of these two compounds are such that both are susceptible to rapid hydrolysis and, consequently, inactivation. Nature appears to have devised in this delicately balanced system a mechanism whereby blood vessels are protected under normal conditions from the harmful deposition of platelet aggregates, but, in case of injury, are able to promptly form such aggregates in the area of damage.

The structure of prostacyclin has been determined and is

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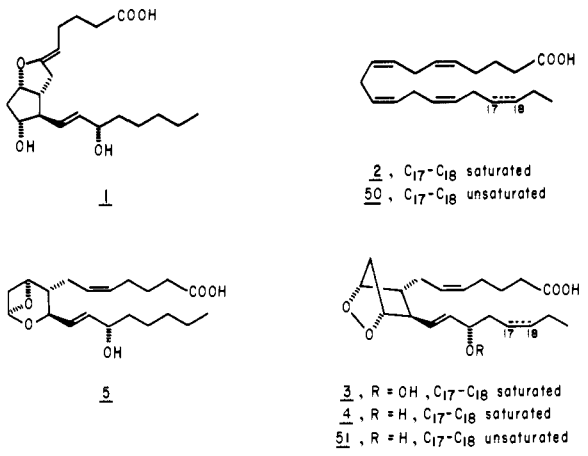


defined by formula **1**.[12] The structure proof depended upon comparison of the biological and chemical properties of natural prostacyclin with those of the sodium salt of synthetic **1**. Our synthesis of prostacyclin sodium salt has been reported in preliminary form.[13] Several other groups have also reported syntheses of prostacyclin salts[14] or derivatives,[15] but none has compared their synthetic material with an authentic sample of natural prostacyclin. We now report details of (a) the synthesis of several examples of the prostaglandin I_1 (PGI_1) series, which serve both as precursors to prostacyclin and as chemically stable analogues of prostacyclin, (b) the synthesis of prostacyclin sodium salt and other closely related molecules, (c) the synthesis of Δ^6-prostaglandin I_1, a double bond isomer of prostacyclin, (d) the synthesis of 6-ketoprostaglandin $F_1\alpha$, the hydrolysis product of prostacyclin, (e) an assay for determining the purity of prostacyclin samples, and (f) the synthesis of prostaglandin I_3 (PGI_3).

Prostaglandin I_1 (PGI_1). The 5,6-dihydroprostacyclin structure has been defined as prostaglandin I_1 with the epimeric C-6 center designated by absolute configuration as 6*R* or 6*S* [e.g., (6*S*)-PGI_1].[16] We have found that compounds of this type may be easily prepared from the $PGF_2\alpha$ series.

In our first experiments, prostaglandin $F_2\alpha$ methyl ester (**6**) was found to react with iodine in the presence of solid sodium carbonate to give the two cyclization products **7** and **8** in yields of 1 and 40%, respectively. Unreacted starting material (54%) also was recovered from the reaction. When attempts were made to increase the conversion of **6** to the iodo ethers **7** and **8** by adding more iodine, the reaction instead gave a complex mixture of products in which the yield of **7** and **8** was greatly diminished. Other modifications, however, significantly improved the yield of **7** and **8** from the reaction. For example, reaction of **6** with aqueous iodine/iodide in the presence of sodium carbonate gave a total 87% yield of **7** and **8** in a ratio of 1:12.4. Whittaker has also reported a modification that gives **7** and **8** in greater than 90% yield.[14b]

The structure of the major diastereomer **8** was determined in the following way. The mass spectrum (see Experimental Section) showed the addition of one iodine atom and the apparent loss of one hydroxyl functionality during the reaction, since the compound formed only a bis(trimethylsilyl) ether. The nuclear magnetic resonance spectrum of the compound (**8**) has only two vinyl protons, indicating that one of the double bonds of the starting prostaglandin $F_2\alpha$ methyl ester (**6**) was lost during the reaction. The hydroxyl group at C-9 was shown to be involved by the fact that prostaglandin $F_2\alpha$ methyl ester 11,15-bis(tetrahydropyranyl) ether (**9**) also formed an iodo ether (**10**), which after removal of the tetrahydropyranyl groups was identical with iodo ether **8**. The C_5-C_6 double bond was shown to be involved by the fact that prostaglandin $F_1\alpha$ methyl ester (**11**) *did not* react with iodine under the same conditions used to prepare **7** and **8** from **6**. The evidence presented to this point allows the conclusion that ether formation has occurred between the C-9 hydroxyl group and either carbon 5 or 6 with the iodine attached at the other carbon. Precedent for haloetherification reactions of this type exists in the chemical literature.[17]

Reductive removal of iodine from **8** was achieved with tri-*n*-butyltin hydride.[18] The resultant "dihydroprostacyclin" or (6*S*)-prostaglandin I_1 (**12**, mp 43 °C) was further reduced with hydrogen over platinum to produce two new compounds, **13** and **14**. From combined gas chromatography-mass spectral (GC-MS) analysis of the trimethylsilyl ether derivatives of **13** and **14**, it was clear that the more polar product resulted from reduction of the C_{13}-C_{14} double bond and therefore must have structure **13**. Likewise, the mass spectrum of the less polar product was consistent with an assignment of structure **14** in which hydrogenolysis of the allylic alcohol followed by reduction of the C_{13}-C_{14} double bond has occurred. The mass spectrum of **14**-Me_3Si contained strong signals at *m/e* 311 and 221, consistent with cleavage of the upper, carboxyl containing

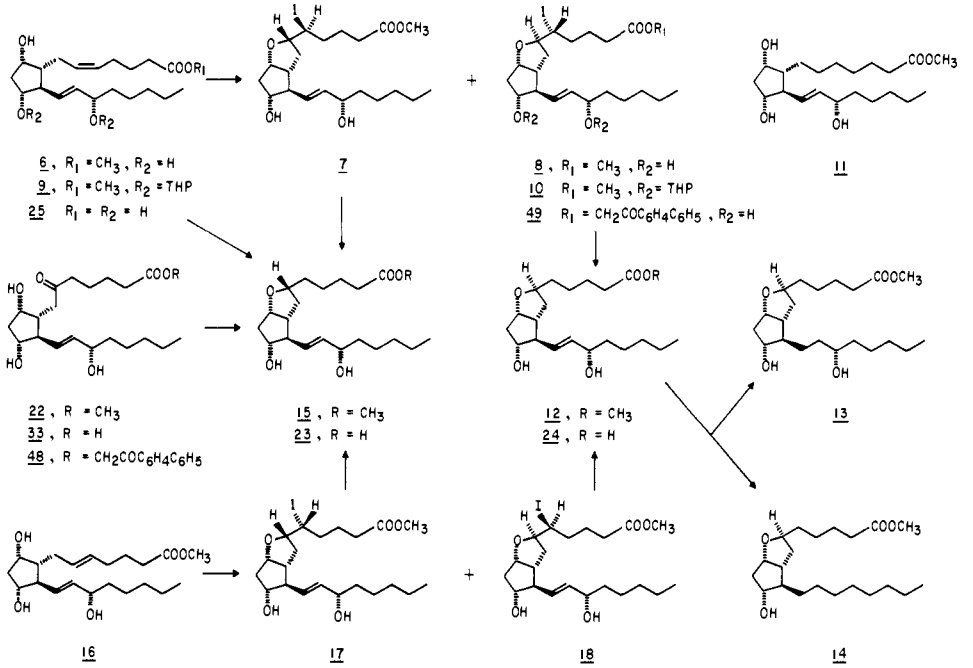


Table I. Chemical Shifts for C-9 Protons in the NMR Spectra of PGI_1 Isomers Having the 6-Exo Configuration

compd	chemical shift, δ	compd	chemical shift, δ
8	4.55	**24**	4.45
12	4.46	**30**	4.47
12-*d*	4.45	**36**	4.52
18	4.52	**49**	4.56
21	4.56	**56**	4.54

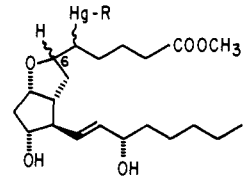
side chain between carbons 5 and 6 upon electron impact. Such a cleavage requires that the ether oxygen be attached at C-6 and, consequently, the iodine must be at C-5 in compound **8**. Reductive removal of iodine from minor iodo ether **7** gave the isomeric (6*R*)-prostaglandin I_1 (**15**, mp 71-73 °C). The possibility that iodo ether **7** was the result of cyclization between C_5 and C_9-OH can be ruled out by the fact that, as discussed in a following section, **7** can serve as a precursor to prostaglandin I_2 methyl ester. In the nomenclature developed by Baldwin, the reaction giving **7** and **8** is an example of a 5-exo-trig ring closure.[19]

Turning now to the configurations at C-5 and C-6 in iodo ethers **7** and **8**, there are four diasteromeric structures possible. If we assume that iodo ether formation proceeds via a trans mode of addition[17] and that once the iodine substituent is attached it does not undergo further displacement by iodide, then the configurations 5*R*,6*R* and 5*S*,6*S* are possible for **7** and **8**. Examination of Dreiding molecular models reveals that the molecular conformation leading to the 5*R*,6*R* isomer should be favored because of fewer serious steric interactions during the cyclization reaction. This then leads to the prediction that the major iodo ether **8** should have the 5*R*,6*R* configuration while the minor iodo ether **7** should have the 5*S*,6*S* configuration.[13]

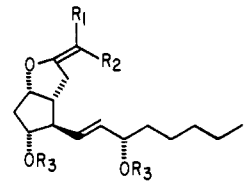
If the preceding rationale is correct, then the reaction of 5-*trans*-prostaglandin $F_{2\alpha}$ methyl ester (**16**) with iodine should lead to one or both of the two diasteriomeric iodo ethers not prepared above. Accordingly, when 5-*trans*-prostaglandin $F_{2\alpha}$ methyl ester (**16**)[20] was allowed to react with iodine in the presence of sodium carbonate, two new iodo ethers, **17** and **18**, were isolated in yields of 10 and 62%, respectively. Reductive removal of iodine from **17** and **18** gave (6*R*)-prostaglandin I_1 methyl ester (**15**) and (6*S*)-prostaglandin I_1 methyl ester (**12**), respectively. The minor iodo ether **17** should be, therefore, the 5*R*,6*S* diastereomer and the major iodo ether **18** should be the 5*S*,6*R* diastereomer.

Confirmation that the preceding predictions were correct was obtained from two sources. First, Nelson has unequivocally assigned the 6*S* configuration to the prostaglandin I_1 methyl ester isomer (**12**) having mp 43 °C.[21] Consequently, the prostaglandin I_1 methyl ester isomer (**15**) having mp 71-73 °C must have the 6*R* configuration. Since the iodo ethers have all been correlated with **12** or **15**, their configurations at C-6 are thereby established and are as predicted. Second, as discussed in a following section, elimination of the elements of HI from these iodo ethers clearly proceeds by a trans elimination mechanism and leads to olefins of defined stereochemistry. The stereochemical requirements of these results confirm the assignments of configuration given to C-5 of the iodo ethers.

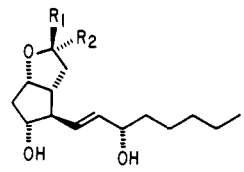
An alternate route to compounds **12** and **15** was found in the reaction of prostaglandin $F_{2\alpha}$ methyl ester (**6**) with mercuric acetate followed by reductive removal of the mercury substituent with alkaline sodium borohydride.[22,23] This reaction sequence gave **15** and **12** in a total yield of 37% and a ratio of about 1:2. The intermediate mercuriacetates (**19**) can be isolated, if desired, and used for other purposes.[24] Reaction of **19** in methanol with aqueous sodium chloride[22,25] gives the corresponding mercurichlorides (**20** and **21**) which partially separate on the basis of C-6 configuration when chromatographed over silica gel.

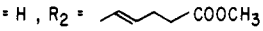
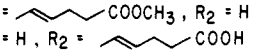
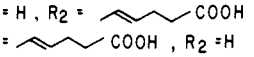
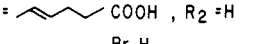


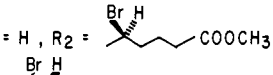
19, R = OAc
20, R = Cl, Beta H_6
21, R = Cl, Alpha H_6



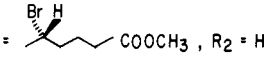
26, $R_1 = (CH_2)_3COOCH_3$, $R_2 = R_3 = H$
31, $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOCH_3$
32, $R_1 = (CH_2)_3COONa$, $R_2 = R_3 = H$
34, $R_1 = R_3 = H$, $R_2 = (CH_2)_3COONa$
41, $R_1 = (CH_2)_3COOCH_3$, $R_2 = H$, $R_3 = Ac$
42, $R_1 = H$, $R_2 = (CH_2)_3COOCH_3$, $R_3 = Ac$
47, $R_1 = (CH_2)_3COOCH_2C_6H_4C_6H_5$, $R_2 = R_3 = H$



27, $R_1 = H$, R_2 =  COOCH3
28, R_1 =  COOCH3, $R_2 = H$
29, $R_1 = H$, R_2 =  COOH
30, R_1 =  COOH, $R_2 = H$



35, $R_1 = H$, R_2 =



36, R_1 = , $R_2 = H$

We have observed a consistent difference between the NMR spectra of compounds in the (6*S*)-PGI_1 series and of compounds in the (6*R*)-PGI_1 series.[13] This difference may be used for the empirical assignment of configuration to new analogues having the PGI_1 structure. As summarized in Table I, all compounds that have been stereochemically related to (6*S*)-PGI_1 (i.e., the carboxylic acid side chain has the β or exo configuration) have a broad multiplet (appearing as a poorly defined quartet in most spectra obtained at 60 MHz) in the range of δ 4.45-4.55. This distinct signal is absent in the (6*R*)-PGI_1 isomers and presumably is grouped at higher field with the other signals found in that region. Perhaps least likely to give this signal are the protons at C-11 and C-15. Both are

in an environment similar to the C-11 and C-15 protons of the classical prostaglandins, which generally do not have a signal in this region of their NMR spectra. Our first inclination was to believe that the C-6 proton was giving this signal since this is the position most different in the two isomer series. However, the fact that the coupling for this signal is exactly the same whether or not there is a substituent, such as iodine, at C-5 suggested that the C-6 proton could not be giving this signal. This was confirmed as follows.

By using deuterated reagents in the reaction of 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**) with sodium cyanoborohydride to prepare **12** and **15,** it should be possible to have the C-6 proton replaced by deuterium in these products. This was done and the NMR spectrum obtained from **12-*d*** (>90% incorporation of one deuterium) retained the signal at δ 4.45. From this we conclude that it is not the proton at C-6 but most likely the proton at C-9 that is giving the characteristic NMR signals for the (6*S*)-PGI series of compounds.

The oxymercuration reaction could be reversed simply by treatment of the mercuriacetates with aqueous hydrochloric acid in methanol. The regenerated $PGF_2\alpha$ was entirely of cis configuration at the C-5 double bond. Reaction of iodo ether **8** with zinc in acetic acid also served to convert this material into prostaglandin $F_2\alpha$ methyl ester and 5-*trans*-prostaglandin $F_2\alpha$ methyl ester (3:2).

The parent molecules of the PGI_1 series, (6*R*)-PGI_1 (**23**) and (6*S*)-PGI_1 (**24**), can be prepared easily either by saponification of the corresponding methyl esters, **15** and **12,** or by application of the oxymercuration reaction to $PGF_2\alpha$ (**25**). By the latter method, **23** and **24** are obtained in a total yield of 83% and in a ratio of 1:2.6. Both compounds are crystalline, **23** having mp 97-99 °C and **24** mp 79-81 °C.

Synthesis of Prostacyclin (PGI_2). The synthesis of prostaglandin I_2 methyl ester (**26**) from iodo ether **7** or **8** via base-catalyzed elimination of HI can be achieved with a variety of bases. Particularly convenient is the reaction of **8** with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in benzene or toluene at 40 °C. From this reaction, essentially pure **26** can be obtained in 90% yield by simply washing the reaction solution with ice-water, drying, concentrating, and crystallizing from ether-hexane at −10 °C. The only impurities detected in the product obtained under these conditions are traces of (6*S*)- and (6*R*)-Δ^4-prostaglandin I_1 methyl esters (**27** and **28**). The reaction under these conditions is slow, requiring 30 h or more to reach completion. The progress of the reaction can be easily followed by taking advantage of the reactivity of **26** to acidic conditions. By simply placing a sample of **26** on a silica gel TLC plate and then developing the plate in the acidic A-IX solvent system,[27] **26** is completely converted to 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**). The latter is a much more polar compound than **26** and is clearly separated from any unchanged starting iodo ether **8** under these conditions.

Reaction of iodo ether **8** with potassium superoxide (KO_2) in dimethylformamide containing dicyclohexyl-18-crown-6[28] also led to the synthesis of **26**. Under these conditions, the reaction is complete within minutes, but chromatographic purification is required to remove the crown ether from the product. Following purification by chromatography over Florisil, this reaction gave **26** in 41% yield.

Chromatography of **26** must be done with care. As noted above, development of silica gel TLC plates in the acidic A-IX[27] systems results in complete conversion of **26** to 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**). TLC of **26** on silica gel in neutral solvents can be done successfully if the TLC plate is developed immediately after spotting. Pretreatment of the silica gel plate with triethylamine or addition of triethylamine to the solvent system further protects **26** from hydrolysis during chromatography. Column chromatography of **26** on silica gel has not been successful in our experience, even when triethylamine was added to the solvent. 6-Ketoprostaglandin $F_1\alpha$ methyl ester (**22**) was isolated from such attempted chromatography. Column chromatography over Florisil has been successful and has been used when chromatographic purification was necessary.

Prostaglandin I_2 methyl ester (**26**) is obtained as a soft, crystalline compound (mp ~30-33°C) after recrystallization from ether-hexane. Recrystallization removes traces of by-products **27** and **28**. Compounds **27** and **28** have been isolated as the free acids, (4*E*,6*S*)-Δ^4-PGI_1 (**29**) and (4*E*,6*R*)-Δ^4-PGI_1 (**30**),[29] following acidification of the mother liquors from large-scale preparations of PGI_2 sodium salt (see Experimental Section for details). The total yield of **29** and **30** is 6% and in a ratio of 1:3.

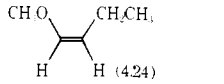
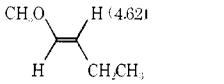
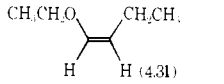
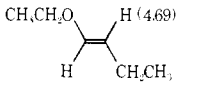
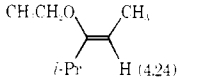
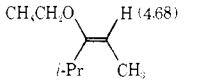
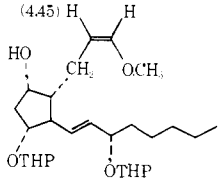
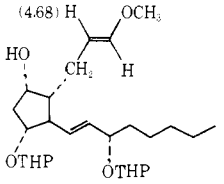
The structures of **29** and **30** follow from their NMR spectral properties and their mode of formation. The presence of two additional vinyl protons in the NMR spectra of the compounds indicates the presence of new, disubstituted olefinic bonds. The compounds are stable and it is reasonable to assume that elimination of HI from carbons 4 and 5 in compounds **7** and **8** has led to these minor products. It also is reasonable to assume that the elimination will have occurred in a trans manner, giving olefins of trans configuration. The presence of a signal at δ 4.48 in the NMR spectrum of **30** is consistent with the assignment of the 6*R* configuration to this product. Compound **29** then must have the 6*S* configuration.

If we assume that **26** is formed via trans elimination of HI from **8,** then the enol ether double bond in **26** should have the 5*Z* configuration. We have confirmed this in the following way. The isomeric iodo ether **18,** obtained from 5-*trans*-prostaglandin $F_2\alpha$ methyl ester (**16**), was subjected to the elimination reaction with potassium superoxide. A new compound, mp 68-70 °C, was obtained from the reaction following workup, chromatography over Florisil, and crystallization and it was assigned the structure (5*E*)-prostaglandin I_2 methyl ester (**31**). Rapid hydrolysis of **31** to **22** confirmed the presence of the enol ether functionality and its stereochemistry was revealed by NMR spectroscopy. In compound **26** the C-5 vinyl proton is at δ 4.16 while in **31** it is at δ 4.67. The downfield signal must be assigned to the proton cis to the vinyl ether oxygen because of the deshielding effect of the latter atom. Therefore, **26,** and **31** must have the 5*Z* and 5*E* configurations, respectively. Such downfield shifts of cis vinyl ether protons relative to the trans isomers are amply illustrated by examples from the literature, some of which are summarized in Table II.[30]

Prostacyclin sodium salt (**32**) is easily prepared from PGI_2 methyl ester (**26**) by saponification with, for example, sodium hydroxide or sodium carbonate in aqueous methanol. Isolation by lyophilization or by crystallization from acetonitrile-water gives prostacyclin sodium salt (**32**) having biological properties identical with those of natural prostacyclin.[12] Recrystallized samples of prostacyclin sodium salt are essentially pure by various assay techniques (see below for one chemical method), but may contain traces of water and sodium carbonate that are exceedingly difficult to remove. Attempts have been made to isolate the free acid from prostacyclin sodium salt (see Experimental Section) but so far it has not been possible to obtain a sample free of 6-ketoprostaglandin $F_1\alpha$ (**33**). Prostacyclin sodium salt (**32**) is completely hydrolyzed to **33** when a sample is applied to a silica gel TLC plate and the plate is developed in the acidic A-IX[27] solvent system. The sodium salt is, of course, converted to the free acid immediately upon contact with the acid solvent. We have also examined the chromatographic behavior of **32** in the acidic systems used by Pace-Asciak and Wolfe in their work with incubations of arachidonic acid with rat stomach homogenates[31] and find that **32** is completely converted to **33** in these systems as well.

We have further compared radiolabeled PGI_2 methyl ester, obtained by the enzymatic conversion of PGH_2 into prosta-

Table II. Chemical Shift Data for Vinyl Ether Protons

proton trans to oxygen	proton cis to oxygen	ref
		*a*
		*b*
		*b*
		*c*

[a] Reference 34. [b] Reference 33. [c] E. G. Nidy and R. A. Johnson, *J. Org. Chem.*, **40**, 1415 (1975).

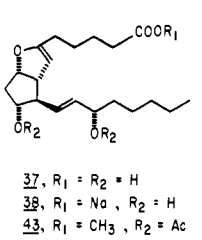
cyclin followed by esterification,[12] with **26** and **31** by TLC. Compounds **26** and **31** have R_f values of 0.69 and 0.65, respectively, on silica gel plates developed in 1:1 acetone-hexane. When the radiolabeled sample is applied to the plate in a mixture with **26** and **31**, the radioactivity moves almost exclusively (>95%) with **26** (see Table III, Experimental Section).

The sodium salt of (5*E*)-PGI_2 was prepared by saponification of the methyl ester **34**. Significantly, the ability of this compound to inhibit PGH_2-induced aggregation of platelets in human platelet rich plasma was only 20% ($\frac{1}{5}$) of that of prostacyclin sodium salt (**32**).[32]

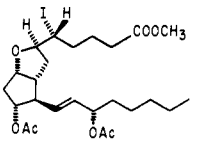
We have briefly examined the synthesis of PGI_2 methyl ester (**26**) via the intermediate bromo ethers **35** and **36**.[14a] The reaction of prostaglandin $F_2\alpha$ methyl ester (**6**) with *N*-bromosuccinimide in methylene chloride gave (5*S*,6*S*)-5-bromoprostaglandin I_1 methyl ester (**35**) and (5*R*,6*R*)-5-bromoprostaglandin I_1 methyl ester (**36**) in a total yield of 60% and a ratio of about 1:9. The isomer **36** is assigned the 5*R*,6*R* configuration on the basis that (a) it is the major product from the reaction, (b) it is the more polar of the two products on silica gel TLC and (c) it gives the characteristic NMR signal for the C_9 proton at δ 4.52. Elimination of HBr from a sample of the 1:9 mixture of **35** and **36** with DBN gave **26** together with a small amount of the Δ^4 isomers **27** and **28** (the latter detected by TLC).

Δ^6-Prostaglandin I_1. A third, isomeric variant of the enol ether structure of prostacyclin is the molecule Δ^6-prostaglandin I_1 (**37**). The possible existence of this substance should be considered for several reasons. First, the equlibration of enol ether isomers catalyzed by either acids[33] or iodine[34] is well established and there is the possibility that such catalysis could also occur enzymatically. Secondly, the possibility that Δ^6-PGI_1 is the structure of a prostaglandin first isolated by Pace-Asciak and Wolfe in 1971[31] has recently been suggested by Sih and colleagues.[35] We describe below the synthesis of a Δ^6-PGI_1 derivative that allows us to determine if Δ^6-PGI_1 is present in biosynthetic samples of prostacyclin and from which Δ^6-PGI_1 sodium salt (**38**) has been obtained.[36]

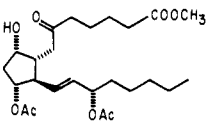
We were curious as to whether or not the hydration of prostacyclin leading to 6-ketoprostaglandin $F_1\alpha$ (see following section) could be reversed in order to permit synthesis of prostacyclin (or isomers) from the latter substance. In order to examine this possibility we prepared 6-ketoprostaglandin $F_1\alpha$ 11,15-diacetate methyl ester (**39**) in the following way.



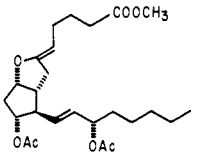
37, $R_1 = R_2 = H$
38, $R_1 = Na$, $R_2 = H$
43, $R_1 = CH_3$, $R_2 = Ac$



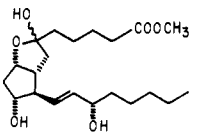
40



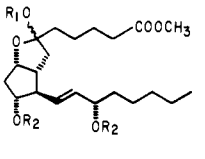
39



41



44



45, $R_1 = CH_3$, $R_2 = H$
46, $R_1 = R_2 = Si(CH_3)_3$

Acetylation of iodo ether **8** gave **40**. The reaction of **40** with DBN gave PGI_2 diacetate methyl ester (**41**), which upon hydrolysis gave the desired diacetate **39**.

When diacetate **39** was heated in refluxing benzene for 2 h with concomitant removal of water, the formation of three new compounds was detected by TLC (silica gel, 25% ethyl acetate in hexane). The properties of these three compounds clearly indicated that they were the three enol ethers, **41, 42,** and **43**. All three gave nearly identical mass spectra and each was readily hydrolyzed to 6-ketoprostaglandin $F_1\alpha$ 11,15-diacetate methyl ester (**39**). Furthermore, samples of the individual products were each isomerized to the same mixture containing all three by a dilute solution of iodine in hexane.[34] The most polar (TLC) of the three products had the same R_f as (5*E*)-PGI_2 diacetate methyl ester (**42**), which was prepared by acetylation of **31**. The product of intermediate polarity had the same R_f as PGI_2 diacetate methyl ester (**41**). The remaining, least polar, product is assigned the structure Δ^6-PGI_1 diacetate methyl ester (**43**). This assignment is confirmed by the presence of a signal for an enol ether proton at δ 4.66 (doublet, J = 2.5 Hz) in the NMR spectrum of **43**. The infrared spectrum of **43** has an absorption band for the enol ether double bond at 1660 cm^{-1}.

The possibility that enzymatically prepared prostacyclin may contain Δ^6-PGI_1 was then examined. A sample of radiolabeled prostacyclin methyl ester prepared from PGH_2 by incubation with hog aorta microsomes and then esterification[12] was acetylated. The radiolabeled material was compared by TLC with **41, 42,** and **43**. As shown in Table IV (see Experimental Section), less than 1% of the radioactivity migrated with an R_f the same as that of Δ^6-PGI_1 diacetate methyl ester (**43**). We conclude that incubation of PGH_2 with hog aorta microsomes does not produce Δ^6-PGI_1.

Δ^6-PGI_1 sodium salt (**38**) was prepared from **43**. The addition of 1 equiv of sodium methoxide to **43** in methanol served to remove the acetate groups by catalysis of ester exchange. Then, the addition of water to the reaction resulted in saponification of the methyl ester. Removal of solvent gave the sodium salt **38**.

6-Ketoprostaglandin $F_1\alpha$. 6-Ketoprostaglandin $F_1\alpha$ (**33**) was first described by Pace-Asciak as an enzymatic product derived from arachidonic acid and PGH_2 by incubation with rat stomach homogenates.[37] Other groups have subsequently reported finding **33** present in a variety of biological tissues.[38]

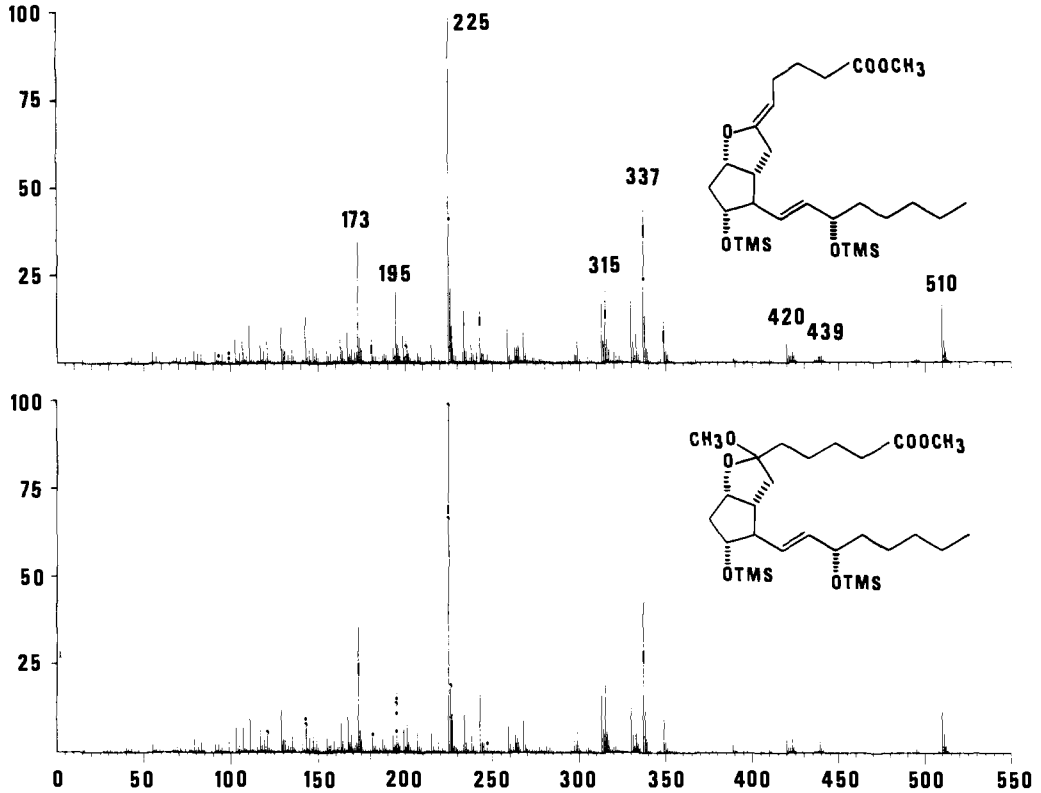


Figure 1. Top: mass spectrum of prostacyclin methyl ester bis(trimethylsilyl) ether. Bottom: mass spectrum of 6-methoxyprostaglandin I_1 methyl ester bis(trimethylsilyl) ether.

The discovery that prostacyclin is (a) the key biosynthetic intermediate between PGH_2 and **33** and (b) rapidly hydrolyzed to **33** has provided an explanation for the existence of **33** in these different locations. The assumption is now often made that if **33** is found in a biological system, then prostacyclin also was present in that system. There is, as yet, no evidence for formation of **33** by any means other than through the intermediacy of prostacyclin. We describe here the chemical synthesis of 6-ketoprostaglandin $F_1\alpha$ (**33**).

6-Ketoprostaglandin $F_1\alpha$ methyl ester (**22**) was first synthesized directly from iodo ether **8** by reaction with silver carbonate in aqueous acid. The compound **22** can also be easily prepared from prostacyclin methyl ester (**26**) by hydrolysis as has been mentioned above. The compound **22** crystallizes with difficulty and even after repeated recrystallization it does not attain a sharp melting point (68–74 °C). The methyl ester of **22** may be removed by alkaline hydrolysis and, following acidification, 6-ketoprostaglandin $F_1\alpha$ (**33**) is obtained as a crystalline product. Compound **33,** like methyl ester **22,** also fails to melt sharply (97–100 °C) even after repeated recrystallizations. The failure of these compounds to melt sharply is likely the result of an equilibrium between the keto alcohol form of the molecules and the hemiketal form (**44**). The hemiketal (**44**) has been captured and isolated as the methyl ketal **45** by reaction with methanol under acid catalysis.

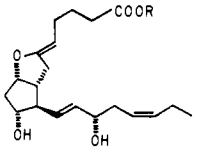
The mass spectra of various derivatives of 6-ketoprostaglandin $F_1\alpha$ are important to the identification of this compound in biological extracts. The spectra of a number of such derivatives have already been reported in the literature, including those of 6-ketoprostaglandin $F_1\alpha$ methyl ester methyloxime tris(trimethylsilyl) ether,[39] 6-ketoprostaglandin $F_1\alpha$ methyl ester *n*-butyloxime tris(trimethylsilyl) ether,[39c] and 6-ketoprostaglandin $F_1\alpha$ methyl ester tris(trimethylsilyl) ether.[12] The fact that the hemiketal form may be derivatized with a Me_3Si group has also been reported and illustrated.[12,39d] The mass spectrum of this Me_3Si derivative (**46**) is identical with that of prostacyclin methyl ester bis(trimethylsilyl) ether (shown in Figure 1). We presume that Me_3SiOH is easily lost from the C-5 and C-6 positions of **46** during electron impact in the mass spectrometer with the result that prostacyclin methyl ester bis(trimethylsilyl) ether is formed as a transient intermediate. It is of interest that the bis(trimethylsilyl) ether of the methyl ketal **45** also gives a mass spectrum (on the LKB mass spectrometer) that is identical with that of prostacyclin methyl ester bis(trimethylsilyl) ether (see Figure 1). The molecular ion has been detected, however, in the high-resolution mass spectrum of **45** (see Experimental Section). Because of the propensity for these derivatives to fragment to prostacyclin methyl ester bis(trimethylsilyl) ether in the mass spectrometer, the interpretation of the ensuing spectra must be made with care.

Assay for Purity of Prostacyclin. Because of the sensitivity of prostacyclin to hydrolysis and the resulting uncertainty as to the purity of various samples, a simple analysis based on thin layer chromatography has been developed. A sample of the sodium salt of prostacyclin is converted to the *p*-phenylphenacyl derivative (**47**) by reaction with excess α-bromo-*p*-phenylacetophenone in the presence of diisopropylethylamine. Any contaminating 6-ketoprostaglandin $F_1\alpha$ is also converted to its *p*-phenylphenacyl ester (**48**) under these conditions as are other possible impurities such as the Δ^4-PGI_1 isomers **29** and **30**. These derivatives are stable to the conditions of silica gel TLC analysis and furthermore are easily separated from one another in this form. Examination of the derivatized prostacyclin sample by TLC thereby provides a convenient measurement of the purity of the sample.

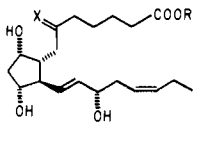
Derivatization of a pure sample of prostacyclin sodium salt results in the formation of a pure sample of **47**. This control experiment demonstrates that prostacyclin is stable to the derivatization procedure. Pure **47** has also been obtained from dehydroiodination of derivative **49,** followed by purification and crystallization.

Prostaglandin I_3. The role of the endoperoxides PGG_2 and PGH_2 as biosynthetic intermediates between arachidonic acid

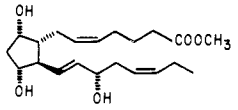
and prostaglandins of the "2 series" is clearly established. The existence of the analogous endoperoxide, PGH_3, is implied by the presence of prostaglandins of the "3 series", such as PGE_3[40] and $PGF_3\alpha$,[41] in mammalian systems. In fact, a crude preparation of the enzyme that converts arachidonic acid into PGH_2 can also convert all *cis*-5,8,11,14,17-eicosapentaenoic acid (**50**) into PGH_3 (**51**).[42] By analogy, the enzyme(s) that convert PGH_2 into prostacyclin may also be able to convert PGH_3 into PGI_3 (prostaglandin I_3). We have carried out a synthesis of PGI_3 sodium salt (**52**) and the related hydrolysis product 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ (**53**) as described below.[43]



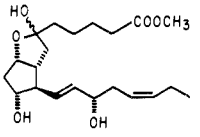
52, R = Na
57, R = CH_3



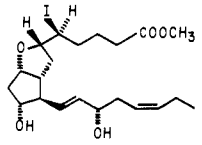
53, R = H, X = O
58, R = CH_3, X = O
60, R = CH_3, X = $NOCH_3$



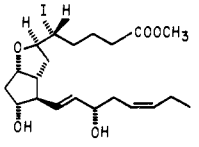
54



59



55



56

The same synthetic approach used for the synthesis of prostacyclin was used for the preparation of PGI_3. The reaction of prostaglandin $F_3\alpha$ methyl ester (**54**)[44] with iodine proceeded smoothly and with no involvement of the C-15 hydroxyl group in a possible cyclization with the 17,18-olefinic functionality. The reaction gave two iodo ethers, **55** and **56**, in a 59% total yield and in a ratio of 1:30. By analogy to the iodocyclization reaction with prostaglandin $F_2\alpha$ methyl ester, the minor and major products here are assigned the structures of (5*S*,6*S*)-5-iodo-*cis*-Δ^{17}-prostaglandin I_1 methyl ester (**55**) and (5*R*,6*R*)-5-iodo-*cis*-Δ^{17}-prostaglandin I_1 methyl ester (**56**), respectively. Supporting these assignments is the presence of a broad quartet (J = 6 Hz) for the C-9 proton at δ 4.54 in the NMR spectrum of **56** and the absence of such a signal in the spectrum of **55**. The NMR spectra also show clearly that both compounds retain the 17,18-olefinic group because the signal for the terminal C-20 methyl group is a much sharply defined triplet under these conditions.

Reaction of major iodo ether **56** with DBN in benzene, followed by chromatographic purification over Florisil, gave prostaglandin I_3 methyl ester (**57**) in 62% yield. The exact position of the C-5 vinyl proton in the NMR spectrum of **57** could not be determined because of other overlapping signals. It was clear, however, that this signal was not between δ 4.5 and 5.0, where it would be expected (see above discussion of (5*E*)-PGI_2 methyl ester) if **57** had the *E* configuration. A weak absorption band for the enol ether in **57** was present at 1695 cm^{-1} in the infrared spectrum. The presence of the enol ether group also was apparent from the ease with which **57** was hydrolyzed to a more polar product (**58**). This product was characterized as 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ methyl ester (**58**).

Prostaglandin I_3 sodium salt (**52**) was prepared from **57** by saponification with 1 equiv of sodium hydroxide. Lyophilization gave **52** as a white powder having infrared absorption at 1690 cm^{-1} for the enol ether functionality.

Hydrolysis of sodium salt **52** in 1:1 tetrahydrofuran–pH 1.5 buffer gave 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ (**53**). Likewise, hydrolysis of methyl ester **57** gave 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ methyl ester (**58**). The latter compound was particularly susceptible to a mobile equilibrium, either neat or in solution, that produced many products upon TLC examination. This mixture presumably included the hemiketal **59**. This equilibrium mixture reverted almost exclusively to **58** upon treatment with the above-mentioned hydrolysis conditions. Because of this tendency to form other products, **58**, was characterized as the methoxime derivative **60**. The mass spectrum of this derivative, as the tris(trimethylsilyl) derivative, has been presented previously.[43]

Summary

(a) The stereospecific synthesis of prostacyclin (PGI_2) sodium salt from $PGF_2\alpha$ methyl ester in three steps is presented. Prostacyclin free acid may be obtained from the sodium salt, but to do so without contamination by 6-ketoprostaglandin $F_1\alpha$ is exceedingly difficult. A chemical assay that can be used to determine the purity of prostacyclin samples is presented.

(b) Prostacyclin is rapidly hydrolyzed to 6-ketoprostaglandin $F_1\alpha$. Thin layer chromatography (silica gel) of prostacyclin in acidic solvent systems results in complete conversion to 6-ketoprostaglandin $F_1\alpha$. These results contradict the claims that prostacyclin may be purified by thin layer chromatography with acidic solvents.

(c) The synthesis and characterization of 6-ketoprostaglandin $F_1\alpha$, the hydrolysis product of prostacyclin, is presented. 6-Ketoprostaglandin $F_1\alpha$ exists in equilibrium with the hemiketal, 6-hydroxyprostaglandin I_1. The latter may be derivatized or trapped as the Me_3Si or methyl ether. Mass spectra of these derivatives are identical with the mass spectrum of prostacyclin (suitably derivatized).

(d) Various examples of the prostaglandin I_1 ("dihydroprostacyclin") nucleus have been prepared by synthesis. In conjunction with Nelson's unequivocal assignment of configuration to (6*R*)- and (6*S*)-PGI_1, we have presented an empirical method, based on NMR, for the assignment of configuration to related molecules.

(e) The double bond isomers of prostacyclin, (5*E*)-PGI_2 and Δ^6-PGI_1, have been prepared by synthesis. Comparison with radiolabeled prostacyclin, obtained biosynthetically from hog aorta microsomes, reveals that these two isomers are not produced in the hog aorta.

(f) In anticipation of the possibility that PGI_3 may be produced enzymatically, we have prepared this substance by synthesis. The hydrolysis product of PGI_3, 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$, has been characterized.

Experimental Section

General. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were recorded with either a Perkin-Elmer Model 137 or a Digilab Model FTS-14D spectrophotometer as Nujol mulls or as neat liquids between salt plates. The ^1H NMR spectra were obtained with a Varian A-60A or a Varian XL-100 spectrometer as solutions in chloroform with tetramethylsilane as an internal standard unless indicated otherwise. The ^{13}C NMR spectra were obtained with a Varian CFT-20 spectrometer in chloroform solution and are reported in parts per million from tetramethylsilane. First-order analyses of the NMR spectra are given. High-resolution mass spectra were obtained with a CEC 21-110B spectrometer. Other mass spectra were obtained with a Model 9000 LKB gas chromatograph–mass spectrograph at 70 eV. Specific rotations were determined at 25 °C. Brine refers to a saturated aqueous solution of sodium chloride.

(5*S*,6*S*)-5-Iodoprostaglandin I_1 Methyl Ester (7) and (5*R*,6*R*)-5-

Iodoprostaglandin I_1 Methyl Ester (8). A. To a stirred solution of 5.0 g (13.5 mmol) of prostaglandin $F_2\alpha$ methyl ester (**6**) in 250 mL of methylene chloride was added 3.05 g of anhydrous sodium carbonate. The mixture was cooled in an ice bath to 5 °C and treated portionwise during 1 min with 3.44 g (13.5 mmol) of iodine. The reaction was allowed to proceed for 50 min, then quenched with the addition of 125 mL of a 10% aqueous solution of sodium sulfite. The organic phase was separated, and the water layer washed once with chloroform. The combined organic extract was washed with brine, dried over magnesium sulfate, and evaporated. The oily residue, which showed heavy product (R_f 0.40) and heavy starting material (R_f 0.20) spots on TLC with 33% acetone in methylene chloride, was chromatographed over 300 g of silica gel. Elution with 10–75% acetone in methylene chloride afforded 2.75 g of product (5.6 mmol, 41%) as a pale yellow oil and recovered prostaglandin $F_2\alpha$ methyl ester (2.70 g, 54%). Close examination of the product revealed the presence of a small amount (about 2%) of a minor isomer having the same R_f as (5*S*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**7**), described below, and a major isomer (97%) having the same R_f as (5*R*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**8**), described in detail in the following section. When attempts were made to increase the conversion of prostaglandin $F_2\alpha$ methyl ester to products by addition of more iodine, the product mixture became very complex and the yield of desired products diminished markedly.

B. A mixture of prostaglandin $F_2\alpha$ methyl ester (**6**, 3.0 g, 8.1 mmol) and water (60 mL) was stirred and cooled in an ice–water bath. Solid sodium carbonate (0.9 g) was added and the stirring rate was increased. Potassium iodide (2.7 g, 10 mmol) was added first, then iodine (4.14 g, 16.3 mmol) was added. The mixture was stirred with ice-bath cooling for up to 3 h. The progress of the reaction may be checked by thin layer chromatography (TLC) using silica gel plates and ethyl acetate to develop the chromatogram. When the starting material was essentially gone, sodium sulfite (2.5 g) and sodium carbonate (0.8 g) were added and the mixture was stirred until decolorized. The mixture was extracted twice with methylene chloride. The combined extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a yellow oil. The oil was chromatographed over a column of silica gel (120 g) using increasing portions of acetone in methylene chloride (from 0 to 50%) to elute the column. Fractions of 50-mL volume were collected. Fractions 19–23 contained a mixture (1.50 g) of isomers (**7** and **8**). Fractions 24–50 contained the more polar isomer **8** (2.15 g). The mixed fractions were rechromatographed over silica gel (100 g) using the same solvent system and collection of 50-mL fractions. Fractions 32–37 gave 0.16 g of pure **(5*S*,6*S*)-5-iodoprostaglandin I_1 methyl ester** (**7**) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.55 (m, 2 H, $C_{13,14}$ vinyl), 3.4–4.5 (m, protons at $C_{5,6,9,11,15}$), 3.65 (s, 3 H, $-OCH_3$), 0.88 (t, 3 H, $J = 5$ Hz, $-CH_3$). Fractions 38–40 gave 0.36 g (ratio of **7** to **8** was about 1:2, total **7** was 0.28 g, 0.567 g, 7%) of mixed **7** and **8**. Fractions 41–60 contained 0.80 g (total 3.19 g, 6.46 mmol, 80%) of **8**. The pure, more polar isomer, **(5*R*,6*R*)-5-iodoprostaglandin I_1 methyl ester (8)**, was a colorless oil: $[\alpha]_D$ + 24° (*c* 0.9585, $CHCl_3$); R_f 0.42 in ethyl acetate; NMR ($CDCl_3$) δ 5.5 (m, 2 H, $C_{13,14}$ vinyl), 4.55 (m, 1 H, C_9 H), 3.4–4.2 (m, 5 H), 3.65 (s, 3 H, $-COOCH_3$), 0.90 (t, 3 H, $J \simeq 5$ Hz, terminal CH_3); mass spectrum (bis(trimethylsilyl) ether) 638.2340 (calcd for $C_{27}H_{51}Si_2O_5I$, 638.2322), other ions at 623 ($M^+ - CH_3$), 567 ($M^+ - C_5H_{11}$), 548 ($M^+ - Me_3SiOH$), 477 ($567^+ - Me_3SiOH$), 421 ($511^+ - Me_3SiOH$), and 173 mass units.

C. When the previous reaction (section A) was modified in the manner described by Whittaker, i.e., water was used to solubilize sodium bicarbonate, the total yield of iodo ethers was in excess of 90% and the ratio of **7** to **8** was estimated to be 5:95.

(5*R*,6*R*)-5-Iodoprostaglandin I_1 Methyl Ester 11,15-Bis(tetrahydropyran-2-yl) Ether (10). Hydrolysis to Give 8. A mixture of prostaglandin $F_2\alpha$ methyl ester, 11,15-ditetrahydropyranyl ether (**9**, 2.0 g), water (23 mL), and sodium bicarbonate (0.70 g) was cooled in an ice bath and stirred. Potassium iodide (1.93 g) and iodine (2.82 g) were added and the resulting mixture was stirred overnight at ice-bath temperature. Sodium sulfite (1.66 g), sodium carbonate (0.76 g), and water (10 mL) were added to the reaction mixture. The mixture was stirred (10 min) and then chloroform (100 mL) was added. The chloroform layer was separated and the aqueous layer was extracted with more $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to give 2.2 g of an oil. TLC (A-IX) indicated a major new, more polar spot (R_f 0.75, starting material R_f 0.63).

A small portion (0.025 g) of the above reaction product was hydrolyzed by reaction with 1 mL of a 20:10:3 solution of acetic acid–water–THF for 2 h at 45 °C. The solvent mixture was removed azeotropically (twice) by addition of toluene and evaporation under reduced pressure. TLC of the reaction product revealed a major spot having the same R_f (0.31 in A-IX, 0.34 in ethyl acetate) as an authentic sample of **8**.

(6*R*)-Prostaglandin I_1 Methyl Ester (15) and (6*S*)-Prostaglandin I_1 Methyl Ester (12). A. From Prostaglandin $F_2\alpha$ Methyl Ester via Oxymercuration. A solution of 0.95 g (3.0 mmol) of mercuric acetate in 10 mL of water was treated with 10 mL of tetrahydrofuran causing a fine yellow precipitate to form. With stirring a solution of 0.73 g (2.0 mmol) of prostaglandin $F_2\alpha$ methyl ester in 10 mL of tetrahydrofuran was added, and the reaction mixture allowed to stir for 2 h. There was some diminution of precipitate. A solution of 200 mg of sodium borohydride in 10 mL of 1 N KOH solution was then added in portions over a 3-min period. The reaction mixture immediately became gray colored and fine droplets of mercury were seen. After 20 min ether and brine were added. The ether layer was separated, washed with brine, dried over magnesium sulfate, and evaporated. The oily residue (0.66 g) was chromatographed over 50 g of silica gel wet packed with 40% ethyl acetate in Skellysolve B. The column was eluted with 40, 60, 75, and 100% ethyl acetate in Skellysolve B. From the 75 and 100% fractions there was obtained 70 mg of pure, less polar (6*R*)-prostaglandin I_1 methyl ester (**15**), 112 mg of mixed fractions, and 250 mg of pure, more polar (6*S*)-prostaglandin I_1 methyl ester (**12**). The mixed fractions were rechromatographed and separated into 35 mg of **15** and 70 mg of **12**. The combined less polar fractions (0.105 g, 0.27 mmol, 13%) were recrystallized from ethyl acetate–hexane to give **15** as silky needles: mp 71–73 °C; $[\alpha]_D$ +13° (*c* 0.8245, $CHCl_3$); NMR ($CDCl_3$) δ 5.55 (m, 2 H, $C_{13,14}$ vinyl), 3.7–4.5 (m, 4 H, protons at C_6, C_9, C_{11}, C_{15}), 3.7 (s, 3 H, $-COOCH_3$), 0.90 (t, 3 H, $J = 5$ Hz, terminal CH_3); R_f 0.40 (1:1 acetone–CH_2Cl_2).

The more polar fractions (**12**) were combined 0.182 g, 0.49 mmol, 24%) and recrystallized from ether–hexane, giving (6*S*)-prostaglandin I_1 methyl ester: mp 43 °C; $[\alpha]_D$ +23° (*c* 0.8715, $CHCl_3$); NMR ($CDCl_3$) δ 5.55 (m, 2 H, $C_{13,14}$ vinyl), 4.46 (m, 1 H, C_9 H), 3.7–4.2 (3 H, m, protons at C_6, C_{11}, C_{15}), 3.7 (s, 3 H, $-COOCH_3$), 0.88 (t, 3 H, $J = 5.5$ Hz, $-CH_3$); R_f 0.37 (1:1 acetone–CH_2Cl_2); mass spectrum (bis(trimethylsilyl) ether) 512.3356 (calcd for $C_{27}H_{52}Si_2O_5$, 512.3353), other ions at 497 ($M^+ - CH_3$), 481 ($M^+ - OCH_3$), 441 ($M^+ - C_5H_{11}$), 422 ($M^+ - Me_3SiOH$), 391 ($422 - C_5H_{11}$), and 173 mass units.

B. From Prostaglandin $F_2\alpha$ Methyl Ester via Iodo Ethers 7 and 8. A solution of (5*R*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**8**, 0.247 g, 0.5 mmol) in absolute ethanol (3 mL) was treated with tri-*n*-butyltin chloride (0.12 g, 0.38 mmol). Then, with stirring under nitrogen, a freshly prepared solution of sodium borohydride (0.050 g, 1.3 mmol) in absolute ethanol (3 mL) was added. After 45 min the reaction mixture was diluted with ethyl acetate and water and the organic phase was separated, washed with water, dried, and evaporated. There was obtained 0.14 g (0.38 mmol, 76%) of an oil having an NMR spectrum and TLC mobility identical with those of (6*S*)-prostaglandin I_1 methyl ester (**12**).

Under the same conditions as described in the preceding paragraph, (5*S*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**7**) is converted to (6*R*)-prostaglandin I_1 methyl ester (**15**).

C. From 6-Ketoprostaglandin $F_1\alpha$ Methyl Ester via Reduction with Sodium Cyanoborohydride. 6-Ketoprostaglandin $F_1\alpha$ methyl ester (0.487 g) was dissolved in ether (50 mL). Magnesium sulfate (~2 g) and sodium cyanoborohydride (0.974 g) were added with stirring. After 5 min, 2 drops of 20% aqueous hydrochloric acid was added to the mixture. After 15 min, the reaction mixture was poured into ice–water (50 mL) and the layers were separated after thorough mixing. The aqueous phase was extracted two more times with ether (50 mL each), and the combined ether layers were washed with 5% $NaHCO_3$ (50 mL) and with brine (50 mL) and then dried over $MgSO_4$. After filtration and concentration, the crude product amounted to 0.310 g. This was combined with the extracts from similar previous experiments, all of which contained some product, representing a total of 1.363 g (0.003 56 mol) of 6-ketoprostaglandin $F_1\alpha$ methyl ester. The combined products were chromatographed (high pressure liquid chromatography, LC) over two Merck size B silica gel columns, eluting with solvent ranging from 50% ethyl acetate–hexane to pure ethyl acetate. There was obtained 0.350 g (0.00095 mol, 26%) of **15**, R_f and NMR spectrum identical with those

of authentic (6*R*)-prostaglandin I_1 methyl ester, and 0.067 (0.000 18 mol, 5%) of **12**, R_f and NMR spectrum identical with those of authentic (6*S*)-prostaglandin I_1 methyl ester.

D. From 23 and 24 via Esterification. Esterification of (6*S*)-PGI_1 (**24**) with diazomethane gave material identical with (6*S*)-prostaglandin I_1 methyl ester (**12**) as described above. Likewise, esterification of (6*R*)-PGI_1 (**23**) with diazomethane gave (6*R*)-prostaglandin I_1 methyl ester (**15**).

(6*R*)-Prostaglandin I_1-6-d_1 Methyl Ester (15-d_1) and (6*S*)-Prostaglandin I_1-6-d_1 Methyl Ester (12-d_1). The preceding procedure (part C) was followed, using 6-keto prostanglandin $F_{1\alpha}$ methyl ester (0.608 g, 0.001 58 mol), ether (60 mL), magnesium sulfate (~2 g), sodium cyanoborodeuteride (1.0 g), and 2 drops of 20% DCl in D_2O. The reaction was not complete after 15 min but appeared by TLC to be nearly complete after 4 h. The mixture was worked up as before. TLC now indicated significantly more remaining starting material and a more polar product in addition to the desired products. Chromatography (LC) on a Merck B column gave 0.123 g (0.00 033 mol, 21%) of **15**-d_1, 0.014 g (0.000 038 mol, 2%) of **12**-d_1, 0.043 g of recovered 6-ketoprostaglandin $F_{1\alpha}$ methyl ester, and 0.079 g (0.000 20 mol, 13%) of a product having the same R_f as 6-hydroxyprostaglandin $F_{1\alpha}$ methyl ester. The introduction of one deuterium into each of the two PGI_1 epimers was more than 90% complete as determined by mass spectrometry. The NMR spectrum of **12**-d_1 retained a signal at δ 4.45.

(6*S*)-13,14-Dihydroprostaglandin I_1 Methyl Ester (13) and (6*S*)-13,14-Dihydro-15-deoxyprostaglandin I_1 Methyl Ester (14). Several milligrams of (6*S*)-prostaglandin I_1 methyl ester (**12**) were dissolved in methanol (3 mL). Platinum oxide was added and the mixture was hydrogenated at 25 °C and atmospheric pressure for 20 min. TLC (30% acetone in CH_2Cl_2) revealed two products, a less polar (R_f 0.60) major material and a more polar (R_f 0.24) minor material. The mixture was derivatized by treatment with bis(trimethylsilyl)trifluoroacetamide–trimethylchlorosilane (3:1) for 1 h. Analysis by combined gas chromatography–mass spectrometry gave a mass spectrum of the material of lower GC retention time which was consistent with the structure (6*S*)-13,14-dihydro-15-deoxyprostaglandin I_1 methyl ester (**14**): *m/e* 426 (M^+), 411 ($M^+ - CH_3$), 395 ($M^+ - OCH_3$), 366 ($M^+ - Me_3SiOH$), 311 ($M^+ - (CH_2)_4CO_2CH_3$), 267, 221, 179, 158. The mass spectrum of the product having a higher GC retention was consistent with the structure (6*S*)-13,14-dihydroprostaglandin I_1 methyl ester (**13**): *m/e* 514 (weak M^+), 443 ($M^+ - C_5H_{11}$), 424 ($M^+ - Me_3SiOH$), 309, 267, 219, 199, 173.

(5*R*,6*S*)-5-Iodoprostaglandin I_1 Methyl Ester (17) and (5*S*,6*R*)-5-Iodoprostaglandin I_1 Methyl Ester (18). A solution of 5-*trans*-prostaglandin $F_{2\alpha}$ methyl ester[20] (**16**, 2.58 g, 0.000 70 mol) in methylene chloride (50 mL) was degassed for 20 min with a stream of nitrogen. The solution was cooled in an ice bath. Sodium carbonate (1.48 g, 0.014 mol) and iodine (1.90 g, 0.0075 mol) were added to the solution and the resulting mixture was stirred at ice-bath temperature for 1 h and then was allowed to warm to room temperature for another 1 h. The reaction mixture was poured into ice–water (100 mL) containing sodium thiosulfate and mixed until all color disappeared. The organic layer was removed and the aqueous phase was extracted with chloroform (4 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure, crude weight 3.48 g. The crude product mixture was chromatographed on silica gel (264 g) using high-pressure liquid chromatography (LC). Fractions of 25-mL volume were collected and the column was eluted with 15% acetone–methylene chloride (600 mL) and then with 25% acetone–CH_2Cl_2. Fractions 124–133 contained a minor product (0.170 g), fractions 134–144 contained a mixture (0.715 g), and fractions 145–224 contained the major product (1.737 g). Elution of the column with 1:1 acetone–methylene chloride removed unchanged starting material (0.398 g, 15%). The mixture of products was rechromatographed in a similar manner using 60 g of silica gel, giving additional minor product (**17**), 0.182 g (total 0.352 g, 0.71 mmol, 10%), and major product, (5*S*,6*R*)-5-iodo-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester (**18**), 0.414 g (total 2.151 g, 0.004 35 mol, 62%). Minor product **17** was a colorless oil: ^1H NMR ($CDCl_3$) δ 5.53 (m, 2 H, olefinic protons), 3.5–4.5 (m, 4 H, protons at $C_{6,9,11,15}$), 3.67 (s, 3 H, $-OCH_3$), 0.90 (t, 3 H, J = 5 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether) 638.2327 (calcd for $C_{27}H_{51}Si_2IO_5$, 638.2321), 623 ($M^+ - CH_3$), 607 ($M^+ - OCH_3$), 567 ($M^+ - C_5H_{11}$), 548 ($M^+ - Me_3SiOH$), 517 ($607^+ - Me_3SiOH$), 511 ($M^+ - I$), 510 ($M^+ - HI$), 477 ($567^+ - Me_3SiOH$), 451, 199, 173 mass units. Major product **18** also was a colorless oil: ^1H NMR ($CDCl_3$) δ 5.57 (m, 2 H, olefinic protons), 4.52 (m, 1 H, C-9 proton), 3.6–4.3 (m, 3 H, $C_{6,11,15}$ protons), 3.70 (s, 3 H, $-OCH_3$), 0.92 (t, 3 H, J = 5 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether) 638.2333 (calcd for $C_{27}H_{51}Si_2IO_5$, 638.2321), remainder of breakdown pattern is similar to that of **17**.

(5ξ,6*S*)- and (5ξ,6*R*)-Prostaglandin I_1 Mercurichloride Methyl Esters (20 and 21). A solution of approximately 2.5 g of crude mercuric acetate **19**[24] in 50 mL of methanol was mixed with 25 mL of brine and stirred for 2 h at room temperature. After concentration of about half volume under vacuum the mixture was extracted three times with ethyl acetate, and the combined extract washed with brine, dried, and evaporated. The oily residue (2.2 g) was chromatographed over 100 g of silica gel and the column eluted with 50, 75, and 100% ethyl acetate in Skellysolve B. From the 75% fractions was obtained 0.33 g of pure less polar isomer followed by 0.18 g of mixed fraction, then (100% fractions) 1.00 g of more polar fraction. The less polar isomer was assigned the 5ξ,6*S* configuration **20** and was crystallized from ethyl acetate–hexane to give fine, colorless needles: mp 60–61 °C (slight sintering 56 °C); $[\alpha]_D$ +14° (*c* 0.9015, $CHCl_3$); R_f 0.47 (A-IX); NMR ($CHCl_3$) δ 5.50 (m, 2 H, vinyl), 3.80–4.55 (m, protons at $C_{6,9,11,15}$), 3.67 (s, 3 H, $-COOCH_3$), 0.90 (t, 3 H, J = 5 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether) weak 746 (M^+), 675.1727 ($M^+ - C_5H_{11}$) (calcd for $C_{22}H_{40}Si_2O_5{}^{200}HgCl$, 675.1786), 656 ($M^+ - Me_3SiOH$), 585 ($656 - C_5H_{11}$), 511 ($M^+ - HgCl$), 421, 199, 173 mass units.

Anal. ($C_{21}H_{35}O_5HgCl$) C; H; Cl, 5.29.

The more polar isomer **21** was assigned the configuration 5ξ,6*R* and was a colorless oil: R_f 0.41 (A-IX); NMR ($CDCl_3$) δ 5.57 (m, 2 H, $C_{13,14}$ vinyl), 4.56 (m, 1 H, C_9 H), 3.83–4.32 (m, protons at $C_{6,11,15}$), 3.67 (s, 3 H, $-COOCH_3$), 0.88 (t, 3 H, J = 5 Hz, $-CH_3$).

Anal. Found: Cl, 5.69.

(6*R*)-PGI_1 (23) and (6*S*)-PGI_1 (24). A. From $PGF_{2\alpha}$ via Oxymercuration. To a stirred mixture of 3.7 g (11.5 mmol) of mercuric acetate, 30 mL of water, and 20 mL of tetrahydrofuran was added a solution of 2.0 g (5.65 mmol) of $PGF_{2\alpha}$ in 40 mL of THF. After 2 h the still cloudy, yellow mixture was treated in portions during 2–3 min with a solution of 0.75 g of $NaBH_4$ in 30 mL of 1 N NaOH solution. The mixture warmed somewhat and immediately became gray colored. After 15 min the mixture was cooled somewhat and cautiously acidified with dilute hydrochloric acid (nitrogen atmosphere). Salt and ether were then added, and the ether layer was separated. The aqueous portion was extracted twice more with ether. The combined extract was washed with brine, dried over magnesium sulfate, and evaporated to afford 2.5 g of colorless oil. The material was dissolved in methylene chloride and chromatographed over a column containing 141 g of sized (30–50 μ) silica gel which had been prewashed with methylene chloride containing 10% acetic acid and the solvent replaced with 100% methylene chloride. The column was eluted with 500 mL each of 20 and 40% acetone in methylene chloride, 1000 mL of 50% acetone in methylene chloride, and 500 mL of 65% acetone in methylene chloride using approximately 50 lb pressure. From the 50% acetone fractions there was obtained first 0.26 g (0.735 mmol, 13%) of less polar isomer followed by 0.41 g (20%) of mixed fractions and 1.01 g (2.85 mmol, 50%) of more polar isomer. Approximately 0.2 g of unreacted $PGF_{2\alpha}$ was recovered from the 65% acetone fractions.

Recrystallization of the less polar fraction from ethyl acetate–hexane gave (6*R*)-PGI_1 (**23**): mp 97–99 °C; R_f 0.50 (A-IX system); $[\alpha]_D$ +13° (*c* 1.061, EtOH); NMR ($CDCl_3$) δ 5.51 (m, 2 H, $C_{13,14}$ vinyl), 3.52–4.42 (m, 4 H, protons at $C_{6,9,11,15}$), 0.90 (t, 3 H, J = 5 Hz, $-CH_3$).

Anal. Calcd for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67. Found: C, 67.34; H, 9.93.

Recrystallization of the more polar fraction from ethyl acetate–hexane gave (6*S*)-PGI_1 (**24**): mp 79–81 °C; R_f 0.45 (A-IX system); $[\alpha]_D$ +31° (*c* 1.031, in EtOH); NMR ($CDCl_3$) δ 5.52 (m, 2 H, $C_{13,14}$ vinyl), 4.45 (m, 1 H, C_9H), 3.35–4.24 (m, 3 H, $C_{6,11,15}$ protons), 0.88 (t, 3 H, J = 5 Hz, $-CH_3$).

Anal. ($C_{20}H_{34}O_5$) C, H.

B. From Saponification of 15 and 12. Saponification of **15** and **12** with aqueous sodium hydroxide in methanol, followed by acidification and isolation, afforded (6*R*)-PGI_1 (**23**) and (6*S*)-PGI_1 (**24**), respectively. The spectral properties of these products are identical with those of **23** and **24** prepared in part A, above.

Reversal of the Oxymercuration Reaction with Hydrochloric Acid.

When solutions of the mercuriacetates **20** and **21**, derived from either $PGF_2\alpha$ or $PGF_2\alpha$ methyl ester, in methanol were treated with aqueous 10% hydrochloric acid, they were converted cleanly to $PGF_2\alpha$ or $PGF_2\alpha$ methyl ester, respectively.

Reversal of the Iodocyclization Reaction with Zinc in Acetic Acid. A 0.25-g portion of iodo ether **8** was dissolved in 3 mL of acetic acid and treated with stirring with 0.25 g of zinc dust. After 45 min thin layer chromatography (100% ethyl acetate) revealed no **12** or **15** but instead a clean conversion to a spot with same mobility and exhibiting the same color reactions as $PGF_2\alpha$ methyl ester. Thin layer using a $AgNO_3$-impregnated silica gel plate (EtOAc, twice) showed the spot to be about a 60/40 mixture of 5-cis and 5-trans isomers.

Prostaglandin I_2 Methyl Ester (26). A. From Reaction of (5*R*,6*R*)-5-Iodoprostaglandin I_1 Methyl Ester (8) with DBN or DBU. To a solution of 1.00 g of the iodo ether **8** in 50 mL of dry benzene or toluene was added 2 mL of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). The base 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) can be used for the reaction and gives results essentially the same as those reported here. The solution was swirled, then warmed to 40–45 °C for 30 h. By this time some crystals had separated. A TLC (25% acetone in methylene chloride) showed a heavy new spot (R_f 0.46) slightly less polar than starting material (R_f 0.40). Ice-water was added, the mixture shaken, and the organic phase separated, dried over magnesium sulfate (few drops of triethylamine added), and evaporated. A nearly colorless oil (~0.9 g) was obtained which was dissolved in 5 mL of anhydrous ether (few drops TEA added) and diluted with swirling with 100 mL of hexane. The cloudy mixture was stirred with methanol–ice cooling until crystallization commenced (some oiled out first), then refrigerated at −10 °C overnight. The suspension was rapidly filtered. The mushy cake was washed out of the funnel with dry benzene (+ TEA) and evaporated to 0.46 g of a colorless oil which crystallized on refrigeration. The material, however, melted below room temperature. The mother liquors and oil which remained stuck to the sides of the original crystallization flask were combined (0.39 g) and chromatographed over a column of 50 g of Florisil which had been prepared by slurry with 25% ethyl acetate in hexane containing 0.5% TEA. The column was eluted with 20, 40, 50, and 60% ethyl acetate in hexane containing 0.25% TEA. From the 50 and 60% fractions there was obtained 0.21 g additional enol ether product, total yield 0.67 g (91% of theory). Thin layer with either ethyl acetate or 25% acetone in methylene chloride exhibited a heavy enol ether spot (R_f 0.52 in ethyl acetate) and a trace of material of intermediate polarity (Δ^4 isomers). It was necessary to run such plates immediately after spotting; otherwise rapid decomposition to 6-ketoprostaglandin $F_1\alpha$ methyl ester was observed. Twenty minutes was sufficient time to effect 100% conversion. Prespotting with TEA prevented the conversion. When a TLC plate was run using an acid–water system such as A-IX,[27] only 6-ketoprostaglandin $F_1\alpha$ methyl ester was seen. This was a good way to determine completeness of the dehydrohalogenation reaction since the iodo ether starting material was unchanged by this maneuver. Recrystallization of the product from ether–hexane gave soft crystals, mp 30–33 °C. The prostaglandin I_2 methyl ester (**26**) prepared in this manner has the following physical properties: $[\alpha]_D$ +78° (*c* 0.8820, $CHCl_3$); IR λ_{max} (liquid melt) 3370 (OH), 1740 (C=O), and 1695 cm^{-1} (O-C=C); ^1H NMR ($CDCl_3$) δ 5.54 (m, 2 H, $C_{13,14}$ vinyl), 4.58 (m, 1 H, >CHO), 4.16 (m, 1 H, OC=CH-), 4.00 (m, 1 H, $>C_{15}HO$), 3.75 (m, 1 H, >CHO), 3.65 (s, 3 H, $-OCH_3$), 0.87 (t, 3 H, J = 5 Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$) (ppm from Me_4Si) 174.6 (C_1), 154.6 (C_6), 136.4 (C_{14}), 131.7 (C_{13}), 96.9 (C_5), 83.7 (C_9), 77.2 (C_{11}), 73.0 (C_{15}), 54.9 (C_{12}), 51.5 (C_{21}), 45.8 (C_8), 40.7 (C_{10}), 37.1 (C_{16}), 33.6 (C_2), 33.2 (C_7), 31.8 (C_{18}), 25.3 (C_4), 25.2 (C_{17}), 24.7 (C_3), 22.6 (C_{19}), 14.0 (C_{20}); TLC (silica gel, 1:1 acetone–hexane) R_f 0.69, (ethyl acetate) R_f 0.52; mass spectrum (bis(trimethylsilyl) ether) 510.3223 (calcd for $C_{27}H_{50}Si_2O_5$, 510.3197), other ions at 495 ($M^+ - CH_3$), 479 ($M^+ - OCH_3$), 439 ($M^+ - C_5H_{11}$), 423.2724 ($M^+ - CH_2CH_2COOCH_3$, calcd for $C_{23}H_{43}Si_2O_3$, 423.2751), 349, 327, 323, 315, 313, 199, and 173 mass units.

B. From Reaction of (5*S*,6*S*)-5-Iodoprostaglandin I_1 Methyl Ester (7) with DBN. A solution of (5*S*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**7**, 0.16 g, 0.324 mmol) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN, 0.3 mL) in benzene (20 mL) was warmed at 40 °C for 22 h. Additional DBN (0.2 mL) was added and the solution was kept at 40 °C for 6 h and at room temperature for 66 h. TLC (A-IX) revealed that **7** was entirely consumed. The reaction solution was washed with ice-water, dried ($MgSO_4$), filtered, and concentrated. A colorless oil (0.12 g, 0.32 mmol, 100%) having the same R_f (1:1 acetone–hexane) on silica gel as **26** was obtained. TLC on silica gel in the A-IX system converted the product into 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**).

C. From Reaction of 8 with Potassium Superoxide. A mixture of potassium superoxide (0.427 g, 0.0060 mol) in DMF (10 mL) containing dicyclohexyl-18-crown-6 (0.75 g, 0.0020 mol) was stirred for 15 min at room temperature. A solution of (5*R*,6*R*)-5-iodo-9-deoxy-6,9α-epoxyprostaglandin $F_1\alpha$ methyl ester (**8**, 0.494 g, 0.0010 mol) in DMF (1 mL) was added to the KO_2–DMF mixture. A sample of the reaction was quenched in water–ether 5 min after the addition. Thin layer chromatography (TLC) of the ether layer showed no starting material remaining. The reaction mixture was worked up by pouring into ice-water and extracting with ether (four times). The ether extract was dried over magnesium sulfate. One-half of the total extract was concentrated under reduced pressure and chromatographed on a Florisil column (15 g) packed with 5% triethylamine in methylene chloride and washed with 100 mL of 50% ethyl acetate–hexane containing 0.1% triethylamine. The material was placed on the column in 50% ethyl acetate–hexane containing 0.1% triethylamine and the column was eluted with the same. Fractions of 15-mL volume were collected. The product was collected in fractions 8–68 and amounted to 0.076 g (0.000 208 mol, 41% yield) of an oil having the same R_f as the sample of **26** prepared above in the following five TLC systems: ethyl acetate, 50% acetone–hexane, 30% acetone–methylene chloride, 10% methanol–benzene, and 20% methanol–benzene.

When a freshly developed TLC plate of the crude reaction product was sprayed with ferrous thiocyanate reagent, no significant spots sensitive to this reagent were observed. It was concluded that peroxides or hydroperoxides were not present in the crude reaction product.

D. From (5*S*,6*S*)- and (5*R*,6*R*)-5-Bromoprostaglandin I_1 Methyl Esters (35 and 36) by Treatment with DBU. A solution of 0.59 g (1.31 mmol) of the bromo ethers **35** and **36** in 25 mL of benzene was treated with 1.1 mL of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) and warmed to 40 °C for 8 h. Little, if any, change was noted by TLC (acetone–hexane, 1:1). Additional DBU (1 mL) was added and the solution heated under reflux for 24 h. TLC now showed the reaction to be complete (spot at R_f 0.60 vs. 0.56 for starting material). The mixture was washed with ice-water, dried, and evaporated. The oil was crystallized from ether–hexane to give 0.40 g (1.09 mmol, 83%) of mushy, crystalline PGI_2 methyl ester (**26**). When a sample of this material was placed on a silica gel TLC plate and the plate developed in the A-IX system, the major spot had the same R_f as an authentic sample of 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**). A minor component of the reaction mixture was detected by TLC and had the same R_f as Δ^4-prostaglandin I_1 methyl ester.

(5*E*)-Prostaglandin I_2 Methyl Ester (31). A mixture of potassium superoxide (0.88 g, 0.0124 mol) and DMF (20 mL) containing dicyclohexyl-18-crown-6 (0.76 g, 0.0020 mol) was stirred at room temperature for 30 min and then was cooled in an ice bath. A solution of (5*S*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**18**, 1.74 g, 0.003 52 mol) in DMF (3 mL) was added to the mixture. From TLC examination, the reaction appeared to be complete within 30 min, so it was worked up by pouring into ice-water and extracting with ether. The pooled ether extracts were dried over $MgSO_4$. Further TLC examination at this point revealed the presence of two materials in the reaction, one of which likely was unreacted starting material. The ether extract therefore was filtered, concentrated, and redissolved in DMF. This solution was treated with additional KO_2–DMF mixture (6 mL of a vigorously stirred mixture) at room temperature. After 10 min, TLC clearly showed the reaction to be complete and the mixture again was worked up by quenching in ice-water and extracting with ether. The residue obtained from the ether extract following filtration and removal of the solvent was chromatographed on a Florisil (80 g) column that was first packed in 5% triethylamine in methylene chloride and then was washed with 25% ethyl acetate in hexane containing 0.1% triethylamine (850 mL). The residue was applied to the column and the column was eluted with the same solvent mixture (500 mL) and then with 1:1 ethyl acetate–hexane containing 0.1% Et_3N. Fractions of 25-mL volume were collected. The product was seen in fractions 16–70. Fractions 16–46 (0.258 g, 0.705 mmol, 20%) were pooled and fractions 47–61 (0.051 g, contained traces of a spot having the R_f of 6-ketoprostaglandin $F_1\alpha$ methyl ester) were pooled. A small amount of DMF was detected in pooled fractions 16–46 by the infrared and NMR spectra. Recrystallization of the product four times

Table III. Radioactive Counting Results

zone	total counts, 20 min	cpm	background, cpm	cor cpm	% of total
fast moving	13097	654.8	30	624.8	69.9
middle	5188	259.4	30	229.4	25.6
slow moving	1404	70.2	30	40.2	4.5

from ether–hexane gave **31** as colorless crystals: mp 68–70 °C; ^{1}H NMR ($CDCl_3$) δ 5.53 (m, 2 H, -CH=CH-), 4.67 (m, 1 H, -OC=CH-), 4.52 (m, 1 H, >CHO), 4.02 (m, 1 H, $-HC_{15}O-$), 3.82 (m, 1 H, >CHO-), 3.67 (s, 3 H, $-OCH_3$), 0.88 (t, 3 H, J = 5 Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$, ppm from Me_4Si) 174.3 (C_1), 155.9 (C_6), 136.4 (C_{14}), 131.3 (C_{13}), 95.9 (C_5), 83.0 (C_9), 77.3 (C_{11}), 72.9 (C_{15}), 55.5 (C_{12}), 51.4 (C_{21}), 45.6 (C_8), 40.4 (C_{10}), 37.2 (C_{16}), 33.4 (C_2), 31.7 (C_{18}), 30.5 (C_7), 26.9 (C_4), 25.7 (C_3), 25.2 (C_{17}), 22.6 (C_{19}), 14.0 (C_{20}); infrared (ν, liquid melt) 3420 (OH), 1740 (C=O), 1690 cm^{-1} (-O-C=C); TLC (silica gel, 1:1 acetone–hexane) R_f 0.65.

TLC Comparison of Enzymatically Prepared, Radiolabeled PGI_2 Methyl Ester with Synthetic PGI_2 Methyl Ester (26) and (5*E*)-PGI_2 Methyl Ester (31). A sample of PGI_2 methyl ester (**26**),[12] which contained enzymatically produced, tritium-labeled material, was applied across the full width of a 1 × 4 in. silica gel plate and also onto the plates that had been left attached to each side. Approximately an equal amount of (5*E*)-PGI_2 methyl ester (**31**) was likewise applied to the plates. The plates, still attached, were developed immediately in a 1:1 acetone–hexane system. Visualization with iodine vapor revealed a broad band. This band was divided into three horizontal sections corresponding to fast-moving, middle, and slow-moving material. After removal of the two side plates, these zones were scrapped into scintillation vials. The radioactivity in each vial was counted, using a Packard Tri-Carb scintillation spectrometer (Model 3375). The results of the counting experiment are summarized in Table III.

Prostacyclin Sodium Salt (32, PGI_2 Sodium Salt). A. Saponification Followed by Lyophilization. PGI_2 methyl ester (**26**, 0.150 g, 0.000 41 mol) was dissolved in methanol (5 mL). Aqueous 0.05 N sodium hydroxide (9.0 mL, 0.000 45 mol) was added to the solution. A cloudy solution resulted. After 2 h, the solubility was improved by the addition of methanol (3 mL) and 1:1 methanol–water (8 mL). The mixture was stirred at room temperature for 72 h, after which a clear solution was seen and TLC (ethyl acetate) showed that no starting material remained. Excess methanol was removed under reduced pressure. The remaining aqueous solution was lyophilized. A white solid (0.155 g, 0.000 41 mol, 100%) was obtained.

B. Saponification Followed by Precipitation. Crystalline PGI_2 methyl ester (36.5 g, 0.10 mol) was dissolved in 750 mL of methanol containing a little aqueous sodium carbonate, then further mixed with 360 mL of water and 36 g of sodium carbonate. The mixture was stirred vigorously at room temperature for 50 h or until thin layer (ethyl acetate and chloroform–methanol–acetic acid, 90:5:5) indicated the hydrolysis to be complete. The mixture was filtered, the cake washed with a little fresh methanol, and the filtrate evaporated on a rotating evaporator to approximately 350 mL volume. An equal volume of acetonitrile was added. The hazy solution was treated with decolorizing charcoal, then filtered and diluted with 3500 mL of acetonitrile with mixing and gentle warming. A milky mixture resulted which soon set up nearly solid with a cotton-like solid. The mixture was refrigerated at −20 °C overnight, then collected on a filter, washed with room temperature acetonitrile, and dried under house vacuum at 45–50 °C for 24 h, yield 29.1 g (0.0778 mol, 77%) of white powder. This material was essentially a single spot when examined by the *p*-phenylphenacyl ester assay (see below) and was 99.6% pure by GLC assay, but the sodium analysis was 8.68% (vs. 6.14% theory) indicating the presence of 7–8% sodium carbonate. The material (less 0.3 g) was taken up in 250 mL of water and diluted with 500 mL of acetonitrile. Upon standing the hazy mixture deposited an insoluble watery layer on the bottom of the flask. The supernatant solution was carefully decanted, decolorized with charcoal, and filtered. The near-water-white filtrate was diluted with swirling and gentle warming with 2750 mL of acetonitrile and refrigerated at −20 °C. The white product was collected on a filter, washed with acetonitrile, and dried in a vacuum oven at 50 °C for 24 h, yield 22.8 g of white powder, mp 166–168 °C (block), 116–124 °C (capillary). When this sample was exposed to 50% humidity, the material absorbed water (Karl Fisher water analysis, 6.9%). An acid titration showed a break in the titration curve from which it was calculated that the sample contained 2.7% sodium carbonate. This sample of prostacyclin sodium salt (**32**) had the following physical properties: $[\alpha]_D$ +88° (*c* 0.8080, $CHCl_3$), +97° (95% EtOH); IR (ν, mull) 3320 (OH), 1693 (O-C=C), 1555, 1470 cm^{-1} (CO_2^-).

Anal. Calcd for $C_{20}H_{31}O_5Na$: C, 64.15; 8.34; Na, 6.14. Found (corrected for water and Na_2CO_3 content): C, 64.46; H, 8.20; Na, 6.84.

Isolation of (4*E*,6*S*)-Δ^4-PGI_1 (29) and (4*E*,6*R*)-Δ^4-PGI_1 (30) from the Reaction of 8 with DBN. The elimination of HI from **8** (50.0 g, 0.101 mol) with DBN was carried out as described above. The crude crystallized product (**26**) from this reaction was saponified as described above and the PGI_2 sodium salt (**32**, 27.0 g, 71%) was obtained by crystallization from acetonitrile–water. The acetonitrile was removed from the filtrate under reduced pressure and the aqueous phase was acidified and extracted with ethyl acetate. The filtrate from crystallization of **26** was also evaporated, saponified with methanol–3 N aqueous NaOH, acidified, and extracted with ethyl acetate. The ethyl acetate extracts from both procedures were combined (crude weight, 12.6 g) and chromatographed over silica gel (CC-4, acid washed, 600 g + 15% H_2O) packed in 50% ethyl acetate–hexane. Eluted first (with increasing amounts of ethyl acetate in hexane as the solvent) was a mixture (2.3 g, 6%) of the two Δ^4-PGI_1 isomers, **29** and **30**. Eluted second was crystalline 6-ketoprostaglandin $F_{1\alpha}$ (**33**, 5.5 g, 15%). Assuming that the latter compound (**33**) arises via **26**, the reaction of **8** with DBN yields 86% of **26** and 6% of the two byproducts, **29** and **30** (as the methyl esters **27** and **28**, respectively). Rechromatography of the mixed fractions of **29** and **30** over acid-washed silica gel (CC-4, 200 g + 15% H_2O) with the same solvent as above gave 1.14 g of mixed fractions that were rich in the less polar isomer and 1.11 g of essentially pure more polar isomer **30**. The remaining mixed fractions of **29** and **30** were rechromatographed over acid-washed silica gel (50 g, 230–400 mesh) by LC. The column was eluted with increasing amounts of acetone in methylene chloride (beginning with 33% and ending with 50%). Eluted first was the less polar isomer **29** (0.46 g). Eluted next was a mixture (0.09 g) followed by the more polar isomer **30** (0.24 g, total 1.34 g). The ratio of the two Δ^4 isomers is therefore about 3:1 in favor of the more polar **30**. Recrystallization of the less polar isomer from acetone–Skellysolve B gave (4*E*,6*S*)-Δ^4-PGI_1 (**29**) as colorless crystals: mp 115–117 °C; $[\alpha]_D$ +10° (*c* 0.9735, EtOH); ^{1}H NMR ($CDCl_3$ + acetone-d_6) δ 5.58 (m, 4 H, olefinic protons), 3.34–4.67 (br m, 4 H), 0.88 (t, 3 H, J = 5 Hz $-CH_3$); mass spectrum (bis(trimethylsilyl) ether, trimethylsilyl ester) 568.3450 (calcd for $C_{29}H_{56}Si_3O_5$, 568.3435), 553($M^+ - CH_3$), 497 ($M^+ - C_5H_{11}$), 478 ($M^+ - Me_3SiOH$), 407 (497 − Me_3SiOH), 239, 173, 117 mass units.

Anal. Calcd for $C_{20}H_{32}O_5$: C, 68.15; H, 9.16. Found: C, 67.82; H, 9.48.

The *p*-phenylphenacyl ester of **29** was prepared and was crystallized from ethyl acetate–hexane: mp 96–100 °C; IR (mull) 3450, 1735, 1715, 1610, 1460, 1375, 1235, 1170, 1050, 965, 890, 835, 765, and 725 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 2 H, J = 8 Hz, aromatic protons ortho to carbonyl), 7.72 (d, 2 H, J = 8 Hz, aromatic protons meta to carbonyl), 7.53 (m, 5 H, $-C_6H_5$), 5.42–5.87 (m, 4 H, olefinic protons), 5.38 (s, 2 H, $-OCH_2CO-$), 3.58–4.48 (m, 4 H), 0.90 (t, 3 H, J = 5 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether) 690.3749 (calcd for $C_{40}H_{58}Si_2O_6$, 690.3772).

The more polar isomer, (4*E*,6*R*)-Δ^4-PGI_1 (**30**), was an oil: ^{1}H NMR ($CDCl_3$–acetone-d_6) δ 5.58 (m, 4 H, olefinic protons), 4.47 (m, 1 H, C_9H), 3.37–4.27 (m, 3 H), 0.89 (t, 3 H, J = 5 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether, trimethylsilyl ester) 568.3454. The *p*-phenylphenacyl ester of **30** was prepared and crystallized from ethyl acetate–hexane: mp 106–108 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 2 H, J = 8.5 Hz, aromatic protons ortho to carbonyl), 7.72 (d, 2 H, J = 8.5 Hz, aromatic protons meta to carbonyl), 5.72 (m, 2 H, C_4 and C_5 olefinic protons), 5.55 (m, 2 H, C_{13} and C_{14} olefinic protons), 4.48 (m, 1 H, C_9H), 3.40–4.30 (m, 3 H), 0.89 (t, 3 H, J = 5 Hz, $-CH_3$); infrared (mull) 3450, 1740, 1715, 1615, 1460, 1375, 1240, 1170, 970, 840, 765, 725, 690 cm^{-1}; mass spectrum (bis(trimethylsilyl) ether) 690.3790.

Prostacyclin (1, PGI_2). A solution of 100 mg of prostacyclin sodium salt in 5 mL of water was covered with 10 mL of ether and chilled in an ice bath. With stirring a dilute solution of $KHSO_4$ was added slowly until the pH of the aqueous layer reached 4–5. The ether layer was

separated and a portion was assayed by the *p*-phenylphenacyl ester assay described below. The assay showed the material to be essentially pure prostacyclin at this point. The ether layer was washed rapidly with cold brine, dried ($MgSO_4$), filtered, and evaporated to a colorless oil. Assay of a sample of this material revealed contamination of the prostacyclin by about 10% 6-ketoprostaglandin $F_1\alpha$. The oil was not very soluble in ether but did not crystallize after limited manipulation. Assay now showed about 25% 6-ketoprostaglandin $F_1\alpha$.

Thin Layer Chromatography of Prostacyclin (PGI_2) in Chloroform–Methanol–Acetic Acid–Water (90:9:1:0.65). A solution of prostacyclin sodium salt (20.6 mg) in methanol (1.0 mL) containing triethylamine (4 drops) was prepared. A 5-μL (0.1 mg of prostacyclin sodium salt) sample of this solution was placed on a 1 × 4 in. silica gel TLC plate (Analtech Uniplate, silica gel GF, 250 μm in thickness). Also placed on the plate as a control was an authentic sample of 6-ketoprostaglandin $F_1\alpha$. The plate was developed in chloroform–methanol–acetic acid–water (90:9:1:0.65 v/v)[31] immediately after application of the samples. Following development, the plate was visualized by spraying with 1:1 methanol–sulfuric acid followed by charring on a hot plate. A heavy, elongated spot (R_f 0.19–0.47) was seen for the prostacyclin sample. An elongated spot of the same R_f was also seen for the control sample of 6-ketoprostaglandin $F_1\alpha$. No other spots were visible on the plate.

To determine if the elongated spot, corresponding in R_f to 6-ketoprostaglandin $F_1\alpha$, was covering any material due to unchanged prostacyclin, the following experiment was performed. The methanol solution (120 μL) (2.5 mg of prostacyclin sodium salt) was applied across the width of a 1 × 4 in. silica gel GF TLC plate to which was attached another such plate on either side. The side plates were also spotted with control samples (5 μL each) of the prostacyclin sodium salt solution. The plates were developed as above, the side plates were removed and visualized, and the zone of the center plate corresponding to the elongated spot was scraped into acetone. The acetone was filtered to remove the silica gel and then was evaporated under a stream of nitrogen. The residue was dissolved in 0.05 mL of 5% diisopropylethylamine in dimethylformamide and derivatized by the addition of 3.5 mg of *p*-phenylphenacyl bromide in DMF (120 μL). After 45 min at room temperature, the reaction mixture was diluted with saturated aqueous sodium bicarbonate solution and with brine. Ether was added to extract the products. TLC of the extract on silica gel (ethyl acetate or 75% ethyl acetate in hexane) showed the presence of 6-ketoprostaglandin $F_1\alpha$ *p*-phenylphenacyl ester and the absence of the *p*-phenylphenacyl ester of PGI_2 (prostacyclin).

In a control experiment using the TLC plates and the chloroform–methanol–acetic acid–water (90:9:1:0.65) system described above, the R_f valves of several standard prostaglandins were found: $PGF_2\alpha$, R_f 0.26; PGE_2, R_f 0.34; PGA_2, R_f 0.58. Pace-Asciak and Wolfe[31] refer to PGF, PGE, and PGA regions having R_f values of 0.15, 0.33, and 0.50, respectively.

Thin Layer Chromatography of PGI_2 in the Upper Phase from a Mixture of Ethyl Acetate–Methanol–Acetic Acid–2,2,4-Trimethylpentane–Water (110:35:30:10:200 v/v). The solution of prostacyclin sodium salt described in the preceding experiment was used. A 2-μL sample of this solution was placed on a 1 × 4 in. silica gel GF TLC plate. A control sample of 6-ketoprostaglandin $F_1\alpha$ also was placed on the plate. The plate was developed in the upper phase from a freshly prepared mixture of ethyl acetate–methanol–acetic acid–2,2,4-trimethylpentane–water (110:35:30:10:200 v/v).[31] The plate was visualized by spraying with 1:1 methanol–sulfuric acid followed by charring on a hot plate. A heavy, elongated spot (R_f 0.48–0.71) was seen for the prostacyclin spot. An elongated spot (R_f 0.48–0.66) was also seen for the 6-ketoprostaglandin $F_1\alpha$ control sample. No other spots were visible on the plate.

Silica gel plates impregnated with silver ions were prepared by dipping them into a saturated solution of silver nitrate in ethanol and then dried at room temperature for at least 30 min. When prostacyclin sodium salt was placed on such a plate and developed in the above solvent system, the only detectable spot again had the same R_f (0.51) as 6-ketoprostaglandin $F_1\alpha$.

In a control experiment using the above solvent system and a silver impregnated silica gel plate PGE_2 was found to have R_f 0.47. Pace-Asciak and Wolfe report isolation of their major fraction from a zone of R_f 0.50 on a silver-impregnated TLC plate developed in the above solvent system.[31]

R_f of 15-Ketoprostaglandin $F_2\alpha$ in the Systems of Pace-Asciak and Wolfe. The R_f values for 15-ketoprostaglandin $F_2\alpha$ on silica gel TLC plates in the solvent systems reported in the two preceding experiments were determined. The R_f in the chloroform–methanol–acetic acid–water system (1 × 4 in. plates) was 0.35. In the upper phase from ethyl acetate–methanol–acetic acid–2,2,4-trimethylpentane–water and using a silver nitrate treated 1 × 4 in. silica gel plate, the R_f was 0.58.

(5*E*)-Prostaglandin I_2 Sodium Salt (34). Aqueous 0.050 N sodium hydroxide (2.5 mL, 0.125 mmol) and water (2.5 mL) were added to a solution of (5*E*)-PGI_2 methyl ester (**31**, 0.041 g, 0.111 mmol) in methanol (5 mL). The resulting solution was stirred at room temperature for 20 h. TLC in 1:1 acetone–hexane showed that no starting material remained. The product (**34**) was obtained as a viscous gum following lyophilization.

(5*R*,6*R*)-5-Bromoprostaglandin I_1 Methyl Ester (35) and (5*S*,6*S*)-5-Bromoprostaglandin I_1 Methyl Ester (36). A solution of 3.68 g (10 mmol) of prostaglandin $F_2\alpha$ methyl ester in 25 mL of methylene chloride was cooled in an ice bath and treated in portions with stirring with 1.78 g (10 mmol) of *N*-bromosuccinimide. The clear solution was stirred 15 min longer (total reaction time 20 min), then washed with aqueous sodium sulfite solution and water, dried over magnesium sulfate, and evaporated. The colorless oily residue was chromatographed over 200 g of silica gel, eluting with 50% ethyl acetate–hexane (1 L), 75% 75% ethyl acetate–hexane (2 L), and finally with pure ethyl acetate. Fractions (110 mL volume) 30–32 contained 0.113 g (0.25 mmol, 2.5%) of the less polar (5*S*,6*S*)-5-bromoprostaglandin I_1 methyl ester (**35**), fractions 33–36 contained 0.40 g (9%, the ratio of **35** to **36** in this mixture is estimated from TLC to be 2:3) of mixed products, and fractions 37–65 contained 2.21 g (4.94 mmol, 49%) of more polar (5*R*,6*R*)-bromoprostaglandin I_1 methyl ester (**36**). The less polar **35** has the following properties: R_f 0.39 (ethyl acetate); ^1H NMR ($CDCl_3$) δ 5.51 (m, 2 H, $C_{13,14}$ vinyl), 3.50–4.38 (m, 5 H, protons at $C_{5,6,9,11,15}$), 3.67 (s, 3 H, OCH_3), 0.88 (t, 3 H, J = 5 Hz, $-CH_3$). The more polar **36** has the following physical properties: R_f 0.36 (ethyl acetate); ^1H NMR ($CDCl_3$) δ 5.52 (m, 2 H, $C_{13,14}$ vinyl), 4.52 (m, 1 H, C_9 H), 3.46–4.33 (m, 4 H, protons at $C_{5,6,11,15}$), 3.67 (s, 3 H, $-OCH_3$), 0.89 (t, 3 H, J = 5 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether) 590 (weak M^+), 575.2203 ($M^+ - CH_3$, calcd for $C_{26}H_{48}Si_2O_5Br$, 575.2224), 559 ($M^+ - OCH_3$), 519 ($M^+ - C_5H_{11}$), 511 ($M^+ - Br$), 510 ($M^+ - HBr$), 500 ($M^+ - Me_3SiOH$), 469, 429, 403, 199, 173 mass units.

PGI_2 Diacetate Methyl Ester (41). A solution of 5 g of iodo ether **8** in 25 mL of pyridine and 10 mL of acetic anhydride stood at room temperature for 4.5 h. Then ice was added, and the product was extracted with ethyl acetate which was washed several times with water, cold 3 N HCl, then saturated $NaHCO_3$, and saturated salt, dried with Na_2SO_4, and evaporated. To the residue was added some benzene which was removed in vacuo, then 50 mL of dry benzene and 10 mL of 1,5-diazabicyclo[5.4.0]undec-5-ene were added and the mixture was stirred at 25 °C for 3 days. Water was added, and the organic phase was washed several times with more water, then with saturated salt, dried with Na_2SO_4, and evaporated. The residue by TLC was largely **41**, giving a yellow spot on a silica gel plate when developed with 16% acetone–methylene chloride and sprayed with vanillin–H_3PO_4 spray, R_f 0.83. A small spot seen at R_f 0.37 corresponds to the 6-ketoprostaglandin $F_1\alpha$ methyl ester 11,15-diacetate (**39**).

A sample of crude **41** from a similar run starting with 600 mg of iodo ether **8** was chromatographed on 50 g of silica gel which had been packed wet in the column in Skelly B containing 5% triethylamine, then prewashed with Skelly B containing 0.1% triethylamine. Elution was with 1 L of a gradient of 0–50% EtAc in Skelly B + 0.1% Et_3N, collecting 25-mL fractions. Fractions 12–23 contained 393 mg of prostacyclin diacetate methyl ester (**41**).

In another run starting with 1.6 g of iodo ether **8**, but reversing the order of reactions, DBU treatment followed by acetylation, and then chromatography as above, except using Florisil instead of silica gel, 999 mg of colorless oil of compound **41** was obtained. The mass spectrum showed ions at *m/e* 450 (M^+), 390, 363, 359, 330 (very strong), 299, 259, 247, 245, 243, 143, and 111, reasonable for the above structure. The IR spectrum showed strong ester absorptions at 1735 and 1240 cm^{-1} and the enol ether double bond at 1695 cm^{-1}. The NMR spectrum showed one double bond at δ 5.7–5.5, three protons (C-9,11,15) as multiplets between δ 5.5 and 4.5, one proton, triplet (C-5) at δ 4.18, OCH_3 at δ 3.67, and two acetates at δ 2.01 and 1.98.

6-Ketoprostaglandin $F_1\alpha$ 11,15-Diacetate Methyl Ester (39). A solution of 499 mg of **41** in 5 mL of tetrahydrofuran was diluted with

Table IV. Counting Results

zone (compd)	total counts, 20 min	cpm	background, cpm	cor cpm	% of total
41	5777	228.8	32	196.8	89.2
42	1283	64.1	32	32.1	13.9
43	684	34.2	32	2.2	0.9

1.5 mL of water and stirred for 68 h at 25 °C. TLC still showed essentially all starting material. About 15 drops of acetic acid was added and stirring continued for an additional 9 h, when TLC showed substantially complete conversion to a more polar spot. The mixture was worked up by adding ethyl acetate, washing with saturated $NaHCO_3$ and saturated salt, drying with Na_2SO_4, and evaporation. The residue was chromatographed on 50 g of silica gel, eluting with 1 L of 50–100% EtAc–Skelly B. Fractions 8–16 (25 mL) contained 405 mg of one-spot material (R_f 0.38 in 16% acetate–CH_2Cl_2). The TLC sample, on standing in CCl_4, gradually produces two new spots on TLC, R_f 0.79 and 0.89, perhaps two isomeric hemiketals. The mass spectrum contained ions at *m/e* 450 (M − 18), 407, 390, 347, 330 (large), 307, 299, 259, and 247. The IR spectrum shows OH at 3550 cm^{-1} and both ester (1740 cm^{-1}) and ketone (1725 cm^{-1}) carbonyls. The NMR spectrum was consistent with structure **39** showing a one-proton multiplet at δ 4.5–4.2 for C-9 and enhanced absorption in the δ 2.8–2.2 region (C-4,6). The ^{13}C NMR spectrum indicated a mixture of ketone and hemiketal forms showing the ketone carbon at 201.7 ppm and the hemiketal carbon at 108.7 ppm.

Preparation of PGI_2 Diacetate Methyl Ester (41), (5*E*)-PGI_2 Diacetate Methyl Ester (42), and Δ^6-PGI_1 Diacetate Methyl Ester (43) from 6-Ketoprostaglandin $F_{1\alpha}$ 11,15-Diacetate Methyl Ester (39). A solution of 1.09 g of **39** in 50 mL of benzene was refluxed in a Soxhlet extractor which contained anhydrous $MgSO_4$ in its thimble for about 1.5 h, at which time starting **39** was largely gone, being replaced by three very close spots, R_f 0.44, 0.40, and 0.37 on silica gel plates developed twice in 25% EtAc–hexane containing a drop of Et_3N. The least polar spot was the most intense, evidently the predominant product. The middle spot (R_f 0.40) had the same mobility as did compound **41** described earlier. The more polar product spot (R_f 0.37) had the same mobility as the diacetate methyl ester of (5*E*)-PGI_2 (**42**), prepared by reaction of **31** with acetic acid–pyridine (1:3) at 25 °C for 2.5 h, then adding water, extracting with ethyl acetate, washing with water, drying, and evaporating.

A sample (500 mg) of the crude product above was chromatographed on two Merck B silica gel columns, eluting with a gradient (1 L) of 10–20% EtAc–Skelly B containing 0.1 Et_3N, collecting 10-mL fractions. Fractions 36–53 contained 163 mg of the least polar product; fractions 56–58 contained 103 mg of compound **41**, identical in IR and NMR spectra with the previous sample, and fractions 72–81 contained 29 mg of the (5*E*)-PGI_2 diacetate methyl ester (**42**). All three fractions gave nearly identical mass spectra, and samples of all three were hydrolyzed to 6-ketoprostaglandin $F_{1\alpha}$ diacetate methyl ester (**39**) when treated for 1 h at room temperature in a small amount of a solution made up of 4 mL of THF, 1 mL of H_2O, and 0.3 mL of HOAc. Samples of each of the three products were isomerized to the same mixture containing all three enol ethers (the least polar predominating) when treated in Skelly B with a very dilute solution of iodine in Skelly B and compared by TLC after 0.75 h.

The new enol ether in fractions 36–53 was assigned the Δ^6 structure **43** and was characterized in the NMR spectrum by a close doublet with long-range coupling centered at δ 4.66 (J = 2.5 Hz) due to the enol ether proton on C-6, and in the IR spectrum by a band due to the enol ether double bond at 1660 cm^{-1} instead of at 1695 cm^{-1} as PGI_2 and (5*E*)-PGI_2 have.

Comparison of Radiolabeled, Biosynthetic Prostacyclin, as the Diacetate Methyl Ester, with Synthetic Enol Ethers 41, 42, and 43. A small sample of tritium-labeled biosynthetic PGI_2 methyl ester[12] was acetylated with 5 drops of a 1:3 solution of acetic anhydride in pyridine. After 2.75 h at room temperature the mixture was worked up as in the preceding experiment and the product was dissolved in a little ethyl acetate and applied in a series of closely spaced spots to the middle two plates of a four-plate sequence of silica gel plates. TLC samples of **41**, **42**, and **43** diacetate methyl ester reference compounds were applied to the outer two plates and the whole was developed twice in 25% EtOAc in hexane. The two outer plates were then broken off and sprayed with vanillin–H_3PO_4 spray and heated to visualize the spots. Using these outer plates as a guide, along with the color produced by exposing the two inner plates to iodine vapor, the zones corresponding to compounds **41**, **42**, and **43** diacetate methyl esters were scraped off the plates into scintillation vials and counted for tritium radioactivity. The results of the counting experiment are summarized in Table IV.

The stability of **41**, **42**, and **43** to the acetylation conditions was checked by exposing each to 1:3 acetic anhydride–pyridine for 2.0 h and then working them up as described in a previous section. TLC comparison of these samples with the standards showed that they were not appreciably changed by the acetylation conditions.

Δ^6-PGI_1 Sodium Salt (38). A solution of 383 mg of **43** in 8.5 mL of MeOH (Burdick and Jackson) under N_2 was treated at 25 °C with 8.8 mL (1 equiv) of 0.1 N NaOMe in MeOH for 5 h to remove the acetates by ester exchange. The solution was concentrated in vacuo to a small volume to remove the methyl acetate produced, then the residue was redissolved in 8.5 mL of MeOH and 1.5 mL of water was added. It was stirred under N_2 overnight, then 10 mL of H_2O was added and the MeOH removed in vacuo. The aqueous solution was then freeze dried to leave a solid residue, 0.259 g, of **38**. The IR spectrum showed no appreciable amount of ester absorption remaining and the product was easily and completely water soluble.

6-Ketoprostaglandin $F_{1\alpha}$ Methyl Ester (22). A. From (5*R*,6*R*)-5-Iodoprostaglandin I_1 Methyl Ester (8) with Silver Carbonate and Aqueous Acid. A mixture of 0.45 g of iodo ether **8**, 20 mL of tetrahydrofuran, 250 mg of silver carbonate, and 5 drops of 70% perchloric acid was stirred vigorously for 24 h. The mixture was then filtered, and the cake washed twice with ethyl acetate. The combined filtrate was concentrated to 1/3 volume, additional ethyl acetate was added, and the solution was washed twice with brine, dried over magnesium sulfate, and evaporated. The oily residue (0.41 g) was chromatographed over 25 g of silica gel. The column was eluted with 75 and 100% ethyl acetate with hexane. From the latter was obtained 0.32 g of TLC-homogeneous product as a nearly colorless oil which, with some difficulty, was crystallized from acetone–hexane, mp 50–65 °C. Recrystallizations did not improve the melting point beyond a range of 68–74 °C. It is assumed that the keto alcohol is in equilibrium with the hemiketal form of this molecule with the result that a sharp melting point cannot be attained. 6-Ketoprostaglandin $F_{1\alpha}$ methyl ester had the following spectral properties: IR (ν, mull) 3380 (OH), 1740 (ester C=O), 1710 cm^{-1} (ketone); NMR ($CDCl_3$) δ 5.5 (m, 2 H, $C_{13,14}$ vinyl), 3.3–4.8 (~6 H, including OH protons), 3.7 (s, 3 H, $-OCH_3$), 2.1–2.7 (~6 H), 0.90 (t, 3 H, $-CH_3$); mass spectrum (tris(trimethylsilyl) ether) 600.3669 (calcd for $C_{30}H_{60}Si_3O_6$, 600.3698), other ions at 585 ($M^+ - CH_3$), 569 ($M^+ - OCH_3$), 529 ($M^+ - C_5H_{11}$), 510 ($M^+ - Me_3SiOH$), 495, 485, 420, 349, 217, 173 mass units.

B. From PGI_2 Methyl Ester (26) by Hydrolysis. A solution of PGI_2 methyl ester (**26**, 0.10 g, 0.273 mmol) in tetrahydrofuran (10 mL) was stirred with an aqueous pH 1.5 buffer solution (10 mL, KCl–HCl was used to prepare this buffer) at room temperature for 0.5 h. Brine (10 mL) was added and the resulting mixture was extracted with ethyl acetate (four times). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was chromatographed on analytical grade silica gel (22 g) using LC techniques. Fractions of 10-mL volume were collected and the column was eluted with 30% acetone–hexane (through fraction 24) and then with 40% acetone–hexane. Fractions 33 and 34 (0.020 g) contained product plus a minor impurity. Fractions 35–44 contained pure product (0.065 g, 0.169 mmol, 62%) which was recrystallized twice from ether–hexane to give 6-ketoprostaglandin $F_{1\alpha}$ methyl ester (**22**), mp 65–70 °C, identical with the material prepared in the preceding section.

C. From Hydrolysis of (5*E*)-PGI_2 Methyl Ester (31). A solution of (5*E*)-PGI_2 methyl ester (**31**, 0.096 g, 0.262 mmol) in tetrahydrofuran (10 mL) and aqueous buffer (pH 1.5, prepared from 25 mL of 0.2 M KCl and 6.5 mL of 0.2 M HCl) was stirred for 90 min at room temperature. The reaction mixture was worked up by addition of brine (10 mL) and extraction with ethyl acetate (4 × 15 mL). The organic extract was dried ($MgSO_4$), filtered, and concentrated to give 0.088 g of crude product. The product was chromatographed on 22 g of silica gel using LC. Elution was with 30% acetone–hexane and fractions of 10-mL volume were collected. The fractions containing product were pooled (0.044 g) and crystallized from ether–hexane to give 0.031 g

(0.0807 mmol, 31%) of 6-ketoprostaglandin $F_1\alpha$ methyl ester, mp 70–74 °C; other properties were identical with those of the sample prepared in the preceding section A.

6-Ketoprostaglandin $F_1\alpha$ (33). A solution of 6-ketoprostaglandin $F_1\alpha$ methyl ester (0.52 g, 1.35 mmol) in 20 mL of methanol was cooled in an ice bath and treated with stirring under nitrogen with 10 mL of 1 N aqueous potassium hydroxide. After 10 min the ice bath was removed and the reaction allowed to proceed for 2 h longer. With cooling aqueous potassium bisulfate solution was added until acidic, and the mixture extracted three times with ethyl acetate. The combined extract was washed with brine, dried over magnesium sulfate, and evaporated to an oil. Crystallization from acetone–Skellysolve B using charcoal afforded 0.36 g (0.97 mmol, 72%) of colorless crystals, mp 75–78 °C. Various lots of this material have melted broadly in the 60–105 °C range, presumably owing to partial existence in the hemiketal form. The crystalline 6-ketoprostaglandin $F_1\alpha$ (**33**) has the following physical properties: R_f 0.26 (A-IX); IR (ν, mull) 3400 (OH), 2640 (acid OH), 1695 cm^{-1} (C=O); mass spectrum (tris(trimethylsilyl) ether trimethylsilyl ester) 658.3914 (calcd for $C_{32}H_{66}Si_4O_6$, 658.3936), 657, 656, 643 ($M^+ - CH_3$), 640, 587 ($M^+ - C_5H_{11}$), 568 ($M^+ - Me_3SiOH$), 553, 497, 485 ($M^+ - (CH_2)_4CO_2Me_3Si$), 478 ($M^+ - 2\,Me_3SiOH$), 407, 395, 201, 173, 111 mass units.

Anal. ($C_{20}H_{34}O_6$) C, H.

6-Methoxyprostaglandin I_1 Methyl Ester (45). 6-Ketoprostaglandin $F_1\alpha$ methyl ester (0.320 g) was dissolved in methanol and allowed to stand overnight. Thin layer (acetone–methylene chloride, 1:1) revealed the formation of a heavy, less polar spot (R_f 0.45 vs. R_f 0.26 for the starting material). The solvent was evaporated and the residue chromatographed over 25 g of silica gel. The column was eluted with 50–100% ethyl acetate in Skellysolve B. From the 50% fractions there was obtained 100 mg of the oily methyl hemiketal. Later fractions were mixed with a slightly more polar unknown and unreacted starting material. The sample of 6-methoxyprostaglandin I_1 methyl ester had the following spectral properties: ^1H NMR ($CDCl_3$) δ 5.5 (m, 2 H, $C_{13,14}$ vinyl), 4.35 (m, 1 H, >CHO), 4.0 (m, 1 H, >CHO), 3.68 (s, 3 H, $-COOCH_3$), 3.12 (s, 3 H, OCH_3), 0.9 (t, 3 H, J = 5 Hz, terminal CH_3); ^{13}C NMR ($CDCl_3$) (ppm from Me_4Si) 111.5 (C_6), 47.8 (OCH_3); mass spectrum (bis(trimethylsilyl) ether) 542.3498 (calcd for $C_{28}H_{54}Si_2O_6$, 542.3459), 527 ($M^+ - CH_3$), 511 ($M^+ - OCH_3$), 510 ($M^+ - CH_3OH$), 471 ($M^+ - C_5H_{11}$), 452 ($M^+ - Me_3SiOH$), 427 [$M^+ - (CH_2)_4CO_2CH_3$], 439 ($M^+ - C_5H_{11}$ and CH_3OH) mass units.

PGI_2 *p*-Phenylphenacyl Ester (47). A. From (5*R*,6*R*)-5-Iodoprostaglandin I_1 *p*-Phenylphenacyl Ester. A solution of 0.20 g of the iodo ester **49** in 15 mL of dry benzene was treated with 0.4 mL of DBN and warmed at 40–45 °C for 22 h. Ice–water was added, the mixture was shaken, and the benzene layer was separated, dried over magnesium sulfate (added a few drops of triethylamine), and evaporated to afford a yellow oil. After some only partial success with crystallization, everything was recombined and chromatographed over 20 g of Florisil. The column was prepared by slurry using 20% ethyl acetate in hexane containing 0.5% TEA. The column was eluted with 20–100% ethyl acetate in hexane containing 0.25% TEA. From the 80–100% ethyl acetate fractions there was obtained 65 mg of an oil showing a heavy spot on TLC for product and light less polar and more polar (6-keto) spots. Earlier fractions showed some product plus a heavy less polar material not observed in a TLC made of the total crude prior to chromatography.

The 65-mg fraction (colorless oil) was crystallized twice from ether–hexane to afford 16 mg as fine leaflets (?), sintered at 65–67 °C, melted at 71–74 °C.

B. From Prostacyclin Sodium Salt. A solution of 0.50 g (1.33 mmol) of prostacyclin sodium salt in 5 mL of dimethylformamide (dried over molecular sieves) was treated with 0.50 g of α-bromo-*p*-phenylacetophenone and stirred for 2.5 h at room temperature. The mixture was then diluted with ice–water and extracted twice with ethyl acetate containing a little triethylamine. The combined extract was washed with cold brine, dried with $MgSO_4$ (Et_3N), and evaporated to an oil which solidified on standing. A methylene chloride solution of the substance was applied to a column of 20 g of Florisil which had been prepared by slurry with 50% ethyl acetate–Skellysolve B containing 1% Et_3N. The column was further eluted with 50 and 75% ethyl acetate–Skellysolve B containing 0.25% Et_3N. The product (R_f 0.70 on TLC using EtOAc) was combined (0.7 g) and crystallized from ether–hexane as a gelatinous material which dried to an off-white solid: mp 76–83 °C; $[\alpha]_D$ +64° (c 0.8580, $CHCl_3$); IR (ν, mull) 3400, 3310 sh (OH), 1740 (ester C=O), 1690 (OC=C), 1605, 1585, 1560, 1515, 1490 cm^{-1} (aromatic C=C); ^1H NMR ($CDCl_3$) δ 8.00 (d, 2 H, J = 8 Hz, protons ortho to C=O), 7.68 (d, 2 H, J = 8 Hz, protons meta to C=O), 7.53 (m, 5 H, $-C_6H_5$), 5.53 (m, 2 H, $C_{13,14}$ vinyl), 5.36 (s, 2 H, $-OCH_2CO-$), 4.60 (m, 1 H, >CHO–), 3.43–4.42 (m, protons at $C_{5,6,11,15}$), 0.88 (t, 3 H, J = 5 Hz, $-CH_3$).

Anal. ($C_{34}H_{42}O_6$) C, H.

6-Ketoprostaglandin $F_1\alpha$ *p*-Phenylphenacyl Ester (48). A mixture of 100 mg of 6-ketoprostaglandin $F_1\alpha$, 5 mL of acetonitrile, 0.2 mL of diisopropylethylamine, and 0.25 g of *p*-phenylphenacyl bromide was stirred at room temperature for 40 min. Dilute citric acid solution and brine were added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried, and evaporated to a crystalline residue which was chromatographed from methylene chloride over 5 g of silica gel. Elution with 60% acetone in methylene chloride afforded a 0.13-g cut of TLC homogeneous product which crystallized from ethyl acetate–hexane as fine nuggets: mp 98–100 °C with previous sintering; mass spectrum (tris(trimethylsilyl) ether) 780 (M^+), 779, 778, 600, 529, 517, 510, 504, 495, 405, 225 mass units.

Anal. ($C_{34}H_{44}O_7$) C; H, 8.29.

(5*R*,6*R*)-5-Iodoprostaglandin I_1 *p*-Phenylphenacyl Ester (49). A solution of 0.20 g of the iodo acid **8** in 10 mL of acetonitrile was treated with 0.4 mL of diisopropylethylamine and 0.5 g of *p*-phenylphenacyl bromide. The mixture was stirred at room temperature for 40 min, then diluted with 10% citric acid solution sufficient to make acid. Brine was added, and the mixture extracted three times with ethyl acetate. The combined extract was washed with brine, dried over magnesium sulfate, and evaporated. The oily residue was chromatographed over 25 g of silica gel to afford 0.20 g of TLC homogeneous phenacyl ester (**49**) (eluted 80 and 100% ethyl acetate in hexane) as a colorless oil: $[\alpha]_D$ +18° (c 0.9880, $CHCl_3$); IR (ν, neat) 3380 (OH), 1740 (C=O, ester), 1700 (ketone), 1605, 1580, 1560, 1515, and 1490 cm^{-1}; mass spectrum m/e 638 ($M^+ - 2H_2O$), 181 ($O^+{=}C{-}C_6H_4{-}C_6H_5$), 128 ($HI^+$) mass units; ^1H NMR ($CDCl_3$) δ 7.98 (d, 2 H, J = 8 Hz, aromatic protons ortho to carbonyl), 7.68 (d, 2 H, J = 8 Hz, aromatic protons meta to C=O), 7.53 (m, 5 H, $-C_6H_5$), 5.54 (m, 2 H, $C_{13,14}$ vinyl), 5.37 (s, 2 H, $-OCH_2CO-$), 4.56 (m, 1 H, C_9 H), 3.46–4.33 (m, protons at C_5, C_6, C_{11}, C_{15}), 0.88 (t, 3 H, J = 5 Hz, $-CH_3$).

A *p*-Phenylphenacyl Ester Assay for Purity of Prostacyclin Sodium Salt Samples. A few milligrams of prostacyclin sodium salt are dissolved in 0.5 mL of dimethylformamide containing 5% diisopropylethylamine and a few milligrams of α-bromo-*p*-phenylacetophenone are added. The mixture is swirled, then allowed to stand for 45 min at room temperature. Saturated aqueous sodium bicarbonate (0.5 mL) and ether (0.5 mL) are added, and the mixture is shaken. The upper ether layer is assayed by thin layer using 100% ethyl acetate as the developing solvent. Spots are visualized by spraying with 50% sulfuric acid and heating on a hot plate. PGI_2 *p*-phenylphenacyl ester exhibits an R_f of 0.57 while on the same plate the corresponding esters of 6-ketoprostaglandin $F_1\alpha$ and the Δ^4 isomers (two close spots) show R_f values of 0.25 and 0.45, respectively. All spots, before spraying, are UV visible. Excess reagent is less polar, moving near the solvent front.

(5*S*,6*S*,17*Z*)-5-Iodo-Δ^{17}-prostaglandin I_1 Methyl Ester (55) and (5*R*,6*R*,17*Z*)-5-Iodo-Δ^{17}-prostaglandin I_1 Methyl Ester (56). A solution of $PGF_3\alpha$ methyl ester[44] (**54,** 1.247 g, 3.40 mmol) in methylene chloride (166 mL) was stirred with a saturated aqueous solution of sodium bicarbonate (26 mL). To the mixture was added dropwise, within 35 min, a solution of iodine (2.5%) in methylene chloride (38 mL, 3.74 mmol) at about 25 °C. After 1 h, the reaction mixture was diluted with 600 mL of methylene chloride and was washed with 30 mL of 0.25 M aqueous sodium thiosulfate. The organic phase was washed successively with water (180 mL), pH 2 buffer solution (70 mL), and water (180 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was chromatographed on two Merck B LC silica gel columns (~120 g total). Eluted first was (5*S*,6*S*,17*Z*)-5-iodo-Δ^{17}-prostaglandin I_1 methyl ester (**55,** 0.035 g, 0.071 mmol, 2%) having the following spectral properties: ^1H NMR ($CHCl_3$) δ 5.49 (m, 4 H, $C_{13,14}$ and $C_{17,18}$ vinyl), 3.33–4.49 (m, 5 H, protons at $C_{5,6,9,11,15}$), 3.67 (s, 3 H, $-OCH_3$), 0.97 (t, 3 H, J = 7 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) derivative) 621.1938 ($M^+ - CH_3$, calcd for $C_{26}H_{46}Si_2O_5I$, 621.1930), 567, 515, 477, 451 mass units. Several fractions containing a mixture of **55** and **56** (0.196 g) were eluted next.

Eluted last was (5*R*,6*R*,17*Z*)-5-iodo-Δ^{17}-prostaglandin I_1 methyl ester (**56**, 0.952 g, 1.94 mmol, 57%) having the following spectral properties: IR (ν, neat) 3380 (OH), 3010 (=CH-), 1740 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 5.50 (m, 4 H, $C_{13,14}$ and $C_{17,18}$ vinyl), 4.54 (m, 1 H, C_9 H), 3.47-4.25 (m, protons at $C_{5,6,11,15}$), 3.67 (s, 3 H, $-OCH_3$), 0.97 (t, 3 H, J = 7 Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$, ppm from Me_4Si) 173.4, 135.1, 134.0, 132.4, 124.1, 80.9, 75.9, 72.4, 55.8, 51.5, 47.2, 41.0, 40.3, 35.8, 35.4, 35.0, 33.0, 25.1, 20.7, and 14.2; mass spectrum (bis(trimethylsilyl) ether) 636, 621.1907 ($M^+ - CH_3$, calcd 621.1930), 567, 515, 509, 508, 477, 451, and 171 mass units.

Anal. ($C_{21}H_{33}O_5I$) C; H, 7.12.

Prostaglandin I_3 Methyl Ester (57). A solution of (5*R*,6*R*,17*Z*)-5-iodo-Δ^{17}-prostaglandin I_1 methyl ester (**56**, 224 mg, 0.45 mmol) and 1,5-diazabicyclo[4.3.0]non-5-ene (0.4 mL) in benzene (10 mL) was heated under nitrogen at 40-45 °C for 48 h. The reaction mixture was cooled to room temperature, diluted with benzene, and washed with water (2 × 8 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give 175 mg of residue. The residue was chromatographed on Florisil (10 g), using 25% acetone-hexane (containing 0.1% triethylamine) as the eluting solvent. There was obtained 101 mg (0.275 mmol, 61%) of prostaglandin I_3 methyl ester (**57**) as a colorless oil: R_f 0.71 (TLC on silica gel in acetone-hexane, 1:1); IR (ν, neat) 3390 (OH), 3010 (=CH), 1740 (C=O), 1695 cm^{-1} (O-C=CH-); ^1H NMR (benzene-d_6) δ 5.61 (m, 4 H, $C_{13,14}$ and $C_{17,18}$ vinyl), 4.22 (m), 3.75 (m, 1 H), 3.43 (s, 3 H, $-OCH_3$), 0.99 (t, 3 H, J = 7 Hz, $-CH_3$); mass spectrum (bis(trimethylsilyl) ether) 508.3025 (calcd for $C_{27}H_{48}Si_2O_5$, 508.3040).

Prostaglandin I_3 Sodium Salt (52). A solution of prostaglandin I_3 methyl ester (**57**, 108 mg, 0.30 mmol) in methanol (5 mL) was treated with 6.6 mL of 0.05 N NaOH solution. The reaction was monitored for the disappearance of starting material by TLC on silica gel in ethyl acetate. The reaction was complete after ~20 h. The methanol was partially removed under reduced pressure. The remaining aqueous solution was frozen and subjected to lyophilization to yield prostaglandin I_3 sodium salt (**52**) as a white powder: IR (ν, Nujol mull) 3350 (OH), 1690 (=C-O-), 1650 cm^{-1} (C=C).

(17*Z*)-6-Keto-Δ^{17}-prostaglandin $F_1\alpha$ (53). Prostaglandin I_3 sodium salt (104 mg, 0.28 mmol) in THF (10 mL) was treated with pH 1.5 buffer solution (10 mL) and stirred at room temperature for 3 h. The reaction mixture was then treated with 15 mL of saturated sodium chloride solution plus ethyl acetate and shaken. The layers were separated and the aqueous phase was extracted with ethyl acetate (three times). The pooled organic phases were washed with saturated sodium chloride solution, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give 91 mg of **53** as an oil. The product has the following physical properties: R_f 0.18 (A-IX); IR (ν, film) 3375 (OH), 1710 cm^{-1} (C=O); ^1H NMR (acetone-d_6) δ 5.48 (m, 4 H, $C_{13,14}$ and $C_{17,18}$ vinyl), 3.50-4.80 (m), 0.95 (t, 3 H, J = 7 Hz, $-CH_3$).

(17*Z*)-6-Keto-Δ^{17}-prostaglandin $F_1\alpha$ Methyl Ester (58). A solution of prostaglandin I_3 methyl ester (**57**, 80 mg, 0.22 mmol) in tetrahydrofuran (5 mL) was stirred with 2 mL of pH 1.5 buffer solution diluted with 2 mL of water. The reaction was complete after 45 min by TLC evidence (silica gel, 40% acetone-hexane; R_f product 0.25). Brine and ethyl acetate were added and the resultant mixture was shaken. The layers were separated and the aqueous layer was extracted with ethyl acetate (three times). The pooled organic layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated to give 79 mg of (17*Z*)-6-keto-Δ^{17}-prostaglandin $F_1\alpha$ methyl ester (**58**) as an oil. The product (**58**) was characterized as the methoxime derivative as described below.

(17*Z*)-6-Keto-Δ^{17}-prostaglandin $F_1\alpha$ Methyl Ester (Syn and Anti) Methoxime (60). (17*Z*)-17,18-Didehydro-6-ketoprostaglandin $F_1\alpha$ methyl ester (74 mg, 0.2 mmol) was stirred with 1.5 mL of a 0.5 M methoxyamine hydrochloride-pyridine solution. The reaction was monitored by TLC and appeared to be complete after ~2 h. The reaction mixture was poured into water and extracted with ethyl acetate (four times). The combined ethyl acetate layers were washed with water, ice-cold 0.1 N HCl, and water, dried (Na_2SO_4), filtered, and evaporated to give an oil (70 mg). The product was chromatographed on a Merck B column with ethyl acetate-hexane to give 35 mg of **60** as an oil: R_f 0.38 (TLC on silica gel in 50% acetone-hexane); ^1H NMR signals at δ 5.54 (4 H, m, CH=CH), 4.37-3.62, 3.85 (s, $=NOCH_3$), 3.79 (s, $=NOCH_3$), 3.67 (s, 3 H, $-OCH_3$), 0.97 (t, 3 H, $-CH_3$, J = 7 Hz); IR (ν, $CHCl_3$ solution) 3320 (OH), 1730 cm^{-1} (C=O); mass spectrum (tris(trimethylsilyl) ether) 627 (M^+), 612.3572 ($M^+ - CH_3$, calcd for $C_{30}H_{58}Si_3NO_6$, 612.3541), 596, 558, 486, 468, 396, 378 mass units.

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Porphyrins. 38.[1] Redox Potentials, Charge Transfer Transitions, and Emission of Copper, Silver, and Gold Complexes

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Abstract: While Cu^{II} porphyrins are known to luminesce, Ag^{II} complexes do not. It is shown here that silver(III) octaethylporphyrin has no emission while gold(III) tetraphenylporphyrin has a moderately intense phosphorescence with a nonexponential decay fit with two decay times of 63 and 184 μs. In contrast to Cu^{II} and Au^{III} porphyrins, the Ag complexes have a metal redox potential, II $\rightleftarrows$ III, between that of ring oxidation and ring reduction suggesting that luminescence is quenched by low-energy charge transfer transitions $Ag^{II} \rightarrow$ ring or ring $\rightarrow Ag^{III}$. Near-infrared (1100–700 nm) absorption spectra confirm the presence of weak absorption bands in Ag^{II} and Ag^{III} complexes that are not observed in complexes of Cu^{II} and Au^{III}. The near-IR absorption of Cu^{II}(TPP) and the quenching of its unusually broad emission by pyridine suggest that a charge transfer state is close to the emitting level. Iterative extended Hückel calculations explain these facts by the energy of orbital $b_{1g}(d_{x^2-y^2})$, which rises along the series Cu < Ag < Au.

Introduction

A recent electronic taxonomy of metalloporphyrins provides a framework for understanding the vast array of optical absorption and emission data and highlights the areas where the biggest questions remain.[3] Metalloporphyrins with partly filled d shells (hemes being in this group) exhibit a variety of spectra and are not completely understood. In this paper we present further investigation on the electronic structure of group 1B (Cu, Ag, Au) metalloporphyrins.

In our previous work we have attributed lack of emission in metalloporphyrins to low-energy states not of (π,π*) character: charge transfer (CT),[3,4] (d,d),[5] or (f,f)[6] transitions. We have further hypothesized that if charge transfer states are the cause, their presence at low energy should correlate with metal redox potentials.[4,7]

In this paper we explore this problem for Cu, Ag, and Au complexes of octaethylporphyrin (OEP) and tetraphenylporphyrin (TPP). The copper porphyrins are long known as having metal valence Cu^{II} [8,9] and strong emission from the tripdoublet state.[10-12] The silver porphyrins show a redox reaction $Ag^{II} \rightleftarrows Ag^{III}$ [13] and have no luminescence.[14,15] (A bimetallic Ag^{I} species has also been reported.[8]) Gold porphyrin has been reported to be Au^{III}.[16a] At the time we began this study there were no reports of emission or redox properties of Au^{III} porphyrins; however, a detailed study of their redox properties has recently been published.[16b]

The aim of this paper is to show that in Cu, Ag, and Au porphyrins lack of emission correlates with low-energy charge transfer transitions. We do this through an examination of (1) visible-near-UV absorption spectra, (2) emission spectra, (3) redox potentials, and (4) near-IR absorption data. We shall survey older data and report new data. We shall also report on iterative extended Hückel (IEH) calculations, and show the extent to which they rationalize the data.

Experimental and Calculational Methods

Preparations. Ag^{II}(OEP),[17] $[Ag^{III}(OEP)][ClO_4]$,[18] and $[Au^{III}(TPP)][AuCl_4]$[16] were prepared by literature methods. $[Ag^{III}(OEP)][PF_6]$ was prepared by controlled-potential electrolysis of Ag^{II}(OEP) at 0.65 V vs. Ag/AgCl. Simultaneous coulometric measurement showed $n = 1$, indicative of a one-electron oxidation. Ag^{II}(TPP) was prepared by refluxing 100 mg of TPP in 100 mL of acetic acid. Solid $AgNO_3$ (0.277 g, tenfold excess) was added along with 100 mg of sodium acetate. The suspension was allowed to reflux for 45 min, cooled to room temperature, filtered, and washed with copious amounts of water. The purple crystals were recrystallized from CH_2Cl_2–CH_3OH. The copper complexes had been prepared by the usual methods.[17]

Electrochemistry. All electrochemical measurements were recorded in dried, redistilled CH_2Cl_2, which was stored over 4 Å sieves. The supporting electrolytes were tetra-*n*-butylammonium hexafluorophosphate or tetra-*n*-butylammonium perchlorate, which were recrystallized and dried in vacuo prior to use. The reference electrode used was the Ag/AgCl electrode, and values obtained have been converted to potentials vs. SCE for comparison with previously published values. Bulk electrolyses were performed on a PAR Model 173 using a Model 176 electrometer probe. The cyclic voltammograms were recorded on standard operational amplifier circuitry, as described previously.[19]

It was found that cyclic voltammetric measurements were repro-

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