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Synthesis of a series of 16, 16-dimethyl-prostacyclin and 6-keto prostaglandin analogs

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SYNTHESIS OF A SERIES OF 16,16-DIMETHYL-PROSTACYCLIN AND 6-KETO PROSTAGLANDIN ANALOGS

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ABSTRACT

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SYNTHESIS OF A SERIES OF 16,16-DIMETHYL-PROSTACYCLIN AND 6-KETO-PROSTAGLANDIN ANALOGS

Advisor: Professor Malcolm B. Polk

Thesis dated: December, 1978

A synthesis is described for a number of 16,16-dimethyl analogs of the prostacyclin and related 6-keto prostaglandin types. Included are 16,16-dimethyl-P_GI_2 sodium salt (XLIV), 6α-16,16-dimethyl-P_GI_1 (XLV), 6β-16,16-dimethyl-P_GI_1 (XLVII), 6-keto-16,16-dimethyl-P_GF_1-α (XLIX), and 6-keto-16,16-dimethyl P_GE_1 (LV). Done, but not included, is the activity for these analogs in the blood platelet aggregation inhibition assay. This activity was consistently less than for the corresponding 16,16-dihydro compounds.

ACKNOWLEDGEMENTS

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INTRODUCTION

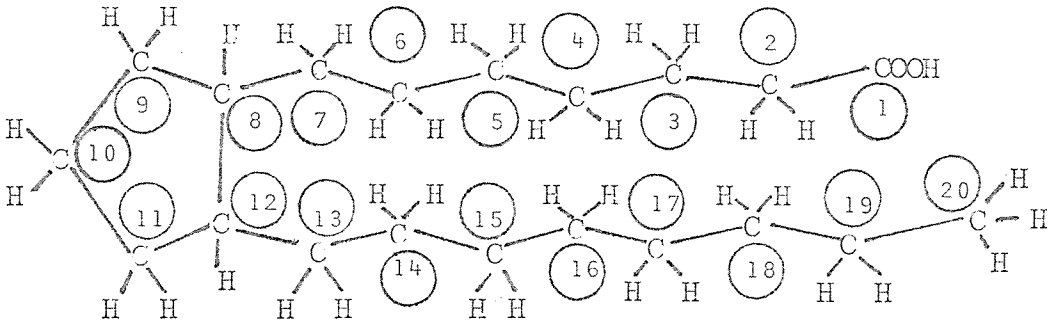
Prostaglandins are hormone-like substances that are ubiquitous in human and animal tissues and are characterized by a highly potent and diverse spectrum of biological activities. Of the fourteen prostaglandins, thirteen occur in man, with the highest concentration in human seminal fluid. In tiny amounts, they have also been found in some non-mammalian tissues such as frog's skin and spinal cord and recently in coral.

These compounds are twenty carbon carboxylic acids synthesized from certain polyunsaturated fatty acids by the formation of a five-membered ring and the incorporation of three oxygen atoms at certain positions. These positions divide prostaglandins into the four main categories E,F,A, and B, with the first three seeming to be the most important therapeutically. All four groups have a trans double bond at the 13 position and a hydroxyl group at C-15 in common. The difference between the aforementioned prostaglandins are that the E and F series possess an additional hydroxyl group at C-11 and are distinguished from each other by the presence of a carbonyl function at C-9 in the E series and a C-9 hydroxyl in the F series. The A and B series may be regarded as dehydration products of the E compounds whereby loss of water has occurred with removal of the C-11 hydroxyl

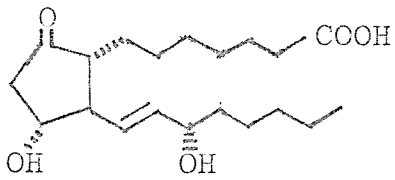
and formation of a double bond in the ring. This dehydration is readily effected chemically and it is considered that some of the A prostaglandins derived from natural sources are in reality artifacts obtained from the E prostaglandins during the isolation procedure.[1]

Some important prostaglandins are represented in the following scheme (Scheme I) by diagrams of their molecular structure. In general, all the prostaglandins are variants of a basic 20-carbon carboxylic (COOH-bearing) fatty acid incorporating a five-member cyclopentane ring. Slight structural changes are responsible for quite distinct biological effects. Prostaglandins of the 1,2 and 3 series respectively incorporate one, two and three double bonds. The molecules designated P_GE and P_GF are called primary prostaglandins; the P_GE structures have an oxygen atom (O) attached to the cyclopentane ring at carbon site 9, whereas the P_GF structures have a hydroxyl (OH) group at the same site. Dehydration of a P_GE molecule leads to either a P_GA or a P_GB compound.

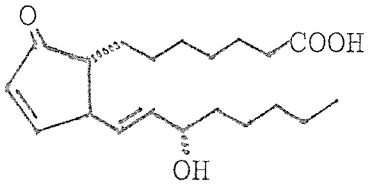
The principal interest in the prostaglandins has focused on their remarkable versatility and the wide range of their effects. The effects themselves may be quite specific: for example, one prostaglandin, P_GE_2 (IV), lowers blood pressure, whereas a closely related member of the family P_GF_2-alpha (V), raises blood pressure. In general,



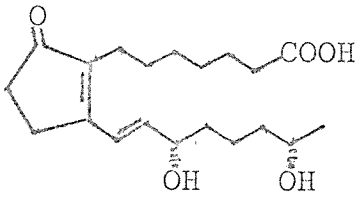
Prostanoic Acid (II)



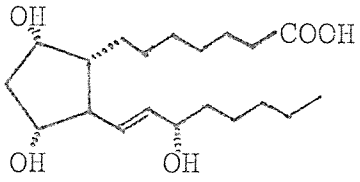
P_GE_1 (III)



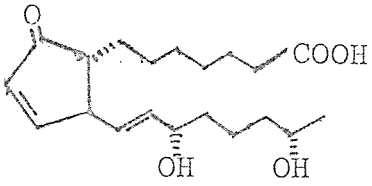
P_GA_1 (VII)



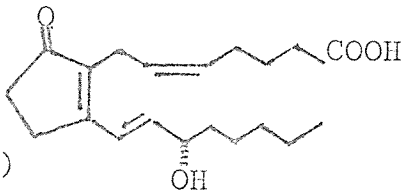
19-Hydroxy-P_GB_1 (XII)



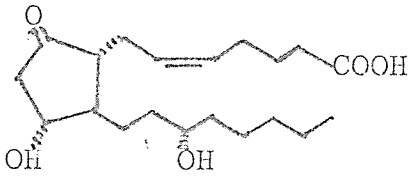
P_GF_1-alpha (I)



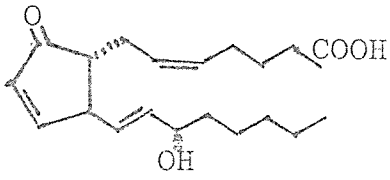
19-Hydroxy P_GA (VIII)



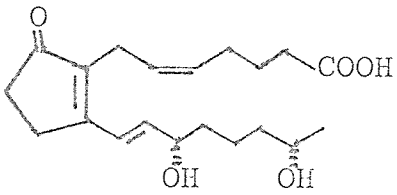
P_GB_2 (XIII)



P_GE_2 (IV)



P_GA_2 (IX)



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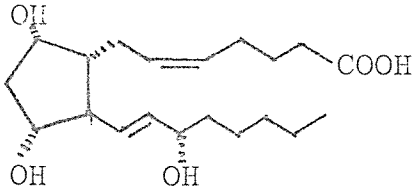
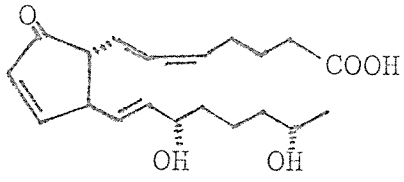
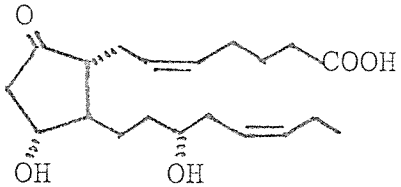


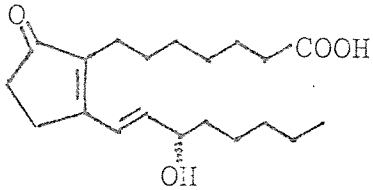
P_GF_2-alpha (V)



19-Hydroxy P_GA_2 (X)



P_GE_3 (VI)



P_GB_1 (XI)

SCHEME I[2]

the effects of the prostaglandins are based on certain broad powers: regulation of the activity of smooth muscles, of secretion, or of blood flow.[3]

Of particular interest are the effects of prostaglandins on the female reproductive system. It has been shown that an intravenous injection of a very low dose of either P_GE_2 (IV) or P_GF_2-alpha (V) stimulates contraction of the uterus. This finding, together with the finding that prostaglandins are present in the amniotic fluid and in the venous blood of women during the contractions of labor, suggested that the prostaglandins may play an important role in parturition. The substances have been used to facilitate childbearing labor in several thousand women.[2] Further studies of the most effective dosage and route of administration of the drug, as well as evaluation in comparison with other methods of inducing labor, may lay a basis for wide adoption of the use of a prostaglandin for this purpose.

The possible use of prostaglandins as agents for abortion and for inducing menstruation is also being investigated. There is evidence that prostaglandins produce these effects by some process more complex than the mere stimulation of uterine contraction. One suggested explanation is that the prostaglandin may induce regression of the corpus luteum, an event that generally does not occur after an ovum is fertilized.[2] There seems to be a strong possibility that prostaglandins or perhaps synthetic analogues may become

important agents in controlling population growth.

As another illustration of their versatility, the prostaglandins seem to hold promise for the prevention of peptic ulcers. Experiments have shown that P_GE_1 (III) or P_GE_2 (IV) can prevent peptic ulcers in dogs and prevent gastric and duodenal ulcers in rats. It seems well established that ulceration of the stomach and the duodenum is generally caused by prolonged exposure of their mucous membrane to gastric juice of high acidity and peptic potency. The stomach normally produces prostaglandins of the E series, and these may serve to regulate gastric secretion under normal circumstances and thus protect the stomach wall against ulceration.[2] If the studies on dogs and rats are confirmed in man, the administration of E prostaglandins may be a helpful treatment for ulcer patients who lack this normal protection. Among other potential biological applications are (1) Asthma: While they shut down gastric secretion, prostaglandins seem to have a special talent for relaxing the smooth muscles of the bronchial tubes and thus opening up the air passages of the lungs. Unlike other bronchodilators, prostaglandins seem to work just in the lungs. The short life-span prevents the spread of side effects and expecially cardiovascular stimulation;[3] (2) High Blood Pressure: Tests have demonstrated that prostaglandin A can lower blood pressure in patients with essential hypertension, apparently by increasing the flow of urine and the excretion of sodium

ions;[3] (3) Arthritis: Zurier and Quagliata of New York University Medical Center have induced arthritis in a group of rats and then treated some with P_GE_1 (III).[3] The untreated rats developed severe arthritis, while those treated showed no symptoms at all. If nothing else, it is clear that prostaglandins play a considerable role in the inflammation process;[3] (4) Clearing the nasal passages: Applied topically to the nose, P_GE_1 (III) has been found effective in widening the passages by constricting the blood vessels;[2] (5) Regulating metabolism: P_GE_1 (III) has been shown to counteract the effects of many hormones in stimulating the metabolic processes, for example, the breaking down of lipids in fatty tissues.[2]

Recently some English scientists discovered that prostaglandins acted as mediators in certain kinds of inflammation and headache.[3] It turns out that the action of aspirin could be at least partly explained in terms of the way aspirin blocked the synthesis of the prostaglandins responsible. Still even more recently, researchers from the University of California reported that prostaglandins produced by certain bacteria on the teeth might be the culprits behind periodontal disease.[3] When administered as a drug, a tiny dose of prostaglandin can either boost or inhibit the body's natural production of prostaglandin.

Much evidence now points strongly to the likelihood that the prostaglandins play a fundamental and critical

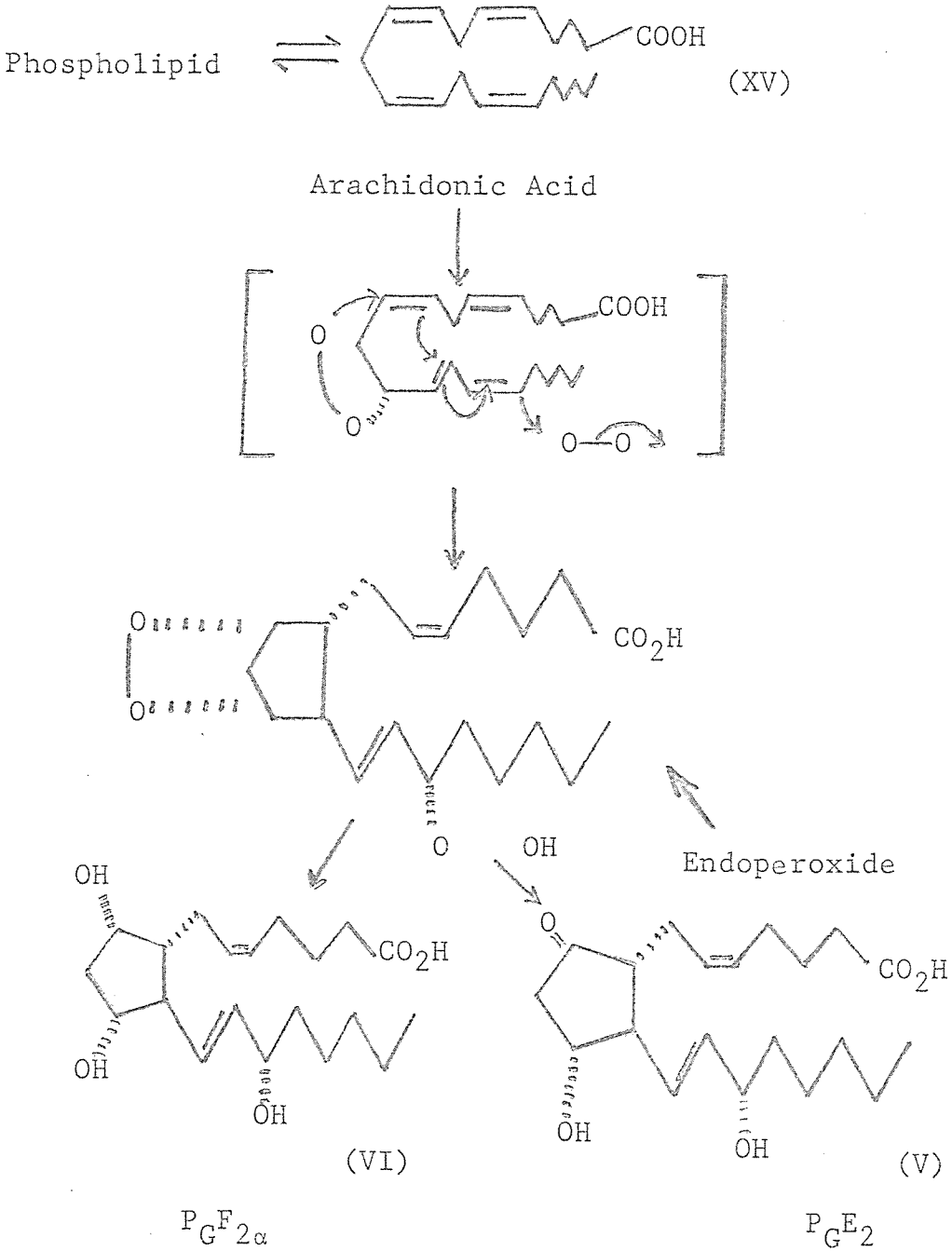
role in the physiology of mammals. Production and release of prostaglandins can be evoked by stimulation of nerves. In recent experiments, testing preparations of the spleen of the cat and the heart of the rabbit, it was found that infusion of the prostaglandin E_2 (IV) markedly inhibited the release of norepinephrine by nerve stimulation.[2]

Prostaglandin formation has been noted in many other apparently unrelated systems. For example, prostaglandins are formed by the lungs during anaphylaxis, by the kidney when its blood supply is restricted, by the surface of the brain when peripheral sensory nerves are stimulated, by the skin during human allergic contact eczema and during certain experimental inflammatory conditions. These diverse situations suggest that prostaglandins may play a fundamental role not only in normal physiological functions, but also in certain pathological conditions.[4]

The prostaglandins are biosynthesized from certain essential fatty acids by a microsomal enzyme system, prostaglandin synthetase (P_GBS), which is widely distributed in mammalian tissues. They do not appear to be stored free in tissues but instead are biosynthesized and released on demand. For example, arachidonic acid (XV), the precursor for P_GE_2 (IV) and P_GF_2-alpha (V), is stored in tissues as a phospholipid and is liberated by activation of a phospholipase "A" by a variety of physiological stimuli. The hydrolyzed arachidonic acid (XV) serves as a substrate for

the P_GBS and is converted to prostaglandins of the "two" series via a key cyclic endoperoxide intermediate as shown in Scheme II.[5]

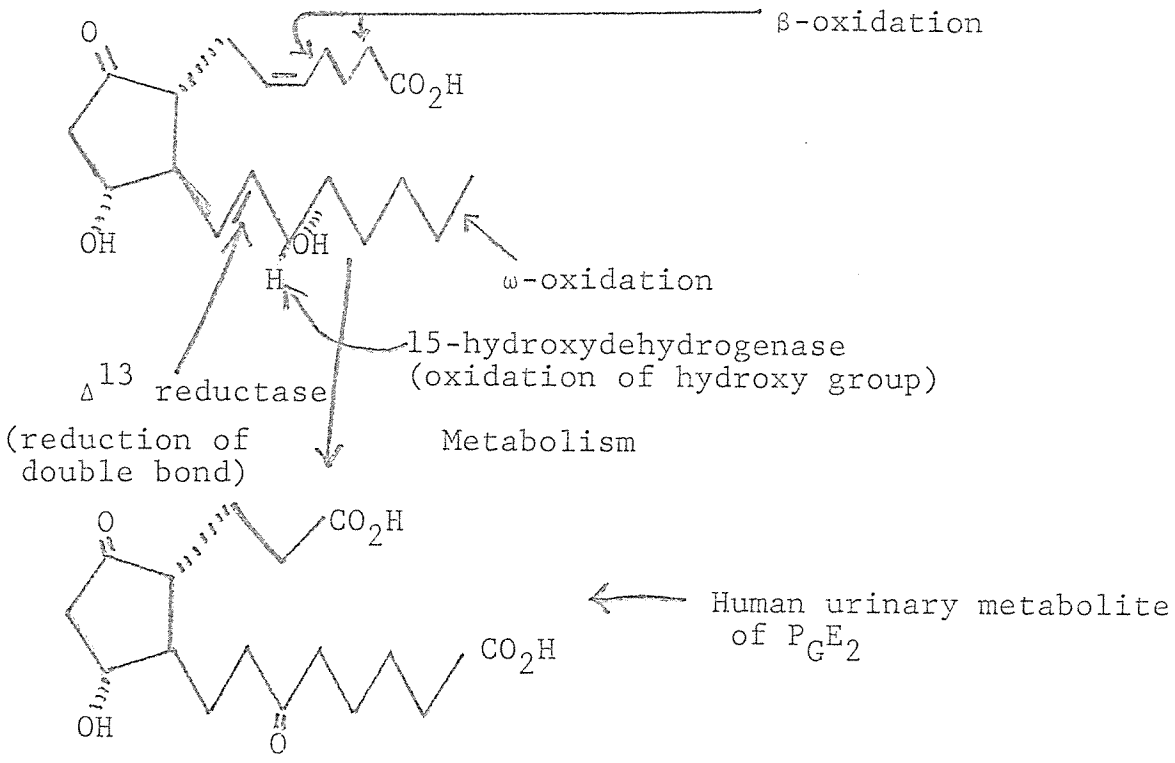
BIOSYNTHESIS OF PROSTAGLANDINS



SCHEME II

Once formed, the prostaglandins are rapidly inactivated by enzymatic reactions which limit their sphere of influence. The primary metabolic step is oxidation of the C-15 allylic hydroxyl by means of 15-hydroxy-prostaglandin dehydrogenase, followed by reduction of the 13,14-double bond by delta-13-prostaglandin reductase. The major metabolic transformations in humans are outlined in Scheme III.[5]

METABOLISM OF P_GE_2 IN HUMANS



SCHEME III

In recent years, total chemical synthesis has assumed major importance as a means of obtaining supplies of prostaglandins and it is now the method of choice. Unlike

biosynthesis, it is not subject to the limitations imposed by availability of an enzyme and it is capable of scale-up to the production of substantial quantities of material. Another advantage of total chemical synthesis is its flexibility which enables the preparation of unnatural stereoisomers and novel structural variants unobtainable by any other means. Some of these have been prepared in an attempt to overcome clinical shortcomings of the natural forms such as their brief duration of action resulting from rapid metabolic inactivation or the presence of side effects as a consequence of their wide spectrum of biological activity. Other novel synthetic forms have the advantage over the natural compounds of greater ease of access owing to the possibility of preparation by shorter synthetic procedures.

Prostacyclin is one of the most recently discovered exciting members of the prostaglandin family. Called prostacyclin because it contains a second five-membered ring in addition to the one common to all prostaglandins, the compound was first discovered in late 1976 by Vane of Wellcome Research Laboratories in the United Kingdom and his co-workers.[7] As a result of its very recent discovery, information on it is very limited.

Prostacyclin is the most potent of the prostaglandins yet discovered in inhibiting aggregation of blood platelets, and it also dramatically dilates blood vessels. As potentially important as prostacyclin is, both its study and its ultimate

usefulness in medicine are limited by a very short biological half-life of one or two minutes. Consequently, several laboratories have been trying to develop more stable analogs that retain the potency of the natural compound.

The work described in this thesis gives attention only to the design of synthesis of the "primary" prostaglandins F and E, and to the prostacyclin I. The starting point for the synthesis is 16,16-dimethyl P_GF_1-alpha (I). It proceeds through various types of functional groups and their placement on the skeleton. The relative positioning of functional groups is the key to understanding their interactions and the design of synthesis.

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Associates A-60A spectrometer using deuteriochloroform solutions. A Beckman IR 424 was used to record spectra (neat) on potassium bromide plates. Removal of solvents in vacuo refers to evaporation at aspirator pressure on a rotary evaporator. Results of thin layer chromatography were determined by spraying plates with a sulfuric acid solution. Mass spectra were run with a Dupont 21-490 mass spectrometer.

Synthesis of 16,16-Dimethyl P_GF_2-α Methyl Ester (XLI).--Diisopropylethylamine (2.5 ml) was added to a solution of 2.42g (6.3 m mol) of 16,16-dimethyl-P_GF_2-alpha (XL) in 50 ml of acetonitrile. After addition of 5 ml of methyl iodide, the mixture was stirred at room temperature for 3 hr. The reaction mixture was checked for the presence of starting material by thin layer chromatography (100% ethyl acetate). Upon determination of the completion of the reaction, the mixture was concentrated to one-fourth of its volume and diluted with 100 ml of ethyl acetate. The solution was then washed with potassium hydrogen sulfate solution (2x50 ml), once with brine, dried over magnesium sulfate, and evaporated to yield 1.78g of a yellowish oil. The oily residue was dissolved in methylene chloride and chromatographed over 100g of silica gel, eluting with 50, 75, and 100% ethyl acetate/skelly solvent B. From the 75%

elutant, there was obtained 1.73g (68%) of the methyl ester: ir (CCl_4) 3400 (OH), 1740 (ester, C=O), 1100 cm^{-1} (methyl ester); NMR ($CDCl_3$) α3.7 (s, ester); mass spectrum (m/e) 396 prominent peaks at 378, 360 ($M^{+}18$, $M^{+}36$).

Synthesis of 5-Iodo-6 R,S,-16,16-Dimethyl PGI_1 Methyl Ester (XLII).--With stirring, a solution of 1.82g (4.6 m mol) of the methyl ester (XLI), 38 ml of methylene chloride and 38 ml of saturated sodium carbonate solution was cooled in an ice-water bath. After 10 min, a solution of 1.52g of iodine in 95 ml of methylene chloride was added over a 15 min period. The reaction was then allowed to progress for 1 hr. Completion of the reaction was determined by thin layer chromatography (100% ethyl acetate). When no starting material was detected, the organic phase was separated and 10% sodium bisulfate solution added until the layer decolorized. The organic phase was then washed with brine, dried over magnesium sulfate, filtered and evaporated. The oily residue was dissolved in methylene chloride and chromatographed over 100g of silica gel eluting with 50, 75, and 100% ethyl acetate/skelly solvent B. Fractions of 50 ml volume were collected. The product (2.40g, 99%) was recovered from the 75-100% ethyl acetate fractions as a yellow colored oil: ir (CCl_4) 3420 (OH), 1735 (ester, C=O), 1090 cm^{-1} (methyl ester); NMR ($CDCl_3$) α5.5 (multiplet), α4.5 multiplet, α3.65 (singlet); mass spectrum (m/e) 651, other prominent peaks at 567 and 545; Anal: Calcd. for

$C_{28}H_{52}Si_2O_5I$: 651.2400. Found: $(M^{+}-CH_3)$ 651.2383.

Synthesis of 16,16-Dimethyl P_GI_1 Methyl Ester (XLV).--Tributyl tin chloride (2.5 ml) and 40 ml absolute ethanol, under nitrogen, were added to a solution of 2.4 g (4.6 m mol) of the iodo ether. With continuous stirring, a solution of 0.5g of sodium borohydride in 20 ml absolute ethanol was added over a 30 min period. The reaction was allowed to progress over a 2.5 hr period. It was followed by thin layer chromatography on silica gel with 33% ethyl acetate/skelly solvent B and 50% acetone/methylene chloride as the developing agents. After acidifying with dilute hydrochloric acid, the solution was concentrated to approximately one-third its volume, diluted with 50 ml water and extracted 3 times with 50 ml portions of ethyl acetate. The combined extracts were washed once with brine, dried over magnesium sulfate and evaporated. The residue was purified by chromatography over 100g silica gel. The column was eluted with 500 ml of 33, 50, 75, and 100% ethyl acetate/skelly solvent B. Fractions of 50 ml volume were collected. Fractions 3-11 (A) afforded 1.68g of less polar material related to the tributyl tin chloride reagent, while fractions 16-27 (B) afforded 1.01g of the desired product. Thin layer chromatographic examination of the product using 50:50 acetone/methylene chloride showed a heavy spot more polar than starting material together with a light,

very slightly less polar spot. The latter is due to the less polar 6-alpha isomer. The following data were obtained for the mixed material: mass spectrum - weak M^+ at 540, prominent ions at (m/e) 509 and 450; ir (CCl_4) 3400 (OH), 1740 (ester, C=O), 1085 cm^{-1} (methyl ester); nmr ($CDCl_3$) α5.5 (m), 4.4 (m), 3.65 (m); tlc (100% ethyl acetate) Rf - 0.37. Anal: Calcd. for $C_{28}H_{53}Si_2O_5$: 525.3411. Found 525.3431.

Synthesis of 16,16-Dimethyl-PGI_1 (XLVIII).--A solution of the methyl ester (XLV)(0.75g, 1.9 m mol), methanol (10 ml) and 3N sodium hydroxide (2 ml) was stirred at room temperature for 3 hr in a nitrogen atmosphere. Thin layer chromatographic examination against starting material was used to determine the completion of the reaction. The reaction mixture was then concentrated under reduced pressure to one-fourth its volume, poured over ice-water and acidified with potassium hydrogen sulfate solution. The organic layer was removed and the aqueous layer was extracted three times with 20 ml portions of ethyl acetate. The combined organic extracts were washed with brine, dried over magnesium sulfate and evaporated (crude weight-0.21g). The crude product mixture was chromatographed over 50g acid-washed silica gel. The column was eluted with 250 ml of 20:80 acetone/methylene chloride, 250 ml of 30:70 acetone/methylene chloride, 500 ml 40:60 acetone/methylene chloride and 1 liter of 60:40 acetone/methylene chloride. Fractions of 50 ml volume were

collected. Fractions 25-28 afforded 140mg (20% yield) of the desired product: ir (CCl_4) 3400 cm^{-1} (OH) ; nmr ($CDCl_3$) α5.48-5.62 (m), 4.55 (m); tlc (A-IX) Rf - 0.33. (The corresponding non-16,16-dimethylated material gave Rf - 0.25 on the same plate). Anal: Calcd. for $C_{30}H_{59}Si_3O$: 585.3670. Found: (for $M^{+}CH_3$) 583.3656.

Synthesis of 16,16-Dimethyl-P_GI_2 Methyl Ester (XLIII).--A solution of 3.3g (6.3 m mol) of the iodo ether, 60 ml toulene, and 6.6 ml of diazabicyclo[4.3.0.]-non-5-ene (DBN) was warmed at 45° for 24 hr. The reaction was followed by thin layer chromatography on silica gel with 1:1 acetone/hexane, A-IX, and ethyl acetate as developers. Completion of the reaction was determined when tlc showed no starting material remaining. The mixture was then transferred to a separatory funnel and washed twice with ice-water. The organic layers were combined and stabilized with triethylamine, dried over magnesium sulfate, filtered and evaporated. A yellowish oil (2.47g) resulted. Thin layer chromatography (ethyl acetate or acetone/hexane 1:1) showed a heavy spot slightly less polar than starting material, a light spot a little more polar than starting material probably due to the delta-4 isomer and a light spot (unknown) less polar than product: ir (CCl_4) 3440 (OH), 1740 (ester, C=O), 1105 cm^{-1} (methyl ester); nmr ($CDCl_3$) α5.53 (m), 4.67 (m), 4.32 (m), 3.67 (s); tlc (ethyl acetate) Rf - 0.55 for product.

Rf - 0.43 for P_G-methyl ester on the same plate.

Synthesis of 16,16-Dimethyl-P_GI_2 Sodium Salt (XLIV).-- A solution of 2.2g of methyl ester (XLIII), 50 ml methanol, 2.2g sodium bicarbonate and 25 ml water was stirred rapidly at room temperature for 48 hr. An additional 24 hr was required for completion of the reaction when thin layer chromatography analysis (100% ethyl acetate) determined that a large amount of starting material remained. The reaction mixture was filtered through charcoal and concentrated under reduced pressure to remove methanol and then diluted with 25 ml of acetonitrile. Upon standing, a small watery layer separated. The organic layer was separated and treated with an additional 125 ml of acetonitrile to induce crystallization. This produced only a very small amount of a white gummy material. Therefore, the mixture was concentrated to an aqueous solution which was frozen and lyophilized to afford 1.68g as a hygroscopic white foam. A p-phenylphenacyl ester assay for the sodium salt was run as follows: a few mgs of prostacyclin sodium salt was dissolved in 0.5 ml dimethyl formamide containing 5% diisopropyl ethyl amine and a few mgs of alpha-bromo p-phenyl acetophenone added. The mixture was swirled, then allowed to stand for 45 min at room temperature. Saturated aqueous sodium bicarbonate (0.5 ml) and ether (0.5 ml) were added to the mixture and both were shaken. The upper ether layer was assayed by thin layer chromatography using 75:25 ethyl acetate/skelly solvent B as developing solvent. Spots were visualized by

spraying with 50% sulfuric acid and heating on a hot plate or viewing under uv light. This assay revealed a heavy spot due to product and a light spot due to a small amount of 6-keto contamination.

Synthesis of 6-Keto-16,16-Dimethyl-P_GF_1-alpha (XLIX).--A solution of the sodium salt (XILV)(1g, 2.48 m mol) in 25 ml water was acidified with 40 ml of dilute hydrochloric acid (10%). A cloudy solution resulted. Approximately 30 ml of ethyl acetate was added to the solution and the resulting mixture was stirred at room temperature for 20 min. The organic layer was removed and the aqueous phase was extracted with ethyl acetate (twice with 25 ml portions). The combined organic extracts were washed with brine, dried over magnesium sulfate and evaporated (crude weight-0.95g). The crude product mixture was applied in methylene chloride and chromatographed over 50g of acid-washed silica gel, eluting with 250 ml of 40:60, 60:40, and 80:20 ethyl acetate/skelly solvent B and 750 ml of 100% ethyl acetate. Fractions of 50 ml volume were collected. The product (0.76g, 77% yield) was obtained from several fractions: tlc (A-IX) Rf - 0.23 (for product), 6-keto $P_GF_{1\alpha}$ (on the same plate) Rf - 0.15; Anal: Calcd. for $C_{34}H_{70}Si_4NO_6$: 700.4280. Found: (for $M^{+}CH_3$, TMS derivative) 700.4307.

Synthesis of p-Phenylphenacyl Ester of 16,16-Dimethyl P_GI_2 (L).--A solution of the sodium salt XLIV (300mg 0.75 m mol) dimethyl formamide (DMF-3 ml) and p-phenylphenacyl

bromide (300 mg) was stirred at room temperature for approximately 3 hr. To this reaction mixture was added ice-water. The mixture was then rinsed in, and extracted twice with ethyl acetate. Additional drops of triethylamine were added, and the mixture was washed in brine and dried over magnesium sulfate. Evaporation of the solvent in vacuo gave 302 mg of a golden oil. The crude product was applied in methylene chloride and chromatographed over 200g of Florisil prepared by slurry with 50:50 ethyl acetate/skelly solvent B containing 1% triethyleneamine. The column was eluted with 100 ml 50:50 ethyl acetate/skelly solvent B and 0.25% triethyleneamine and 200 ml of 75:25 ethyl acetate/skelly solvent B and 0.25% triethyleneamine and 100% ethyl acetate and 0.25% triethyleneamine. Fractions of 25 ml volume were collected. Thin layer chromatographic analysis at this point (100% ethyl acetate) determined the presence of ultra violet visible excess reagent in the earlier fractions with the later fractions containing an unknown mixture. All fractions were recombined and rechromatographed over 200g Florisil in 20:80 ethyl acetate/skelly solvent B and 0.25% triethyleneamine. The column was eluted with 250 ml of 20:80, 30:70, 40:60, and 50:50 ethyl acetate/skelly solvent B and 0.25% triethyleneamine. Thin layer chromatographic analysis in 50:50 ethyl acetate/hexane showed that fractions 2-5 contained excess reagent, fractions 9-11, 0.10g of a clearly less polar substance, and fractions 17-21, the product,

(0.10g, 0.000174 moles, (23%)): mass spectrum - last ion of any significant intensity at (m/e) 684; ir (CCl_4) 3400 cm^{-1} (OH); Anal: Calcd. for $C_{42}H_{62}Si_2O_6$: 718.4085. Found 718.4037.

Synthesis of 6-alpha and 6-beta-16,16-Dimethyl P_GI_1 Isomers via Mercuration of 16,16-Dimethyl-P_GF_2-alpha (XLVII and XLVIII).--A 500 ml, 3-necked flask was charged with 3.6g of mercuric acetate and 30 ml of water. The mixture was stirred until the mercuric acetate dissolved and then treated with 20 ml tetrahydrofuran. A solution of 2.0g of 16,16-dimethyl P_GF_2-alpha (XL) in 40 ml tetrahydrofuran was then added and the mixture stirred for 2 hr at room temperature. A solution of 0.75g of sodium borohydride in 30 ml of 1N sodium hydroxide solution was added in portions over 5 min under a nitrogen atmosphere. After stirring for an additional 20 min the mixture was acidified with dilute hydrochloric acid. Ether (100 ml) was added, then sodium chloride to saturation. The organic phase was separated. The aqueous layer which contained droplets of free mercury was extracted twice more with ether. The combined ether extracts were washed with brine, dried over magnesium sulfate and evaporated to afford 2.49g of oil. Thin layer chromatography (ethyl acetate:cyclohexane:acetic acid - 40:60:2) indicated 3 close spots. The major spot was in the center. The more polar minor spot appeared to be unreacted starting material. Multiple attempts to separate the mixture via chromatography over standard CC-4 silica gel using both methylene chloride/

acetone and ethyl acetate/skelly solvent B systems gave only marginal success. Ultimately, best results were obtained using high pressure liquid chromatography over a 141g acid-washed silica gel column eluting with 20-50% acetone in methylene chloride. This afforded 0.24g of the tlc-homogeneous 6-alpha isomer (Rf-0.36 in 60:40:2) and 0.58g of 6-beta isomer (Rf-0.33 in A-IX system).

Synthesis of 6-Keto-16,16-Dimethyl P_GF_1-alpha Methyl Ester (LI).--A solution of 2.29g (6.0 m mol) of the ether (XLIII) in 100 ml of ether and 25 ml of tetrahydrofuran was treated with 25 ml of 5% hydrochloric acid and stirred for 20 minutes. The organic layer was separated and the aqueous layer was washed once with ether. The combined ether extracts were washed twice with brine, dried over magnesium sulfate, filtered and evaporated. This resulted in a crude weight of 2.70g. The yellow residue was chromatographed over 100g of silica gel and eluted with 250 ml of 40:60 ethyl acetate/skelly solvent B, 500 ml of 80:20 ethyl acetate/skelly solvent B and 1500 ml of 100% ethyl acetate. Fractions 21-25 ((A)-0.80g) and fractions 26-77 ((B)-1.07g) were combined. Fraction A was rechromatographed over 50g silica gel using the same elutants to give more material like cut B. The overall yield was 1.77g (74%). Anal: Calcd. for $C_{34}H_{70}Si_4NO_6$: 700.4280. Found: (for M^+-CH_3 of methoxime TMS derivative) 700.4307.

Synthesis of 11,15-Bis-Tetrahydropyranyl Ether of 6-Keto-16,16-Dimethyl-P_GF_1-alpha Methyl Ester (LII).--

A mixture of 1.77g (4.3 m mol) of 6-keto-16,16-dimethyl P_GF_1-alpha methyl ester (LI), 25 ml methylene chloride, 3.5 ml dihydropyran, and 1.5 ml pyridine hydrochloric acid in methylene chloride solution was stirred at room temperature for 24 hr. Thin layer chromatographic analysis using 50:50 ethyl acetate/cyclohexane demonstrated the completion of the reaction. The mixture was then transferred to a separatory funnel with a small amount of methylene chloride. The organic layer was washed once with saturated sodium carbonate solution, twice with brine, dried over magnesium sulfate and evaporated. The yellow residue was chromatographed over 50g of silica gel using 500 ml of 10:90 and 25:75 acetone/methylene chloride as elutants. The combination of appropriate fractions on the basis of thin layer chromatographic results using 33:67 ethyl acetate/cyclohexane afforded 1.86g of a less polar substance and 0.48g of a more polar substance. The less polar substance was rechromatographed using slurry of 50g silica gel made up with 100% methylene chloride. The column was eluted with 300 ml 100% methylene chloride, 750 ml 5:95 acetone/methylene chloride and 500 ml 20:80 acetone/methylene chloride. By thin layer chromatographic analysis in 33:67 ethyl acetate/cyclohexane, it was determined that an additional 1.11g contained product.

These fractions (total of 1.59g) were shown to be contaminated by thin layer chromatography, but this is typical behavior for a 6-keto-P_GF_1-alpha methyl ester structure.

Synthesis of the 11,15-Bis-Tetrahydropyranyl Ether of 6-Keto-16,16-Dimethyl P_GE_1 (LIV).--A mixture of 1.59g (2.8 m mol) of the crude ester, 35 ml methanol and 10 ml 3N sodium hydroxide was stirred at room temperature for 4 hours under nitrogen. After cooling in an ice-bath, solid sodium chloride and potassium hydrogen sulfate solution were added to saturate and acidify the solution respectively. The solution was then extracted twice with ethyl acetate. The combined extracts were washed with brine, dried over magnesium sulfate, and evaporated. Thin layer chromatographic analysis (A-IX) at this point demonstrated completion of the reaction. An 89% yield of a yellow oil was obtained. With stirring in a methanol-ice bath, a solution of 1.39g of the yellow oil in 50 ml acetone was treated dropwise with 3.5 ml of Jones' reagent over a 5 minute period. The mixture was stirred in a cooling bath for an additional 30 minutes. Thin layer chromatographic analysis in 60:40:2 demonstrated the completion of the reaction. Isopropanol (10 ml) was added to this solution and the reaction mixture was stirred for 10 minutes. The reaction mixture was then concentrated to one-fourth of its volume, extracted three times with ether, washed once with brine, and evaporated. A yellow oil (1.29g)

was chromatographed over 150g of CC-4 silica gel made up with 100% methylene chloride. The column was eluted with 250 ml of methylene chloride, 1 liter of both 5:95 and 10:90 acetone/methylene chloride, and 500 ml 25:75 acetone/methylene chloride. Fractions of 50 ml volume were collected. Thin layer chromatographic analysis at this point in 40:60:2 showed considerable contamination, but fractions 6-8 (A) (0.06g), fractions 9-11 (B) (0.05g) and fractions 12-47 (C) (0.90g) were obtained. Fraction C was determined to contain the desired product (58% yield).

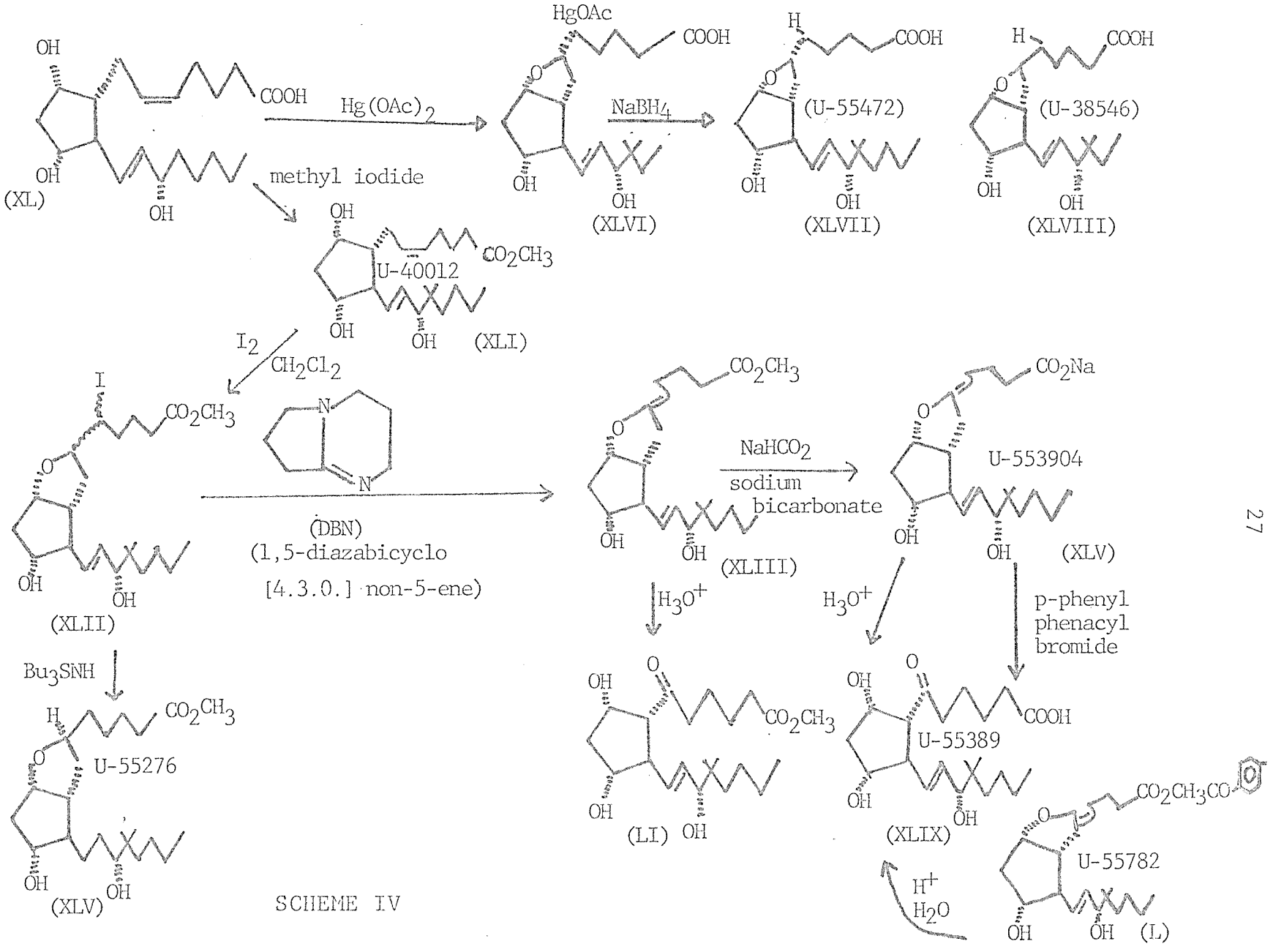
Synthesis of 6-Keto-16,16-Dimethyl P_GE_1 (LV).--With swirling, bis-tetrahydropyranyl ether (0.90g) (LIV) was dissolved in 10 ml acetic acid, 4 ml water and warmed at 40-45° for 4 hr. Thin layer chromatographic analysis in A-IX demonstrated the completion of the reaction. The solution was then diluted with brine, and extracted three times with 25 ml portions of chloroform. The combined organic layers were then washed twice with brine, dried over magnesium sulfate and evaporated at 40-45° to dryness. The yellow residue was chromatographed over a column made up with 50g CC-4 silica gel in 25:75 ethyl acetate/skelly solvent B. Additional elutions were made with 250 ml of 25:75 ethyl acetate/skelly solvent B. Fractions of 50 ml volume were collected. The combination of fractions on the basis of thin layer chromatographic results using A-IX afforded 0.21g (33% yield) of the desired product. The appearance of an

ultraviolet-visible by-product as seen in previous analogs was not evident: mass spectrum (m/e) 612 (weak), and other very weak ions at 522, 513; uv max (95% C_2H_5OH) 278 nm, trace at 378 nm.

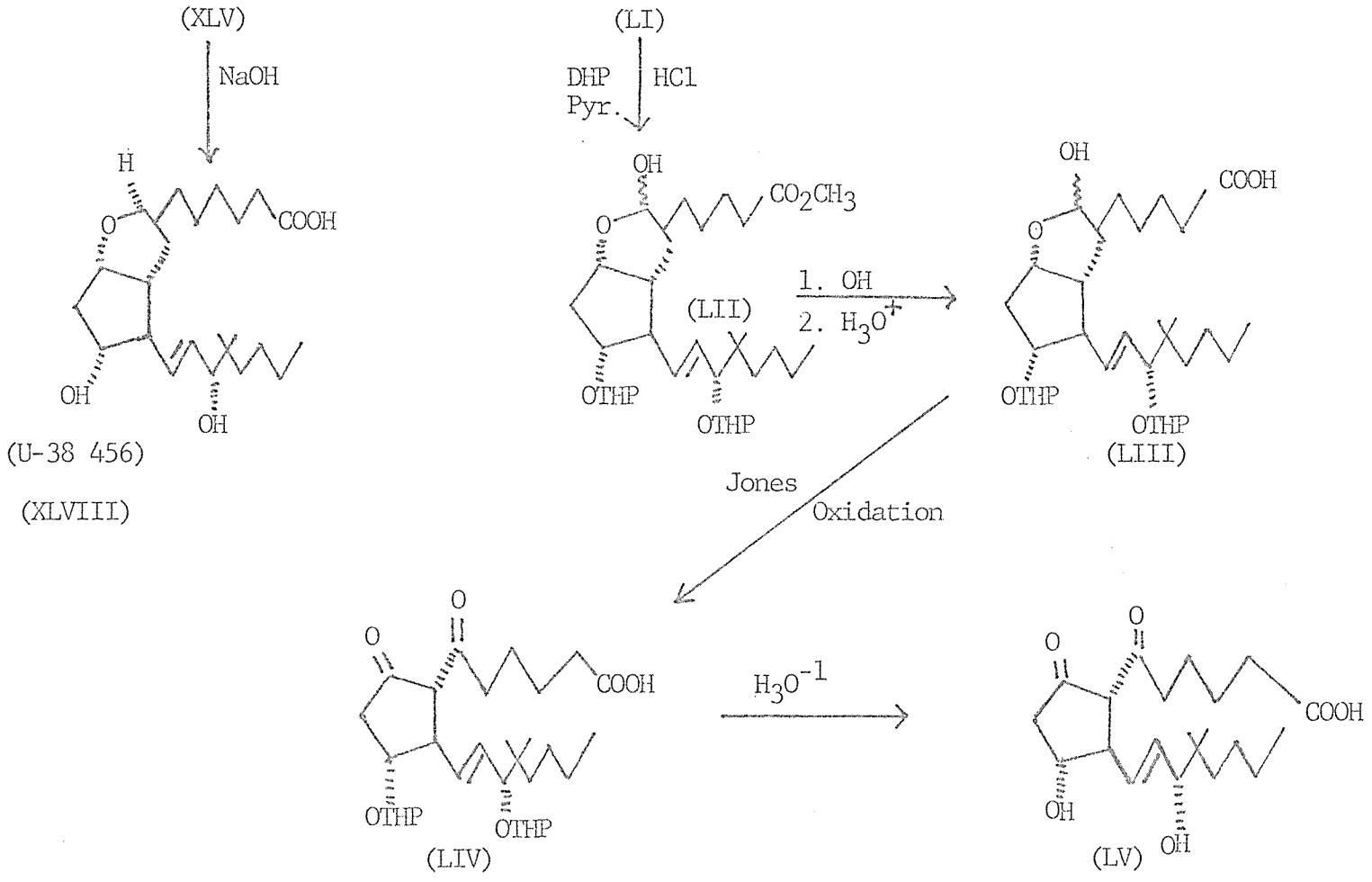
RESULTS AND DISCUSSION

The sequence used is shown in Scheme IV.[8] The reaction of 16,16-dimethyl P_GF_2-alpha (XL), with methyl iodide resulted in a 68% yield of the corresponding methyl ester (XLI). The methyl ester (XLI) was identified by mass spectometry, infrared, and mainly by a singlet in the nmr at α3.7 ppm. Iodination of 16,16-dimethyl P_GF_1-alpha methyl ester (XLI) afforded 5-iodo-6,-R,S,-16,16-dimethyl P_GI_1 methyl -ester (XLII) in nearly quantitative yield. XLII was identified by the appearance of a strong ion at (m/e) 651. No effort was made to separate the isomers. Deiodination of (XLII) with tri-butyl tin hydride prepared in situ from tri-butyl tin chloride and sodium borohydride gave the dominant 6-beta-P_GI_1 methyl ester (XLV) in 33% yield. (XLV) was identified by a weak M+ at 540 in the mass spectrum. Hydrolysis with 3N sodium hydroxide produced the corresponding free acid, 16,16-dimethyl, P_GI_1 (XLVIII), which had been previously prepared.[9]

Dehydrohalogenation of the iodo-ether (XLII) with 1.5 diazabicyclo [4.3.0] non-5-ene (DBN) gave crude 16,16-dimethyl P_GI_2 methyl ester (XLIII) in 88% yield. The nmr spectrum, ($CDCl_3$) α5.53 (multiplet), α4.67 (multiplet), α4.32 (multiplet), and a α 3.67 (singlet) was mainly used to identify this compound. An organic base, 1,5 diaza-bicyclo 4.3.0 non-5-ene (DBN) has been claimed to be the


(XL)
Hg(OAc)2
NaBH4
(XLVI)
(U-55472)
(XLVII)
(U-38546)
(XLVIII)
methyl iodide
U-40012
(XLI)
I2
CH2Cl2
(XLII)
(DBN)
(1,5-diazabicyclo
[4.3.0.] non-5-ene)
(XLIII)
NaHCO2
sodium
bicarbonate
U-553904
(XLV)
H3O+
p-phenyl
phenacyl
bromide
Bu3SNH
U-55276
(XLV)
(LI)
U-55389
(XLIX)
U-55782
(L)
H+
H2O

SCHEME IV



SCHEME IV (CON'T)

most versatile dehydrohalogenating agent known. DBN is made readily and is available commercially. It is a strong proton abstractor and its cation probably forms an unusually stable ion pair with the leaving halide group.

The previously mentioned methyl ester (XLIII) without purification was converted to the more stable salt by hydrolysis with sodium bicarbonate in aqueous methanol. A p-phenyl phenacyl ester assay was used for the identification of the sodium salt. The salt did not crystallize as does P_GI_2 sodium salt, therefore the product was frozen and lypholized to afford a 33% yield as a hygroscopic white foam. Treatment of the salt with p-phenyl phenacyl bromide in dimethyl formamide gave the p-phenyl phenacyl ester of 16,16-dimethyl P_GI_2 (L) in a 23% yield. The mass spectrum showed the last ion of any significant intensity at (m/e) 684. The material exhibited the correct tlc mobility when freshly prepared, but on standing, rapidly decomposed to the stable 6-keto P_GF_1-alpha derivative. Hydrolysis of the sodium salt under acidic conditions (10% HCl) gave 6-keto 16,16-dimethyl P_GF_1-alpha (XLIX) in 77% yield. Thin layer chromatography (A-IX) showed an Rf of 0.15.

The sequence of iodination, dehydroiodination and hydrolysis were repeated to produce the 6-keto 16,16-dimethyl P_GF_1-alpha methyl ester (LI). The reaction of a mixture of 6-keto 16,16-dimethyl P_GF_1-alpha methyl ester

(LI) with dihydropyran and pyridine HCl in methylene chloride solution resulted in positions 11 and 15 being protected as tetrahydropyranyl ethers. Subjecting the corresponding free acid (LIII) to Jones' oxidation by dropwise treatment with Jones' reagent over a 5 minute period and removing the protective groups afforded 6-keto 16,16-dimethyl P_GE_1 (LV).

All 16,16-dimethyl analogs reported were obtained as oils.

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