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Total Synthesis of a Novel Isoprostane $IPF_{2\alpha}$-I and Its Identification in Biological Fluids

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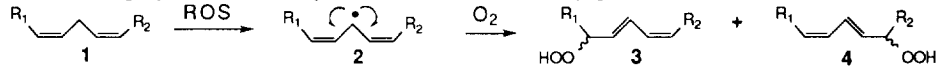
Abstract: The first total synthesis of $IPF_{2\alpha}$-I **25** is described using D-glucose as starting material. This novel isoprostane has been used to establish its presence in human urine. Copyright © 1996 Elsevier Science Ltd

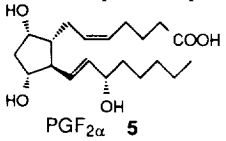
More and more we have come to the realization that, in addition to the necessary enzymatic machinery which keeps us alive, a parallel non-enzymatic free-radical-mediated biochemical system is operative.[1] Reactive oxygen species (ROS), such as •OH, •OOH, ROO•, •OO^-, which are products and by-products of enzymatic reactions, and CH_3•CHOH and CH_3•CO and •CH_3, by-products of alcohol consumption have been known for some time to interact with polyunsaturated fatty acids (PUFA) to form conjugated dienes (Scheme 1).[1,2]

Scheme 1

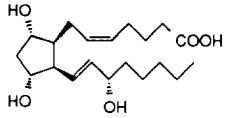


1 2 3 4

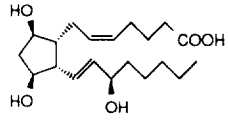
This peroxidation process has been linked to pathological injury in diseases such as heptatorenal syndrome,[3] alcohol-induced liver disease,[4] pulmonary hypertension,[5] myocardial infarction[6] and atherosclerosis.[7] Recently 8-*epi*-$PGF_{2\alpha}$ **6**, a member of a new class of products, the isoprostanes, has been identified in *in vitro* and *in vivo* systems as a product of free-radical peroxidation of arachidonic acid (AA) and has been shown to be a potent vasoconstrictor.[8] It has also been identified as a minor by-product in the enzymatic cyclooxygenase-1 (COX1)[9] and COX2[10] peroxidation of AA. We have performed the first total synthesis of 8-*epi*-$PGF_{2\alpha}$ **6** and its enantiomer **7**,[11] and recently reported the total synthesis of 12-*epi*-$PGF_{2\alpha}$ **8**,[12] an isoprostane potentially formed *in vivo*.



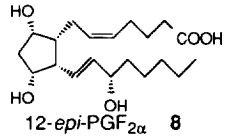
$PGF_{2\alpha}$ **5**



8-*epi*-$PGF_{2\alpha}$ **6**

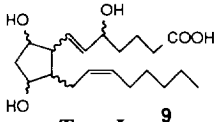


enantiomer of 8-*epi*-$PGF_{2\alpha}$ **7**

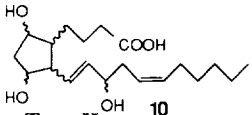


12-*epi*-$PGF_{2\alpha}$ **8**

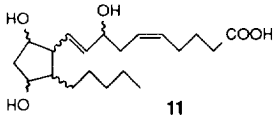
Four types of isoprostanes **9**, **10**, **11** and **12** have been proposed as potential products of ROS-initiated peroxidation of AA.[11-13] They are formed as a result of an initial hydrogen atom abstraction at C_7, C_{10} and



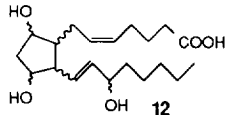
Type I **9**



Type II **10**



Type III **11**

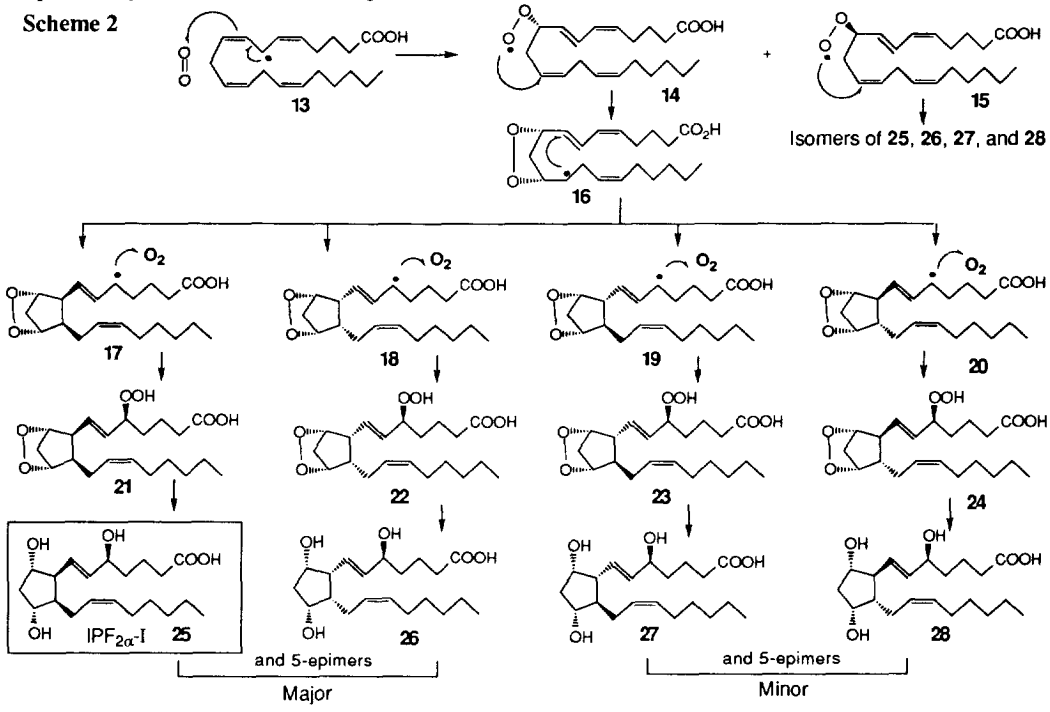


Type IV **12**

C_{13} of AA. Isoprostanes **6**, **7**, and **8** belong to Type IV isoprostanes.

We show in Scheme 2, a detailed analysis of the steps leading to the generation of isoprostanes of Type I from AA by a free-radical peroxidation process. The purpose of this exercise is to help us predict and evaluate the probability of which stereo- and regioisomers are formed *in vivo*.

Scheme 2

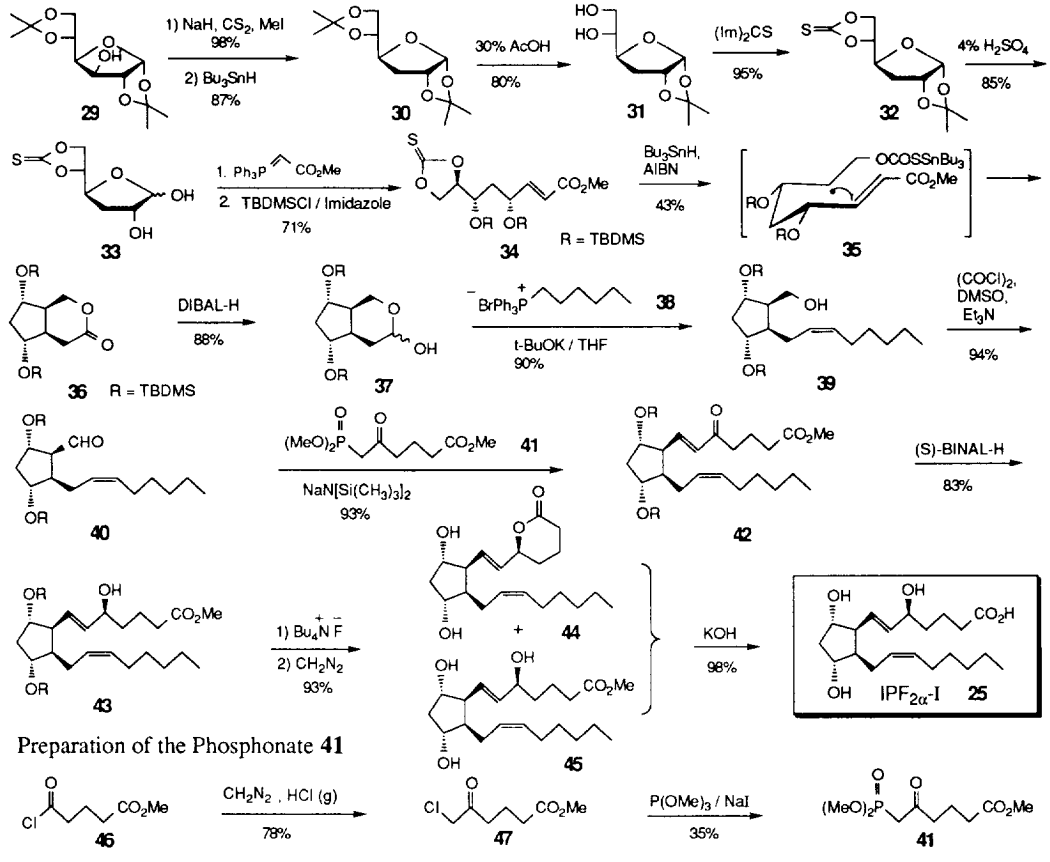


We report here the first proof of the existence *in vivo* of $IPF_{2\alpha}$-I[14] **25**, an isoprostane of Type I **9**, validating our proposal as to the existence of several classes of isoprostanes. This has been achieved by a two-step strategy involving the first total synthesis of $IPF_{2\alpha}$-I **25** and its identification in human urine.

The synthesis of $IPF_{2\alpha}$-I **25** is shown in Scheme 3. The deoxygenation of diacetonide-D-glucose **29** was achieved by first preparing the xanthate derivative in 98% yield using sodium hydride, carbon disulfide and methyl iodide, and then treating it with tri-n-butyltin hydride to give 3-deoxydiacetonide-D-glucose **30** in 87% yield.[15] The chemoselective cleavage of the 5,6-isopropylidene group in **30** with 30% aqueous acetic acid at room temperature afforded the diol **31** in 80% yield. Treatment of diol **31** with thiocarbonylbis(imidazole) in 1,2-dichloroethane at room temperature gave the thionocarbonate derivative **32** in 95% yield. Removal of the 1, 2-isopropylidene group with 4% aqueous sulfuric acid in tetrahydrofuran (THF) at reflux temperature gave the epimeric lactols **33** in 85% yield. The Wittig reaction of **33** with methyl(triphenylphosphoranylidene)acetate in THF was carried out at room temperature and the resulting crude mixture was treated with t-butyldimethylsilyl chloride in dimethylformamide at 60 °C to give **34** in 71% yield. Finally, the cyclization of **34** was achieved using tri-n-butyltin hydride (2 equiv.) and AIBN (0.5 equiv.) in toluene at reflux temperature to afford the *syn-anti-syn* lactone **36** as the major product in 43% isolated yield.[16] The stereoselective formation of the lactone **36** as the major product is probably due to a preferred chair conformation of radical intermediate **35**.

The reduction of lactone **36** with DIBAL-H in methylene chloride at -78 °C, followed by acidic work-up, afforded a mixture of lactol epimers **37** in 88% yield, used as such in the next step. The Wittig reaction with commercial hexyltriphenylphosphonium bromide **38** (4 equiv.) and potassium t-butoxide (3.99 equiv.) at -78 °C

Scheme 3. Synthesis of $IPF_{2\alpha}$-I **25**, *(5S,6E,8β,9α,11α,14Z)-5,9,11- trihydroxyprosta-6,14-dien-1-oic acid*.



Preparation of the Phosphonate **41**

proceeded smoothly to give the *cis* olefin **39** in 90% yield. The Swern oxidation of the alcohol **39** using oxalyl chloride, DMSO, and triethylamine yielded aldehyde **40** in 94% yield. Horner-Emmons reaction of **40** at -78 °C, to introduce the upper side chain using the anion of β-ketophosphonate **41** generated with sodium *bis*(trimethylsilyl)amide in THF at room temperature, afforded the enone **42** in 93% yield. The enantioselective reduction of the C_5 keto group in **42** with chiral reducing agent[17] (S)-BINAL-H proceeded well and afforded the desired pure 5(S) derivative **43** in 83% yield. The deprotection of the *bis*-silyl groups in **43** was carried out using tetrabutylammonium fluoride in THF at room temperature and the crude product was treated with diazomethane to give the lactone **44** and the methyl ester **45**. The mixture of **44** and **45** can be separated by flash column chromatography to afford the pure compounds. Finally, the basic hydrolysis of **44** and **45** with aqueous potassium hydroxide in dioxane at room temperature yielded the desired $IPF_{2\alpha}$-I **25** in 98% yield.[18]

A urine sample from normal volunteers was collected and prepared for GC-MS analysis as described

previously.[9] The TBDMS-pentafluorobenzyl ester (PFB) of the urinary sample was prepared, as well as the *tris*-TBDMS-PFB derivative of synthetic $IPF_{2\alpha}$-I, 8-*epi*-$PGF_{2\alpha}$, and $^{18}O_2$ 8-*epi*-$PGF_{2\alpha}$, used as the internal standard. The retention time of the internal control was 19.655 min. The new $IPF_{2\alpha}$-I peak appears at retention time 19.356 min. This peak was identified by comigration with authentic $IPF_{2\alpha}$-I **25**.

In addition, we converted the urinary $IPF_{2\alpha}$-I to its 6-membered-ring lactone and confirmed its identity by comparison with the synthetic lactone **44** in the following manner. We treated the urinary mixture containing $IPF_{2\alpha}$-I with excess dicyclohexylcarbodiimide. The urinary lactone was purified on TLC using the synthetic lactone **44** as visualization standard. The urinary lactone was isolated, hydrolyzed with KOH/water and the reaction mixture acidified, extracted with ethyl acetate and the solvent evaporated. The GC-MS of the *tris*-TBDMS-PFB ester of the residue was identical to the $IPF_{2\alpha}$-I derivative obtained from the synthetic lactone **44** submitted to the same procedure.

Identification of the new isoprostane, $IPF_{2\alpha}$-I **25**, in biological fluids is a significant step in the effort to evaluate the free-radical-initiated biochemical pathway in disease states.

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14. Assumptions we used to name this isoprostane: IP = isoprostane; F_α = two hydroxyls on the ring with the stereochemistry shown; 2 = two double bonds. The structure as shown provides the basis for naming all isoprostanes of Type I, e.g. the epimer at C_5 in $IPF_{2\alpha}$-I will be called 5-*epi*-$IPF_{2\alpha}$-I.
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18. Spectral data for the $IPF_{2\alpha}$-I **25** : ^{1}H NMR (CD_3COCD_3) δ 5.58-5.48 (m, 2H, C_7-H, C_6-H), 5.46 (m, 2H, C_{14}-H, C_{15}-H), 4.05 (m, 1H, C_5-H), 3.97 (m, 1H, C_9-H), 3.88 (m, 1H, C_{11}-H), 2.68 (m, 1H, C_8-H), 2.4 (q, J = 7.3 and 14.3 Hz, 1H, C_{10}-H), 2.3 (t, J = 7.3 Hz, C_2-H_2), 2.17-195 (m, 5H, C_{12}-H, C_3-H_2, C_{13}-H_2), 1.78-1.6 (m, 2H, C_{10}-H, C_4-H), 1.6-1.48 (m, 3H, C_4-H, C_{16}-H_2), 1.4-1.26 (m, 6H, C_{17}-H_2, C_{18}-H_2, C_{19}-H_2), 0.9 (t, J = 6.8 Hz, 3H, C_{20}-H). ^{13}C NMR (CD_3COCD_3) δ 174.8, 136.7 , 130.9, 129.8, 129.6, 76.2, 76.1, 72.4, 53.7, 51.5, 44.0, 37.9, 34.2, 32.3, 30.2, 29.3, 27.2, 23.3, 22.0, 14.4. HRFAB MS *m/z* calc for $(M+Na)^+$ 377.2304, found 377.2293.

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