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Syntheses and Identification of the Most Abundant Urinary Type VI Isoprostanes

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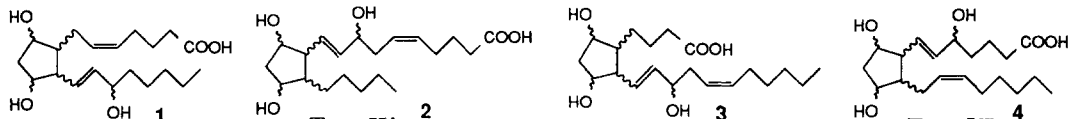
Abstract: The total synthesis of 8-12-*iso*-iPF$_{2\alpha}$ -VI **12** and its 5 epimer **13** is described. With the aid of the synthetic materials these isoprostanes have been identified in and isolated from human urine.

Free radicals, e.g. HO$^{\cdot}$, HOO$^{\cdot}$, ROO$^{\cdot}$, have been implicated in inflammatory and degenerative diseases, e.g. atherosclerosis.[1,2] We and others have shown that isoprostanes (iPs), a new class of natural products isomeric with prostaglandins, are formed *in vivo* by a non-enzymatic free-radical catalyzed peroxidation of polyunsaturated fatty acids (PUFA), e.g. arachidonic acid (AA).[3-5] Four classes of iPs (Scheme 1) can be formed from AA and we have proposed two mechanisms for their formation by a free-radical process.[5,6] iPF$_{2\alpha}$-III and 8,12-*iso*-iPF$_{2\alpha}$-III have been shown to possess potent biological activities.[3,7]

Scheme 1



We report the first syntheses of 8-12-*iso*-iPF$_{2\alpha}$-VI **12** and 5-*epi*-8-12-*iso*-iPF$_{2\alpha}$-VI **13**, all-*syn* isoprostane isomers from Type VI **4** (Scheme 1, 2). With the help of these synthetic compounds, we have isolated and identified the two most abundant isoprostanes discovered to date in human urine. Using the HPLC/MS/MS methodology described in Figure 1, it appears that (isoprostanes) **12** and **13** are each 5 times more abundant than iPF$_{2\alpha}$-VI **14**. Each of **12** and **13** is 20 times more abundant than iPF$_{2\alpha}$-III (not shown). Measurement of group VI isoprostanes described in this paper is preferred to the measurement of iPF$_{2\alpha}$-III because of the higher concentration in urine and also due to the fact that iPF$_{2\alpha}$-III is also produced enzymatically and is a possible source of confusion if one is interested in measuring the free radical peroxidation process contribution only.

The importance we attribute to group VI iPs **4** (Scheme 1) and the individual structures **12**, **13**, **14**, and **15** (Scheme 2) within this group, which is the subject of this report, stems from the unique relationship between the C$_5$-OH and the carboxylic acid. Considering that there is the potential for the formation of a total of 64 isoprostanes from AA by a non-enzymatic free radical peroxidation,[3,5] we sought to exploit this unique property of group VI. For example using GC/MS we have, in the case of iPF$_{2\alpha}$-VI **14** as well as in the present one iPs

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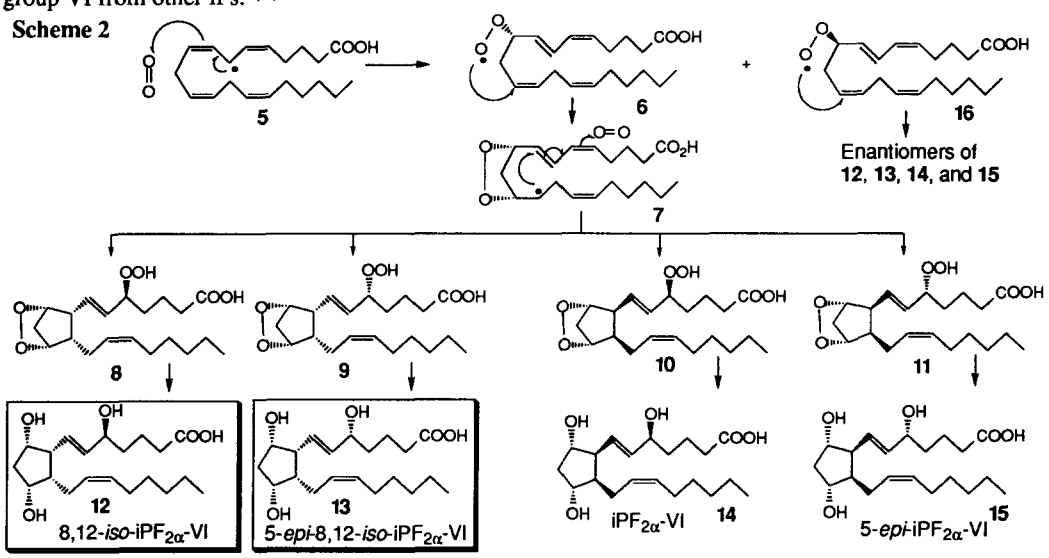
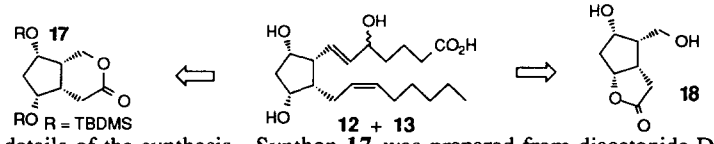
12 and **13**, introduced a lactonization step in the urine isolation procedure which allows the easier separation of group VI from other iPs.[1,2,8]

Scheme 2



The synthetic design for 8-12-*iso*-iPF$_{2\alpha}$-VI and its 5-R-epimer is shown in Scheme 3. We elected in this particular case to use the all-*syn* 6-membered ring lactone **17** in preference to the equivalent 5-membered ring lactone **18**, the synthesis of which we also described recently.[6,9] Using **18** as the starting lactone would have entailed a few extra synthetic steps in the syntheses of **12** and **13**. As can be seen, **17**, which has all the four substituents on the 5-membered ring *syn* or *cis* to each other, contain the exact stereochemical arrangement of the four asymmetric centers necessary for the synthesis of **12** and **13**.

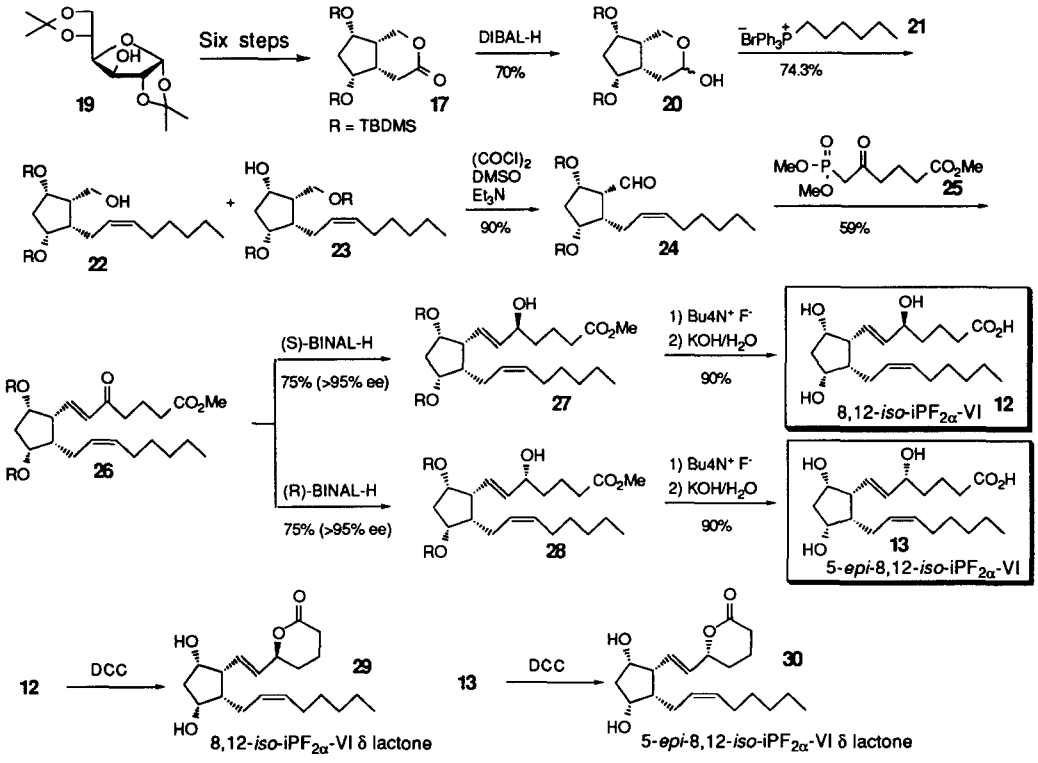
Scheme 3



Scheme 4 shows the details of the synthesis. Synthon **17** was prepared from diacetonide-D-glucose **19** in six steps.[1] The reduction of lactone **17** with DIBAL-H in methylene chloride at -78 °C, followed by acidic work-up, afforded a mixture of lactol epimers **20** in 70% yield, used as such in the next step. The Wittig reaction with commercial hexyltriphenylphosphonium bromide **21** (4 equiv.) and potassium t-butoxide (3.99 equiv.) at -78 °C proceeded to give the *cis* olefin **22** and the compound **23** in 75% yield. Compound **23** can be recycled if required to afford **22.** Compound **23** is formed as a result of the silyl migration from the secondary alcohol to the primary under the basic reaction conditions. The silyl migration is due to the geometry of the all-*syn* substituted bicyclic lactol **20** as we have not observed this type of migration in the Wittig reactions with *syn-anti-syn* lactols.[1,4] The Swern oxidation of the alcohol **22** using oxalyl chloride, DMSO, and triethylamine yielded aldehyde **24** in 90% yield. Horner-Emmons reaction of **24** at -78 °C, to introduce the upper side chain using the anion of β-ketophosphonate **25**[1] generated with sodium *bis*(trimethylsilyl)amide in THF at room temperature, afforded the enone **26** in 59% yield. The enantioselective reduction of the C_5 keto group in **26**

with the chiral reducing agent (S)-BINAL-H [1,4] proceeded smoothly and afforded the desired pure 5(S) derivative **27** in 75% yield. Similarly, reduction of **26** using the (R)-BINAL-H afforded the 5(R)-isomer **28** in 75% yield. The deprotection of the *bis*-silyl groups in **27** using tetrabutylammonium fluoride in THF at room temperature gave a mixture of the lactone **29** and the acid **12**. Finally, this mixture was treated with aqueous potassium hydroxide in dioxane at room temperature to yield the desired 8,12-*iso*-iPF$_{2\alpha}$-VI **12** in 90% yield from **27**.[10] Similarly, **28** afforded **13**, as shown.

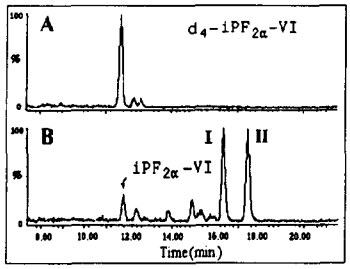
Scheme 4: Syntheses of 8,12-*iso*-IPF$_{2\alpha}$-VI **12** and its 5-epi isomer **13**.



The total synthesis of **12** and **13** described here has been motivated by the following observation. During mass spectrometric experimentation with the synthetic iPF$_{2\alpha}$-VI, we determined its fragmentation pattern using a negative ion (electron capture) chemical ionization mass spectrometer. In particular, we identified a characteristic major fragment ion at *m/z* 115 ($CHO(CH_2)_3COO^-$), which is indicative of an OH at position five in relation to the COOH. Of the four classes of iPs, III **1**, IV **2**, V **3**, and VI **4** (Scheme 1), only class VI has this relationship. Furthermore, examination of the fragmentation pattern of the iPs from human urine, revealed that the two unknown peaks shown in Figure 1 contains the *m/z* 115 ion fragment, an indication that these could be from group VI iPs. We were gratified to find out that our speculation proved to be correct. With the use of the synthetic standards, we assigned the structures **12** to peak II and **13** to peak I in Figures 1, 2, and 3 based on the identity of the biological and synthetic products in two different chromatographic systems. The first is a

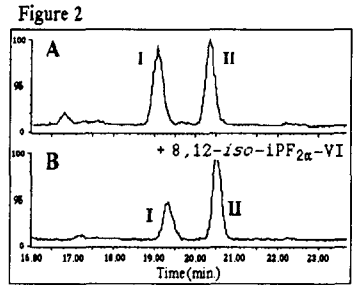
GC/MS comparison and the second is the LC/MS system shown in Figures 2 and 3. In addition, the synthetic lactones **29** and **30** are identical to the biologically derived ones.

Figure 1



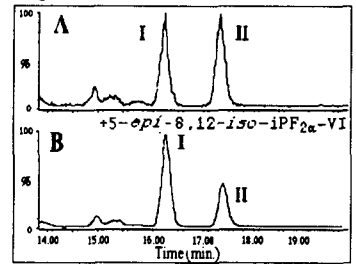
LC/MS/MS of 1 ml urinary extract
Panel A: Product ion *m/z* 357
Panel B: Product ion *m/z* 353
1 ml urine purified as follows:
1) Solid phase extraction using reverse phase cartridges C18 EC, 100 mg eluted with ethyl acetate 2) HPLC using C18, 3μ, 2 x 150 mm reverse phase column.

Figure 2



Panel A: LC/MS/MS of 1 ml urinary extract.
Panel B: Coelution of identical 1 ml urine extract and synthetic (1 ng) 8,12-*iso*-iPF$_{2\alpha}$-VI.

Figure 3



Panel A: LC/MS/MS of 1 ml urinary extract.
Panel B: Coelution of identical 1 ml urine extract and synthetic (1 ng) 5-*epi*-8,12-*iso*-iPF$_{2\alpha}$-VI

We have in this report described the total syntheses of 8-12-*iso*-iPF$_{2\alpha}$-VI **12** and 5-*epi*-8-12-*iso*-iPF$_{2\alpha}$-VI **13** and identified their presence in human urine. The fact that these urinary isoprostanes appear to be the most abundant iPs discovered to date should increase the window of opportunity and provide the basis for a more sensitive noninvasive assay for the measurement of iPs as a lipid peroxidation marker in degenerative diseases. We have used as a first approximation for a quantitative determination the already available 17,17,18,18-tetradeutero iPF$_{2\alpha}$-VI.[8] For a more accurate quantitation the deuterated analogs of 8-12-*iso*-iPF$_{2\alpha}$-VI **12** and 5-*epi*-8-12-*iso*-iPF$_{2\alpha}$-VI **13** will have to be synthesized. Finally, the relative abundance of iPs as measured in urine and described here is not necessarily a reflection of their relative abundance as generated *in situ* in tissues and cells since a metabolic step is involved. Special studies to address this issue are in progress.

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9. Rondot, B.; Durand, T.; Girard, J. P.; Rossi, J. C.; Schio, L.; Khanapure, S. P.; Rokach, J. *Tetrahedron Lett.* **1993**, *34*, 8245-8248.
10. Spectral data for the 8,12-*iso*-iPF$_{2\alpha}$-VI **12** : ^{1}H NMR (CD_3COCD_3) δ 5.9 (dd, J = 10.5 and 15.3 Hz, 1H, C_7-H), 5.45 (m, 2H, C_6-H, C_{14}-H), 5.31 (m, 1H, C_{15}-H), 4.15 (m, 1H, C_5-H), 4.11-4.03 (m, 2H, C_9-H, C_{11}-H), 2.63 (m, 1H, C_8-H), 2.31 (t, *J* = 7.4 Hz, 2H, C_2-H), 2.28-2.2 (m, 2H, C_{10}-H, C_{13}-H), 2.15 (m, 2H, C_{12}-H, C_{13}-H), 1.82 (m, H, C_{10}-H), 1.75-1.65 (m, 3H, C_4-H, C_{16}-H_2), 1.55-1.45 (m, 2H, C_3-H_2), 1.38-1.22 (m, 6H, C_{17}-H_2, C_{18}-H_2, C_{19}-H_2), 0.88 (t, *J* = 6.6 Hz, 3H, C_{20}-H). ^{13}C NMR (CD_3COCD_3) δ 174.8, 138.1, 130.6, 130.3, 129.9, 75.3, 73.0 (2 x C), 51.7, 48.5, 44.2, 37.9, 34.36, 32.4, 30.3, 28.1, 25.2, 23.3, 22.0, 14.3. ESI MS *m/z* calc for (M-1) 353.2, found 353.2.