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SYNTHESIS OF NOVEL PROSTAGLANDIN $F_{2\alpha}$ ISOMERS AND STRUCTURE OF AN ENZYMATICALLY FORMED 13-HYDROXYPROSTAGLANDIN ENDOPEROXIDE

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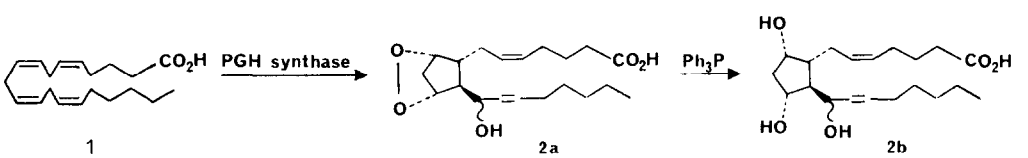
SUMMARY: *The structure of a novel 13-hydroxyprostaglandin endoperoxide (***2a***), which was formed during enzymatic conversion of arachidonic acid (***1***), was elucidated by comparison with four isomeric chemically prepared F-prostaglandins (***6a, 6b, 9a, 9b***). Triphenylphosphine reduction of* **2a** *confirmed identity of the biological material with (5Z, 14Z) (9S, 11R, 13S)-trihydroxyprosta-5,14-dien-1-oic acid,* **6b**.

Although the metabolic fate of arachidonic acid (**1**) has been extensively investigated during the past two decades[1,2], relatively little is known about the short-living radical intermediates of the metabolic cascade and the order of their formation. Recent studies have shown that the oxidative *in vitro* conversion of **1** in the presence of microsomal or purified prostaglandin H synthase not only led to the formation of prostaglandin H_2 (PGH_2) but also yielded other cyclic endoperoxides and monohydroxy acids, which could be partially characterized[3,4]. After reduction (Ph_3P) or isomerization (aqueous buffer) to the corresponding $F_{2\alpha}$ (**2b**) - or E_2-prostaglandins respectively, and mass spectrometric identification, one of the trapped intermediates was tentatively assigned to be a structurally homogeneous 13-hydroxyprostaglandin H_2 (**2a**) (Scheme 1)[4].

Scheme 1

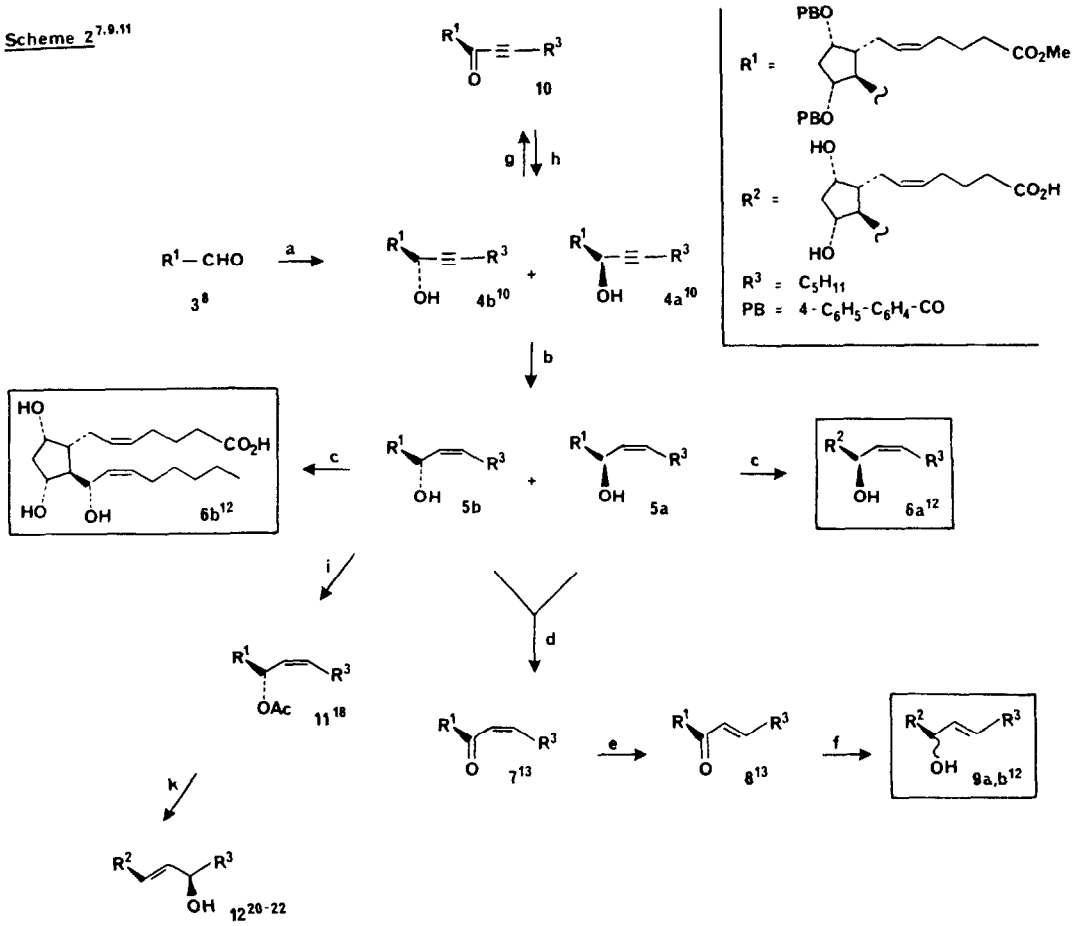


1 **2a** **2b**

In order to establish the absolute configuration of the 13-hydroxy group and the geometry of the olefinic 14,15-double bond of **2a** and to enable unequivocal comparison with the enzymatic product, we decided to prepare the epimeric and cis/trans isomeric $F_{2\alpha}$ -prostaglandins corresponding to the structure of **2b**[5].

The synthetic route is detailed in Scheme 2[6,7]. Addition of 1-lithio-1-heptyne to aldehyde **3**[8] gave a 1:5 mixture of **4a** and **4b**[9-11]. Semi-saturation of the triple bonds, chromatographic separation of the isomers, and protective group removal afforded the epimeric (14Z) F-prostaglandins **6a** and **6b**[12]. For the synthesis of

Scheme 2[7,9,11]



a: 4 equiv. LiC≡C-C_5H_{11}, THF -78°C, 2*h*; the epimers (total yield 65%) were obtained in a ratio 1:5(a:b)[9].- b: 1.H_2, Lindlar's catalyst, quinoline, oxygen-free ethyl acetate (88%) 2. separation of the epimers by silica gel chromatography, mobile phase ethyl acetate/n-hexane 1:2. - c: 1. 10 equiv. K_2CO_3, absol. methanol, 24*h* 2. 10 equiv. 0.1*N* LiOH solution in oxygen-free H_2O, methanol, 24*h*.- d: MnO_2, $CHCl_3$, 7*d* (the reaction was not yet complete), isolation of the product by chromatography.- e: 8 equiv. Pyr·HCl, $CHCl_3$ 40°C, 24*h*.- f: 1. $NaBH_4/CeCl_3 \cdot 6H_2O$, MeOH 2. separation of the epimers by silica gel chromatography 3.= c. - g: 10 equiv. $BaMnO_4$, absol. CH_2Cl_2 24*h*. - h: several reducing agents were employed and the ratio for **4a**/**4b** was determined by HPLC[14].- i : 10 equiv. Ac_2O, absol. Pyr., DMAP. - k: 1. $PdCl_2(CH_3CN)_2$, THF, 48*h* 2. 50 equiv. 0.1 *N* LiOH/ oxygen-free H_2O, methanol, 10*d*.

the 14-trans isomers the allylic alcohols **5a,b** were oxidized to give the cis-enone **7**[13]. After rearrangement of **7** to the more stable trans-enone **8**[13], carbonyl reduction and deprotection, a separable 1:1 mixture of (14E) F-prostaglandins **9a,b**[11,12] was obtained. Chromatographic (GLC, HPLC, TLC) comparison of the enzymatic prostaglandin **2b**, obtained from reductive cleavage of the endoperoxide **2a**, with the four synthetic isomers **6a,b** and **9a,b** proved that the biosynthetic 13-hydroxyprostaglandin was identical with the (14Z) isomer **6** which originates from the major isomer **4b**[9] in the initial addition reaction a (Scheme 2).

In order to establish the absolute configuration of **4,5** and **6** at C-13 the ynone **10** was prepared and then reduced by use of several chiral reagents[14]. However, the results of these reductions were not conclusive[14]. The major isomer **5b**[9] (mp 84-86°C) was therefore acetylated and the resultant acetate **11**[18] was subjected to the palladium (II)- catalyzed allylic acetate rearrangement which is known to proceed under complete chirality transfer[19]. Protective group removal and chromatographic and spectroscopic comparison with authentic material[4] proved complete identity of the rearranged product with 15-epi-$PGF_{2\alpha}$ (15R-$PGF_{2\alpha}$), **12**[20-22]. These results provided clear evidence for the chirality at C-13 of **4b** (13R) and **5b,6b,11**(13S) and completed the structure elucidation of the reduction product **2b** ((5Z,14Z)(9S,11R,13S)-trihydroxyprosta-5,14-dien-1-oic acid,**6b**) of the 13-hydroxyprostaglandin endoperoxide **2a**[23].

Acknowledgments

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6. A similar approach leading to 12,15-epi-11-deoxy-prostaglandins has recently been independently developed: R.Mahrwald, F.Theil,W.I.Melnikova, H.Schick, and K.K.Pivnitsky, *Zh.Org.Khim.* **22**, 1647(1986). We are grateful to Prof.Schick for this notice.
7. The structures shown in Scheme 2 refer to racemic material. All reactions were carried out in an atmosphere of argon.
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9. For the determination of the absolute configuration at C-13 see below.
10. **4b**: m.p. 80-82°C. - High-resolution MS (70eV): calc. for $C_{47}H_{50}O_7$[M]$^+$726.3556, found 726.3554.- MS(DIP,70eV), m/z (%): 726(0.4), 708(1), 533(1), 510(1), 379(1), 330(5), 312(11), 198(90), 181(100), 152(34).

11. The compounds are readily distinguished by silica gel TLC using the solvent systems (I): EtOAc/n-hexane(2:3), (II):EtOAc/n-hexane (1:2), (III): EtOAc/n-hexane(1:3), (IV): EtOAc/AcOH (98:2). R_f values: **4a**, 0.62(I), 0.60(II); **4b**,0.57(I), 0.54(II); **5a**, 0.57(I), 0.51(II), 0.29(III); **5b**, 0.53(I), 0.47(II), 0.23(III); **6a**, 0.48(IV); **6b**, 0.52(IV); **7**, 0.67(II), 0.51(III); **8**, 0.60(II), 0.41(III); **9a**, 0.50(IV); **9b**, 0.53(IV); **10**, 0.63(II), 0.41(III); **11**, 0.60(II); **12**, 0.41 (IV).

$PGF_{2\alpha}$ methyl ester:0.33(IV);15-epi-$PGF_{2\alpha}$ methyl ester:0.50(IV).

12. Rp-HPLC: 5 μm Nucleosil C_{18} (Macherey-Nagel, Düren/FRG), 250 x 4.6(I.D.)mm column, mobile phase water/acetonitrile/methanol/acetic acid (60:37.5:2.5:0.1,v/v), flow rate 1 ml/min, UV detection at 200 nm; t_R(min) =14.9(**6a**), 12.9(**6b**), 15.1 and 15.6(**9a/9b**).- GC/MS (Hewlett-Packard 5985 A): PI/EI, 70eV, source temperature 200°C, 2,3,4,5,6-pentafluorobenzyl ester 9,11,13-tris (trimethylsilyl ether) derivatives (MW=750); m/z(%), **6a**: 660(2), 570(6), 480(5), 421(7),353(6), 263(5), 237(3), 225(33), 217(11), 199(100), 191(8), 181(63); **6b**: 750(7), 679(17), 660(100), 589(22), 570(66), 480(25), 421(17), 353(15), 311(13), 199(48), 181(55); **9a**: 750(0.3), 660(4), 589(3), 570(8), 480(8), 473(2), 421(6), 353(8), 263(5), 237(3), 225(19), 217(8), 199(100), 181(75); **9b**: 750(0.3), 679(1), 660(6), 589(4), 570(6), 480(7), 421(6), 353(10), 263(6), 225(12), 217(8), 199(100), 191(8), 181(89).-

High-resolution MS (70eV), **6b**: calc. for $C_{20}H_{34}O_5[M]^{\cdot+}$ 354.2406, found 354.2410; calc. for $C_{20}H_{32}O_4$ $[M-18]^{\cdot+}$ 336.2300, found 336.2299; calc. for $C_{20}H_{30}O_3$ $[M-2x18]^{\cdot+}$ 318.2195, found 318.2197.- ^{13}C-NMR (75MHz, $CDCl_3$, δ-values in ppm, int.std.TMS) **6b**: 14.01(C-20), 22.52 (C-19), 24.52 (C-3), 26.43/27.05/27.60 (C-4/7/16), 29.32(C-17), 31.52 (C-18), 32.73(C-2), 42.73(C-10), 47.24(C-8), 58.90(C-12), 68.51(C-13), 74.71/75.28 (C-9/11), 129.49/129.52/130.80 (C-5/6/15), 133.45 (C-14), 176.17(C-1).

13. ^{1}H-NMR($CDCl_3$, δ-values in ppm,int.std.TMS) of H-14 and H-15: 7(at 300 MHz), 6.35 (H-14,td, ^{4}J(H-14,16)=1.5 Hz,^{3}J(H-14,15)=11.5 Hz), 6.23(H-15,td, ^{3}J(H-15,16)=7.2 Hz,^{3}J(H-14,15)=11.5 Hz).- **8** (at 80MHz,H-16 decoupled), 6.33(H-14, d,^{3}J=15.6 Hz), 7.00(H-15, d,^{3}J=15.6 Hz).

14. Reductions of **10** by use of sodium borohydride/methanol, (R)-Alpine-Borane[15], (S)-Alpine-Borane[15], K-9-O-DIPGF-9-BBNH[16], NB-Enantrane[17] afforded **4a** : **4b** ratios(%) of 49:51,8:92,6:94,13:87, and 10:90 respectively. Ratios were determined by sp- HPLC on 5 μm silica gel column 300 x 3.9(I.D.)mm, mobile phase EtOAc/n-hexane (1:6) flow rate 1 ml/min, UV detection at 254 nm. Retention times of **4a** and **4b** were 15.3 min and 23.8 min, respectively.

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18. M.p. 74-76°C; calc. for $C_{49}H_{54}O_8$ (771.0) C 76.34, H 7.06; found C 76.16, H 7.04.-
High-resolution MS(70eV) : calc. for $C_{47}H_{50}O_6$ $[M-60]^{\cdot+}$710.3607, found 710.3600.- MS(DIP, 70eV), m/z(%): 710(0.4), 574(0.2), 512(2), 314(31), 199(11), 198(58), 152(40), 117(15), 60(18), 43(30).- ^{13}C-NMR(75MHz, $CDCl_3$ δ-values in ppm, int. std. TMS, selected resonances): 14.04(C-20), 21.21 (CH_3CO), 22.56 (C-19), 24.79(C-3), 29.13(C-17), 31.53(C-18), 33.42(C-2), 39.49(C-10), 45.52(C-8), 51.36(OCH_3), 54.52(C-12), 69.26(C-13), 165.60/165.68(PB-*C*O), 170.13 (CH_3CO), 173.81(C-1).

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23. Further investigations concerning the biological role of **6b** are in progress.

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