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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF 15-OXOPROSTAGLANDINS: CONTRIBUTION OF THE ω-CHAIN ENONE GROUP TO CYTOTOXICITY

Margus Lopp[#*], Annika Lopp[‡], Anne Paju[#], Ülo Lille[‡] and Tõnis Pehk[&].

[#]Department of Organic Synthesis, Institute of Chemistry. Akadeemia tee 15, EE0026 Tallinn, ESTONIA;
[‡]Department of Bioorganic Chemistry, Institute of Chemistry. Akadeemia tee 15, EE0026 Tallinn, ESTONIA;
[&]Department of Physics, Institute of Chemical Physics and Biophysics. Rävala pst 10, EE0001 Tallinn, ESTONIA.

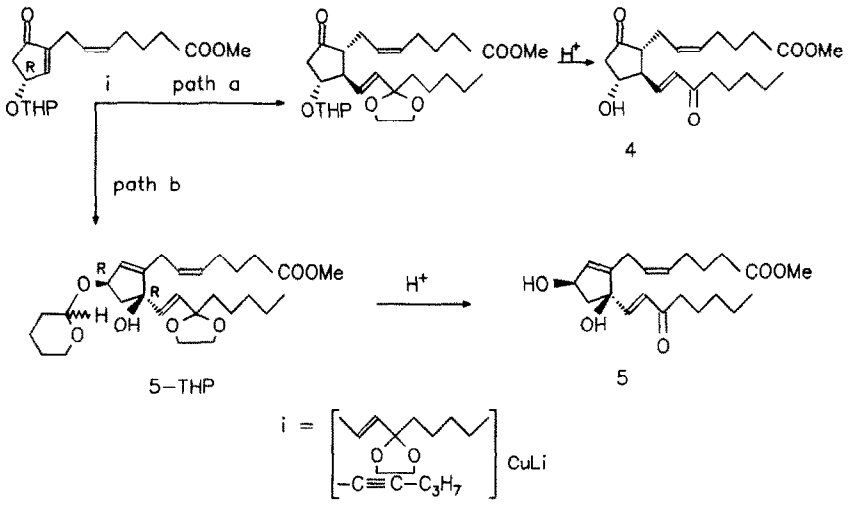
Abstract: 15-Oxo metabolites of prostaglandins E_2, $F_{2\alpha}$ and new 15-oxo prostaglandin analogues **5** and **7** were found to reveal higher antiproliferative activity than the corresponding 15-hydroxy derivatives on human leukemia K562 cells *in vitro*. The responsibility of the enone group in the ω-chain of a prostanoid molecule for this activity is suggested.

Prostaglandins (PGs) of the E, A, and D series as well as marine coral prostanoids (clavulones, punaglandins etc.) have been shown to inhibit the growth of several animal and human tumour cell lines, including various leukemia cell lines.[1-4] Regarding the structure requirements, it is well established that a cyclopentenone unit is essential for the cytotoxic activity of a prostanoid compound.[5,6] Thus, cyclopentenoic prostaglandins, for example, PGA and PGJ, can be transported into cells and accumulated in cell nuclei[7,8], differently from their respective metabolic precursors PGE and PGD. No studies have been carried out on the cytotoxic activity of the prostanoids, containing an enone unit in the ω-chain of the molecule. For example, the 15-oxo derivatives generally considered to be metabolic inactivation products of natural prostaglandins[9] have not been studied on this activity.

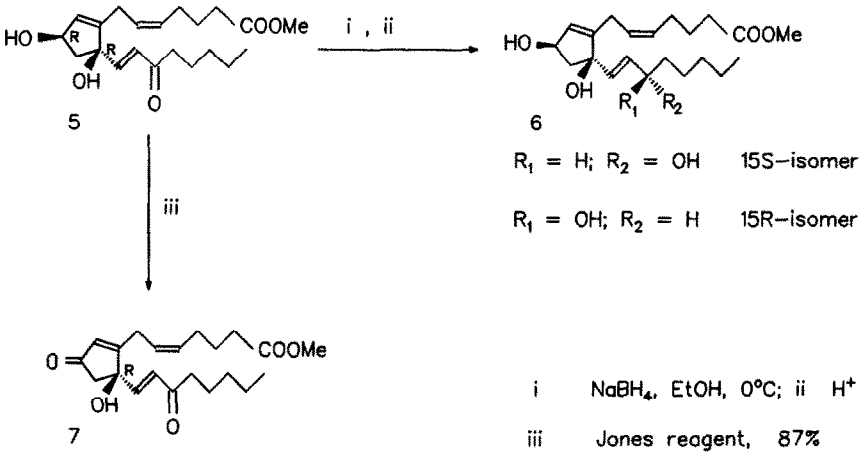
Interestingly, we found that 15-oxo-PGE_2, - the first degradation metabolite of PGE_2, exhibited a higher antiproliferative activity on human leukemia K562 cell line than PGE_2. This finding forced us to study more widely the contribution of the enone group in the ω-chain of a prostaglandin molecule to the antiproliferative activity. We have synthesized 15-oxo-$PGF_{2\alpha}$ **2**, 15-oxo-PGE_2 methyl ester **4** and ring modified 15-oxo prostanoids **5** and **7**, and compared their cytotoxicity with that of the corresponding 15-hydroxy prostaglandins - $PGF_{2\alpha}$ **1**, PGE_2 **3** and 15-hydroxy prostanoids **6** corresponding to the prostaglandin analogue **5**.

15-Oxo-$PGF_{2\alpha}$ **2** was synthesized according to Anggard and Samuelsson.[10] 15-Oxo-PGE_2 methyl ester **4** and a new prostaglandin analogue - 9-deoxy-11-deoxy-10(R), 12(R)-dihydroxy-15-oxo-Δ^8 -PGE_2 methyl ester **5** were prepared starting from (R)-(+)-methyl-7-(3-tetrahydropyranyloxy-5-oxo-1-cyclopentenyl)-5(*Z*)-heptenoate [11] (Scheme 1).

Scheme 1



Scheme 2



The 1,4-conjugate addition (path a) of the ketalic cuprate reagent to this enone gave 15-oxo-PGE_2 methyl ester **4** in 30% yield after deacetalization, while the 1,2-addition of the reagent (path b) led to the diastereomeric THP derivatives **5-THP** in a 3:2 ratio. These diastereomers were easily separable on silica gel before deacetalization. However, after deacetalization a single product, dihydroxy enone **5**, with *cis*-orientated OH- groups, was obtained in 9% yield from the starting enone.[12] Compound **5** represents a new synthetic prostaglandin analogue, characterized by 8,9 double bond and hydroxyl groups at C-10 and at C-12 position.

The corresponding 15-hydroxy compounds were obtained from **5** by reducing it with $NaBH_4$ in EtOH yielding, after chromatographic separation, diastereomeric 15(R)- and 15(S)-hydroxy prostanoids **6** (87%)[13] (Scheme 2). Compound **5** was also oxidized by using Jones reagent into 9-deoxy-11-deoxy-12(R)-hydroxy-10,15-dioxo-Δ^8-PGE_2 methyl ester **7** in 87% yield [14] (Scheme 2).

The antiproliferative activities of $PGF_{2\alpha}$ **1**, PGE_2 **3**, their methyl esters,[15] the corresponding 15-oxo-derivatives **2** and **4**, and the new synthetic prostanoids **5-7** are presented in the Table.

TABLE

The antiproliferative activity of $PGF_{2\alpha}$ **1**, PGE_2 **3** (free acids), their methyl esters, the corresponding 15-oxo derivatives - 15-oxo-$PGF_{2\alpha}$ **2**, 15-oxo-PGE_2 methyl ester **4**, and synthetic prostanoids **5**, **6**[a], and **7** on human leukemia K562 cells *in vitro*[b].

Compound	IC_{50}, μM[c]
1	>700
1 methyl ester	99 ± 4
2	362 ± 7
2 methyl ester	61 ± 5
3	63 ± 4
3 methyl ester	41 ± 1.4
4	28 ± 1.5
5	20 ± 1
6	201 ± 9
7	6.6 ± 0.4

[a] A mixture of 15(R) and 15(S) isomers as well as the diastereomers separately.
[b] Cells were grown in DMEM, supplemented with 10% of fetal calf serum, penicillin (100 U/mL), and streptomycin (0.1 mg/mL) at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were plated in 24-well Nunc multidishes at a density of 2 x 10^5/ mL and incubated in the presence of various concentrations of the investigated compounds for 48 h. The viable cells, determined by trypan blue dye exclusion, were counted on a hemocytometer.
[c] IC_{50} is the dose that, after 48 h, reduces the cell growth *in vitro* by 50% as compared to the control cultures. The data are expressed as means ± SEM (n=4-8).

As can be seen from the data presented in the Table, the methyl esters of PGE_2 and, especially, of $PGF_{2\alpha}$ were more cytotoxic than the parent free acid compounds, which may be explained by their better penetration into the cell. The 15-oxo derivatives revealed an additional increase in the cytotoxic activity. In case of PGE_2 such an increase may be caused by a more rapid serum albumin- catalyzed dehydration of 15-oxo-PGE_2 to 15-oxo PGA_2 when compared to the conversion of PGE_2 to PGA_2 in the culture medium.[16] PGA has been shown to be the ultimate active compound which is transported through the cell membrane and, in the protein-bound form, into the cell nucleus.[17,18]

However, since albumins have no destructive effects on $PGF_{2\alpha}$ and 15-oxo-$PGF_{2\alpha}$,[16] such a mechanism does not explain the cytotoxicity caused by the prostaglandins of F series. Our data demonstrate that the enone group of the ω-chain of the $PGF_{2\alpha}$ molecule clearly enhances the cytotoxicity (cf. compound **2** to its parent compound **1** both as a free acid and as a methyl ester,see Table). This phenomenon may evidence of the better transportation of 15-oxo-$PGF_{2\alpha}$ into cells while no accumulation of $PGF_{2\alpha}$ by murine leukemia cells in culture has been shown[7]. Also, the α,β-unsaturated carbonyl group of compound **2** may react, similarly to cyclopentenone prostaglandins, with some cellular nucleophiles, which ultimately results in the cytotoxicity.

Although compound **5**, because of its structural properties, does not possess the ability to form a cyclopentenone prostaglandin (PGA-type) *via* enzymatic dehydration, it still revealed a higher cytotoxic activity than 15-oxo-PGE_2 methyl ester. The reduction of the 15-oxo group in compound **5** to the trihydroxy compound **6**[19] decreased the antiproliferative activity about 10 times. Thus, the facts presented above indicate that the enone unit in the ω-chain plays an important role in the cytotoxic activity of compound **5**. Oxidation of the hydroxyl group at C-10 in compound **5** to compound **7** bearing enone units in both the ring and ω-chain resulted in the additional increase of the activity about 3 times (Table). This points to the formation of a reactive, rather unusual α,β-unsaturated carbonyl group in the cyclopentane ring (Δ^8 , 10-oxo) which also contributes to the cytotoxic activity of the compound. The antiproliferative activity of compound **7** exceeded the activity of PGD_2 ($IC_{50} = 10\ \mu M$) on the K562 cell line.[20]

In conclusion, the results of the present study demonstrate that the enone unit in the ω-chain of natural prostaglandins of E and F series contributes to the cytotoxic effect of the compounds. So, 15-oxo-PGE_2 and 15-oxo-$PGF_{2\alpha}$, the first metabolites of PGE_2 and $PGF_{2\alpha}$ in most cells[21], reveal higher antiproliferative activity than PGE_2 and $PGF_{2\alpha}$ on the K562 leukemia cell line *in vitro*. The ring modified 15-oxo-prostaglandin analogues **5** and **7** represent a new series of cytotoxic prostanoids. The difference in the activity of **5**, **6**, and **7** shows that both the ω-chain and ring enone groups play an important role in the determination of their biological activity.

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12. The synthesis procedure was as follows: 1.22 mL of n-BuLi (1.27M in hexane) was mixed at -78°C with 503 mg of (*E*)-1-iodo-1-octen-3-one ethylene ketal in 3.5 mL of Et_2O and a complex made from 202 mg of pentynyl copper and 506 mg of $[(CH_3)_2N]_3P$ in 10 mL of Et_2O was added. After 1h at -78°C 437 mg of (R)-(+)-methyl-7-(3-tetrahydropyranyloxy-5-oxo-1-cyclopentyl)-5-(Z)-heptenoatein 3 mL of Et_2O was added. The reaction mixture was stirred at -78°C for 20 min and at -20°C for 1.5 h. After a typical work-up procedure (NH_4Cl solution, extraction with ether) and separation of the reaction products on silica gel (hexane-acetone 10/3) and subsequent deacetalization **4** (150 mg,30%) $[\alpha]_D^{20} = -10.2°$, **5** (44 mg, 9%) were obtained. The structures of the compounds were confirmed by ^{13}C NMR spectra (Bruker AMX-500). The chemical shifts (δ) in $CDCl_3$ from TMS as a reference were the following:
4: 174.0 (C-1), 33.4 (C-2), 24.7 (C-3), 26.7 (C-4), 131.6 (C-5), 126.1 (C-6), 25.5 (C-7), 53.1 (C-8), 213.0 (C-9), 46.4 (C-10), 71.8 (C-11), 54.3 (C-12), 144.5 (C-13), 132.1 (C-14), 200.0 (C-15), 41.1 (C-16), 23.8, (C-17), 31.5 (C-18), 22.5 (C-19), 13.9 (C-20), 51.6 (ester CH_3).
5: 174.8 (C-1), 33.4 (C-2), 24.5 (C-3), 26.5 (C-4), 130.7 (C-5), 126.7 (C-6), 24.2 (C-7), 149.8 (C-8), 130.7 C-9), 74.5 (C-10), 50.1 (C-11), 83.9 (C-12), 148.2 (C-13), 127.2 (C-14), 200.7 (C-15), 41.4 (C-16), 23.8, (C-17), 31.5 (C-18), 22.5 (C-19), 13.9 (C-20), 51.8 (ester CH_3). The *cis*-configuration of hydroxyl groups was confirmed on the bases of H-10 chemical shift at 4.65 ppm. For 4-cyclopentene-*trans*-1,3-diols this shift should be around 4.9 ppm as confirmed by comparing the ^1H chemical shifts of pairs of model compounds - *cis*- and *trans*-isomers of 1-acetylhydroxy-3-hydroxy-4-cyclopentene(**a**) and methyl-7-(3,5-dihydroxy-5-methyl-1-cyclopentenyl)heptenoate (**b**), where *cis*- carbinol protons resonate at 4.65 (**a**) and 4.63 (**b**) and *trans*-carbinol protons at 4.83 (**a**) and 4.95 (**b**) ppm.

13. The isomers of **6** were separated chromatographically on silica gel and their ^{1}H and ^{13}C NMR spectra were recorded. They had too close ^{1}H and ^{13}C chemical shifts and ^{1}H- ^{1}H coupling constants to differentiate between the diastereomers. ^{13}C and (^{1}H) chemical shift assignments confirm the structure of these isomers: 51.70 (3.684) (OCH_3), 174.62 (C-1), 33.27 (2.34) (CH_2-2), 24.53 (1.69) (CH_2-3), 26.46 (2.04 and 2.09) (CH_2-4), 130.51 (5.51) (CH-5), 127.12 (5.60) (CH-6), 24.10 (2.87 and 2.68; other isomer 2.86 and 2.67) (CH_2-7), 150.69 (C-8), 129.72 (5.65; other isomer 5.64) (CH-9), 73.44 (4.66; other isomer 4.65) (CH-10), 50.92 (1.90 and 2.60;) (CH_2-11), 83.72 (C-12), 133.70 (5.589; other isomer 5.582) (CH-13), 131.93 (5.80; other isomer 5.77;) (CH-14), 72.25 (4.16, other isomer at 4.14) (CH-15), 37.44 (1.54) (CH_2-16), 25.08 (1.34) (CH_2-17), 31.75 (1.30) (CH_2-18), 22.60 (CH_2-19), 14.00 (0.90) (CH_3-20). The isomers are relatively unstable at room temperature in chloroform but stable in ethanol.

14. The oxidation procedure was as follows: To a solution of 12 mg of **5** in 0.6 mL of Et_2O/acetone (1/1) 0.01 mL of Jones reagent was added at 0°C. After 0.5 h the reagent excess was destroyed by 0.2 mL of i-PrOH (10 min) and the mixture poured into brine solution. The product was extracted with ether and purified on silica gel (hexane/i-PrOH 25/1) to afford 11 mg of **7** (87%). ^{13}C and (^{1}H) NMR: 51.70 (3.66) (OCH_3), 174.46 (C-1), 33.04 (2.30) (CH_2-2), 24.33 (1.65) (CH_2-3), 26.43 (2.00 and 2.11) (CH_2-4), 132.45 (5.61) (CH-5), 124.49 (5.57) (CH-6), 25.52 (2.84 and 3.28) (CH_2-7), 179.63 (C-8), 130.38 (6.01) (CH-9), 203.49 (C-10), 51.19 (2.70 and 2.76) (CH_2-11), 79.60 (C-12), 145.86 (6.68) (CH-13), 128.36 (6.55) (CH-14), 199.94 (C-15), 41.47 (2.57) (CH_2-16), 23.69 (1.62) (CH_2-17), 31.40 (1.30) (CH_2-18), 22.42 (1.32) (CH_2-19), 13.87 (0.90) (CH_3-20).

15. The methyl esters of $PGF_{2\alpha}$, PGE_2 and 15-oxo-$PGF_{2\alpha}$ were prepared from the corresponding free acids with diazomethane in ether in 88-93% yield.

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19. Our experiments confirm the data[4,6] that the stereochemistry of 15-hydroxyl group is not essential in the antiproliferative effect of the compound since **6**, a mixture of diastereomers, exhibited the same activity as the pure 15(R) or 15(S) isomer of **6**.

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