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LIPASE-CATALYSED ACYLATION OF PROSTANOIDS

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Abstract: Natural prostaglandins (PG) $F_{2\alpha}$ and E_1 as well as (+)-cloprostenol were regioselectively 11-acylated using Novozym® 435 as a catalyst and vinyl acetate as an acyl donor. Unlike the above compounds the 15-OH group of PGE_2 was also acylated with a significant velocity under the same conditions. The enantiospecificity of the lipase-catalysed 11-acetylation of cloprostenol was established by separate treatment of (+)- and (-)-cloprostenols.

A number of prostanoids are continuously used in biological, pharmacological and medical research. The simplest approach to synthesising several prostanoids involves interconversions starting from $PGF_{2\alpha}$, PGE_2, etc. PGs are (poly)hydroxycarboxylic acids of complex structure.[1] Some of them are both acid- and base-sensitive compounds. Consequently, methodologies of the chemo- and regioselective treatment of hydroxyl groups of the parent PG are of crucial importance. The choice of the synthetic methods as well as protecting groups should be in accordance with stability requirements for target compounds.

Lipases[2,3,4] have been proved to allow stereo- and/or regioselective acylation of polyhydroxylated compounds such as steroids,[5] carbohydrates,[6] etc.[7] It is also noteworthy that some modifications of PGs *in vivo* could be related to hydrolase-catalysed reactions.[8,9,10] Nevertheless, the lipase-catalysed acylation of PGs in organic media has not been described. Herein we report our preliminary results of studies on the chemo-, regio- and enantioselectivity of the lipase-catalysed derivatisation of some prostanoids.

1. Acylation of prostanoids of F type catalysed by Novozym® 435 (*Candida antarctica* lipase B (CALB)).

The common rule for enantiopreference for lipases[3] (Figure 1, A) suggests the accessibility of the 11-hydroxyl group of $PGF_{2\alpha}$ (**1**) to the enzyme (Figure 1, B). Indeed, the CALB-catalysed[11] acetylation of (**1**) in the $CHCl_3$/vinyl acetate solution (Table 1, run 1) proceeded smoothly and was highly chemo- and regio-selective: the crude product contained the target 11-acetyl-$PGF_{2\alpha}$ (**2**)[12] (found earlier in soft coral)[8] with over 99 % purity by NMR spectroscopic analysis (Table 2). The esterification of the carboxyl group of PG was suppressed by a large excess of vinyl acetate. However, negligible amounts of several (by NMR) by-products (~1 % after 48 h; ~3 % after 96 h) were detectable on TLC beginning from the 6th h of incubation. 11-Acetyl-$PGF_{2\alpha}$ (**2**) was readily purified by recrystallisation from $CHCl_3$/n-hexane (2/3) at -15°C.

The enantioselectivity of the lipase-catalysed acylation of prostanoids was studied by using cloprostenol, both of its individual optical antipodes being available.[13] (+)-Cloprostenol (**3**), whose configuration (Figure 1, C) is similar to that of natural $PGF_{2\alpha}$ (**1**) reacted just like (**1**) (Table 1, run 2) under catalysis by CALB[14,15] affording the corresponding 11-acetate (**4**)[12] in over 98 % yield. (-)-Cloprostenol (**5**) gave (Figure 1, D) no detectable amount of the 11-acylated product upon the same procedure (Table 1, run 3). Furthermore, the conversion of (**5**; probably acetylation of the 9-hydroxyl group; cf. Figure 1, A and D) was very slow being accompanied by partial degradation of the sample and therefore the product was not investigated further.

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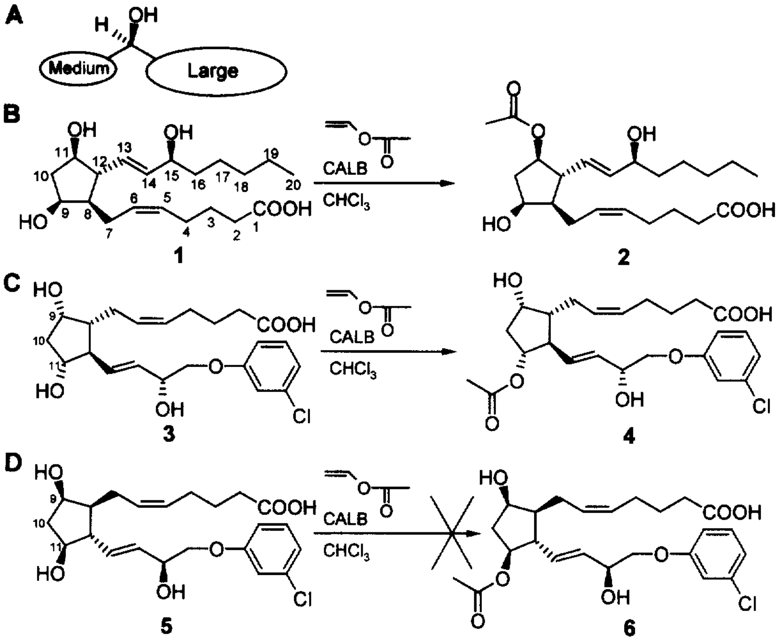

Figure 1. The enantio- and regioselective acetylation of prostanoids of F type catalysed by CALB.
A: the enantiomer of the secondary alcohol acylated preferably by lipases; **B**: acetylation of natural $PGF_{2\alpha}$ (**1**); **C**: acetylation of (+)-cloprostenol (**3**); **D**: no (-)-cloprostenol-11-acetate (**6**) was obtained upon lipase-catalysed acylation.

2. Lipase-catalysed modification of prostanoids of E type in organic media.

The acylation of PGE_2 (**7**) with vinyl acetate in $CHCl_3$ catalysed by CALB proceeded readily affording 11-acetyl-PGE_2 (**8**)[12] as almost a single product detected after incubation for the first 4 h. Unexpectedly, the 15-hydroxyl group of 11-acetyl-PGE_2 (**8**), unlike that of 11-acetyl-$PGF_{2\alpha}$ (**2**), appeared to be accessible to CALB: after 24 h the ratio of mono- to bis-acetylated product (**8**)/(**9**) was 3/1 (Table 1, run 4 and 5). The increasing modest amounts of PGA_2 (**10**)[12] and 15-acetyl-PGA_2 (**11**)[12] were detected as well.[16] The formation of PGA_2 (**10**) was also observed when instead of CALB Lipolase® 100T (*Humicola* (*Thermomyces*) *lanuginosa* lipase (HLL)) was used to catalyse the acetylation of PGE_2 (**7**) (Table 1, run 10). It is noteworthy, that PGA_2 and 15-acetyl-PGA_2 methyl ester have been separated from soft coral tissue.[9,10]

Being concerned about the increase of the content of PGA_2-s (**10**) and (**11**) in the reaction mixture during the acetylation, we decided to study the lipase-catalysed deacylation of (**8**) and (**9**). The mixture containing 11-acetyl-PGE_2 (**8**) and 11,15-diacetyl-PGE_2 (**9**) (ratio: 3/2) together with the little amounts of PGA_2 (**10**) and 15-acetyl-PGA_2 (**11**) (see Table 1, run 4: the crude product) was treated with HLL in methanol (Table 1, run 6) to give a quantitative access to PGA_2 (**10**) and 15-acetyl-PGA_2 (**11**) at a ratio of 3/2.[16,17] The other part of the same starting mixture was incubated with CALB in methanol (Table 1, run 7) to afford a complex mixture of products: carboxylic acids (no PGE_2 was detected) and esters. Indeed, an independent trial showed (Table 1, run 8) that PGE_2 ethyl ester (**12**)[12] was readily formed upon treatment of PGE_2 (**7**) with CALB in ethanolic chloroform.

Thus, the HLL-catalysed cleavage of the 11-acetoxy moiety of 11-acetyl-PGE_2-s (**8**) and (**9**) leads exclusively to the formation of the corresponding PGA_2 via elimination of acetic acid from the acetyl-β-ketol moiety. No PGE_2 was detected in any stage of the deacylation reaction, thus supporting the hypothetical mechanism proposed in Figure 4.[18] Additionally, the fact of acetylation of PGE_2 (7) using HLL (Table 1, run 10) suggests the elimination reaction to be catalysed by free serine as proton donor. Thus, we have stated that PGE_2 (7) can be recovered neither from its acetyl derivatives (8) nor (9) upon lipase-catalysed deacylation using CALB or HLL in methanol.

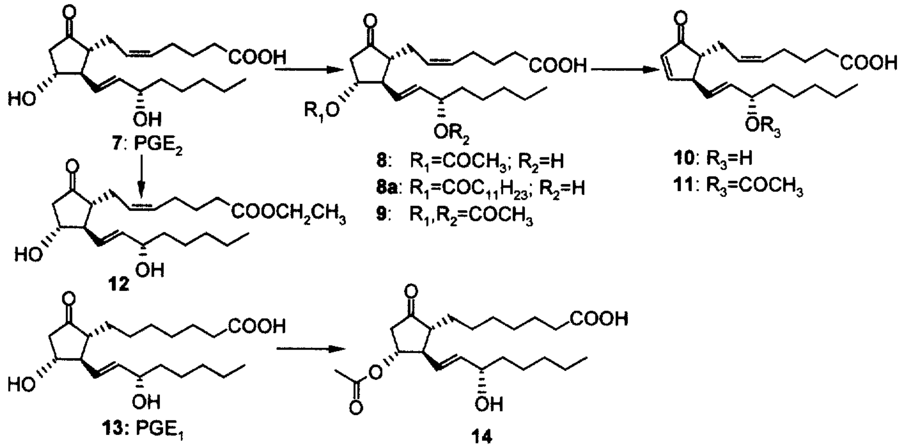

Figure 2. Lipase-catalysed modification of prostanoids of E type.

Table 1. Lipase-catalysed reactions[a,b] of prostanoids.

Run No.	Substrate (amount)	Enzyme (amount)	Medium (volume)	Acyl donor (amount)	Time [h]	Degree of conversion	Products (ratio)
1	$PGF_{2\alpha}$ (1) (0.3 g)	Novozym®435 (0.3 g)	$CHCl_3$ (3.5 ml)	vinyl acetate (1.5 ml)	16	>99 %	11-acetyl-$PGF_{2\alpha}$ (2)/ by-products > 99/1
2	(+)-cloprostenol (3) (50 mg)	Novozym®435 (0.3 g)	$CHCl_3$ (4 ml)	vinyl acetate (2 ml)	24	>99 %	(+)-11-acetyl-cloprostenol (4)/ by-products >99/1
3	(-)-cloprostenol (5) (10 mg)	Novozym®435 (0.15 g)	$CHCl_3$ (2 ml)	vinyl acetate (1 ml)	96	70-80 %	products not identified; formation of (-)-11-acetyl-cloprostenol (6) was not detected
4	PGE_2 (7) (25 mg)	Novozym®435 (0.15 g)	$CHCl_3$ (2 ml)	vinyl acetate (1 ml)	a) 4 b) 24 c) 48	75 % >95 % >99 %	11-acetyl-PGE_2 (8)/ 11,15-diacetyl-PGE_2 (9) = 70/1 (8)/(9) = 3/1 (8)/(9) = 3/2 and PGA_2-s (10) and (11) < 10 %
5	PGE_2 (7) (50 mg)	Novozym®435 (0.3 g)	$CHCl_3$ (4 ml)	vinyl acetate (1 ml)	96	>99 %	(8)/(9) = 2/3 and PGA_2-s (10) and (11) (15-20 %)
6	11-acetyl-PGE_2 (8)/ 11,15-diacetyl-PGE_2 (9) = 3/2 and PGA_2-s (10;11) < 10 % (15 mg)	Lipolase®100T (1.0 g)	CH_3OH (4 ml)	--	72	>99 % conv. of PGE-s	PGA_2 (10)/15-acetyl-PGA_2 (11) = 3/2 (formation of by-products was negligible)
7	Same as previous (15 mg)	Novozym®435 (0.2 g)	CH_3OH (4 ml)	--	72	> 50 %	not identified (a complex mixture of PG-s and their esters)
8	PGE_2 (7) (25 mg)	Novozym®435 (0.3 g)	$CHCl_3$/EtOH 99/1 (5 ml)	--	24	90 %	PGE_2 ethyl ester (12)/ starting material (7) = 9/1
9	PGE_2 (7) (50 mg)	Novozym®435 (0.3 g)	$CHCl_3$ (5 ml)	vinyl laurate (1.3 ml)	96	50 %	11-lauroyl-PGE_2 (8a)/PGA_2 (10) ≅ 20/1 (a significant amount of lauric acid was also detected in the crude product)
10	PGE_2 (7) (50 mg)	Lipolase®100T (1.0 g)	$CHCl_3$ (4 ml)	vinyl acetate (2 ml)	96	20 %	11-acetyl-PGE_2 (8)/PGA_2 (10) > 10/1; no other products were detected
11	PGE_1 (13) (50 mg)	Novozym®435 (0.3 g)	$CHCl_3$ (4 ml)	vinyl acetate (2 ml)	96	75 %	11-acetyl-PGE_1 (14); no other products were detected

a: all reactions were performed at room temperature, no agitation of the reaction mixture was carried out;
b: typically crude products were investigated using TLC and NMR spectroscopy; in some cases NMR spectroscopic studies were repeated after purification of the product.

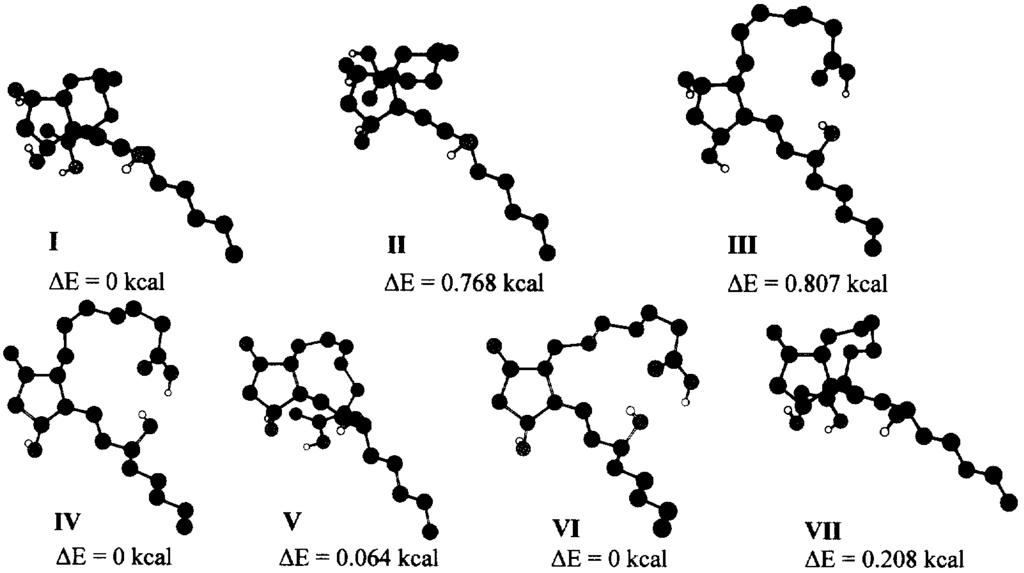

Figure 3. The most favoured conformers of PGs in gas phase: $F_{2\alpha}$ (**I, II, III**), E_2 (**IV, V**), E_1 (**VI, VII**).

Our preliminary studies have shown different factors to influence the regioselectivity of the lipase-catalysed acylation of PGs. However, using a vinyl ester donating a bulky acyl group (vinyl laurate: Table 1, run 9) allowed the regioselective CALB-catalysed acylation of the 11-hydroxyl group of PGE_2 (**7**). Unlike CALB, only the 11-hydroxyl group of PGE_2 was acylated by HLL using vinyl acetate as an acyl donor (Table 1, run 10). In order to study the influence of structural differences between remote from the potential reaction centre parts of the substrate molecules on the regioselectivity we tested also the CALB-catalysed acetylation of PGE_1 (**13**). Exclusively only 11-acetyl-PGE_1 (**14**) was formed (Table 1, run 11) demonstrating the highest degree of sensitivity of the enzyme to a change of structural features of the substrate.

At first glance it seems to be rather difficult to observe large steric differences between PGE_2 (**7**) and $PGF_{2\alpha}$ (**1**) as well as between PGE_2 (**7**) and PGE_1 (**13**). Numerous crystal structures of PGs obtained by X-ray analysis show "hairpin-like" conformations[1,19] for them in a solid state. These conformations are considered arguable for PGs in solution and when interacting with a receptor.[1] Differences in NMR spectra (Table 2) observed between PGs and their esters (corresponding to remote from the structural difference regions of the molecule) prompted us to study the geometry of PGs by using quantum chemical calculations.[20,21] Indeed, our results of conformational analysis of PGs in gas phase show the conformers in which the carboxylic acid moiety has formed hydrogen bonds with the hydroxyl group leading to a six-membered ring coordination of these functional groups to be the most favourable ones (Figure 3).[22] For $PGF_{2\alpha}$ the conformer in which the 11-hydroxyl is engaged into the hydrogen bond formation (Figure 3, **I**) is clearly the most favourable. For PGE_1 the conformer (similar to "hairpin") with the 15-hydroxyl group, being hydrogen-bonded with carboxylic acid moiety (Figure 3, **VI**), is more favourable than that with 11-OH being included into the H-bonding (Figure 3, **VII**). For PGE_2 the energies of these two most favourable conformers (Figure 3, **IV** and **V**) were found to be almost equal. These results correlate with the crystallisability of PGs[23] in the order $PGE_1 > PGE_2 > PGF_{2\alpha}$. The results also correlate with the ability of CALB to produce 11-acylated PGs in the order $PGF_{2\alpha} > PGE_2 > PGE_1$,[24] the phenomenon which could be mediated by the intramolecular coordination of the carboxylic acid functionality to the 11-OH group. However, our results still do not help us to explain differences in the acetylation of 15-OH group. Concerning the regioselectivity it can be expected that the steric differences between PGs are evidently amplified by the coordination of certain functional groups of PG with appropriate groups of the lipase. For instance, the carboxyl group of PG expectedly coordinates with imidazole of histidine 224 for CALB, etc.[25] – thus, allowing the formation of a conformation characteristic just for a certain PG-lipase pair and probably other than calculated for gas phase (Figure 3). Additionally, it seems noteworthy that the results presented in Figure 3 show a higher flexibility of the α-chain corresponding to PGs of 2 series due to the presence of the 5,6-cis double bond.

In conclusion, the regioselectivity of the lipase-catalysed acylation of PGs has been shown to depend on the lipase, substrate and the acyl group to be transferred by the lipase.

Table 2. ^{13}C NMR chemical shifts[a] of prostanoids.

Comp. No. / Atom No.	clo-prostenol (3), (5)	11-acetyl-cloprostenol (4)	$F_{2\alpha}$ (1)	11-acetyl-$F_{2\alpha}$ (2)	A_2 (10)	A_2 methyl ester[26]	15-acetyl-A_2 (11)	E_1 (13)	11-acetyl-E_1 (14)	E_2 methyl ester[26]	E_2 ethyl ester (12)	E_2 (7)	11-acetyl-E_2 (8)	11,15-diacetyl-E_2 (9)	11-lauryl-E_2 (8a)
C-e2											13.8				
C-e1						51.5				51.6	60.2				
C-1	177.2	177.5	177.4	177.6	178.2	174.0	178.4	177.1	177.1	174.2	173.6	178.0	178.1	178.5	179.0
C-2	32.9	32.8	33.3	33.1	33.4	33.5	33.5	33.9	33.9	33.5	33.5	33.2	33.1	33.2	33.1
C-3	24.4	24.4	24.6	24.5	24.6	24.8	24.6	24.5	24.5	24.7	24.5	24.4	24.4	24.4	24.3
C-4	26.3	26.3	26.3	26.3	26.6	26.7	26.6	28.7	28.6	26.6	26.4	26.3	26.3	26.4	26.3
C-5	129.6	129.9	129.4	129.8	131.1	131.0	131.1	29.2	29.1	130.9	130.7	130.8	131.1	131.1	130.9
C-6	129.1	128.5	129.1	128.6	126.7	126.7	126.7	26.5	26.3	126.6	126.3	126.7	126.2	126.1	126.2
C-7	25.2	24.6	25.1	25.0	27.4	27.4	27.4	27.6	27.3	25.2	25.0	25.1	24.5	24.7	24.5
C-8	50.7	49.8	49.9	49.7	52.0	52.0	51.9	54.4	53.7	53.8	53.5	53.6	53.6	53.6	53.6
C-9	72.6	71.7	72.2	71.5	210.5	210.5	210.4	215.0	213.9	214.2	214.1	215.8	213.1	212.9	213.0
C-10	**42.9**	**40.9**	**42.6**	**40.8**	133.2	133.3	133.4	**45.8**	**43.6**	46.1	45.8	**46.1**	**43.8**	**43.8**	**43.7**
C-11	**77.6**	**78.8**	**77.3**	**79.0**	165.3	165.0	165.1	**71.8**	**73.2**	72.0	71.7	**72.1**	**72.3**	**73.0**	**73.0**
C-12	**55.6**	**51.5**	**55.1**	**51.3**	49.5	49.6	49.6	**54.7**	**50.6**	54.5	54.3	**54.5**	**49.1**	**49.2**	**49.2**
C-13	132.2	129.6	132.8	130.7	130.0	130.2	132.5	132.0	129.7	131.7	131.5	131.5	128.9	130.9	129.0
C-14	134.9	133.4	135.1	135.0	**135.0**	**135.1**	**130.6**	136.7	136.4	136.7	136.6	136.5	**136.0**	**132.0**	135.9
C-15	70.8	70.4	73.2	72.5	**72.6**	**72.5**	**74.3**	73.3	72.4	73.1	72.9	73.2	**73.3**	**73.8**	72.1
C-16	71.9	71.7	36.8	36.8	**37.2**	**37.2**	**34.3**	36.9	36.9	37.3	37.0	36.9	**36.8**	**34.2**	36.7
C-17	159.3	159.1	25.2	25.2	25.1	25.1	24.8	25.1	25.0	25.1	24.8	25.2	24.9	24.7	24.9
C-18	115.1	115.1	31.7	31.7	31.7	31.7	31.5	31.6	31.7	31.7	31.5	31.6	31.6	31.4	31.6
C-19	134.8	134.8	22.5	22.6	22.6	22.6	22.5	22.6	22.6	22.6	22.4	22.6	22.6	22.4	22.5
C-20	121.3	121.3	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.1	13.9
C-21	130.3	130.3													
C-22	113.1	113.1													
$C_{11}1$		171.1		171.3					170.9				170.8	170.4	173.5
$C_{11}2$		21.2		21.2					20.8				20.8	20.7	34.0[b]
$C_{15}1$							170.5							170.5	
$C_{15}2$							21.2							21.2	

a) NMR spectra were measured in $CDCl_3$ solution at room temperature on a Bruker AMX500 instrument. ^{1}H-^{1}H and ^{1}H-^{13}C 2D COSY correlation diagrams were used for the full assignment of ^{1}H and ^{13}C chemical shifts. Only ^{13}C chemical shifts are reported with single decimal point precision. Derivatisation results in inequality of nearly all chemical shifts of prostanoid and its acylated derivative. For the unambiguous determination of acylation sites characteristic α(to low field)- and β(to high field)-effects of acylation (see the bold underlined data) were used.

b) Chemical shifts for the remaining atoms of lauryl residue are not given.

Conclusions.

1. The acylation of the 11-hydroxyl group of $PGF_{2\alpha}$ and PGE_1 as well as that of (+)-cloprostenol with vinyl acetate catalysed by Novozym® 435 was found to occur highly chemo- and regioselectively, while both of the hydroxyl groups of PGE_2 were readily accessible to this enzyme under the same conditions.
2. The rate of acylation of the 15-hydroxyl group of the prostanoid was found to depend drastically on negligible differences in prostanoid structure, on the structure of the acyl group to be transferred and on the enzyme used.
3. (-)-Cloprostenol did not give any detectable access to 11-acetyl-derivative upon treatment with vinyl acetate catalysed by Novozym® 435 in chloroform showing the enantiospecificity of the process.
4. The deacylation of 11-acetyl-PGE_2-s (**8**) and (**9**) in methanol catalysed by Lipolase® 100T gave a quantitative access to the corresponding PGA_2-s (**10**) and (**11**).[17]

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12. Characterisation of compounds: ^{13}C NMR spectra of the compounds are given in **Table 2**. (**1**) – $PGF_{2\alpha}$ was purchased from Chinoin Ltd. (Hungary); TLC: R_f=0.069 ($CHCl_3$/EtOH 9/1); (**2**) – TLC: R_f=0.302 ($CHCl_3$/EtOH 9/1); $[\alpha]^{20}_{546}$ +54.2 (c 1.4, $CHCl_3$); mp 79-80.5°C; (**4**) – TLC: R_f=0.328 ($CHCl_3$/EtOH 9/1); $[\alpha]^{20}_{546}$ +33.9 (c 1.1, $CHCl_3$); (**7**) – PGE_2 was purchased from Kemasol Ltd. (Estonia); TLC: R_f=0.094 (EtOAc); (**8**) – TLC: R_f=0.491 (EtOAc); (**8a**) – TLC: R_f=0.362 (C_6H_6/EtOAc 1/1); (**9**) – TLC: R_f=0.679 (EtOAc); (**10**) – TLC: R_f=0.387 (EtOAc); (**11**) – TLC: R_f=0.623 (EtOAc); (**12**) – TLC: R_f=0.377 (EtOAc); (**13**) – PGE_1 was purchased from Kemasol Ltd. (Estonia); TLC: R_f=0.103 (EtOAc); (**14**) – TLC: R_f=0.566 (EtOAc).
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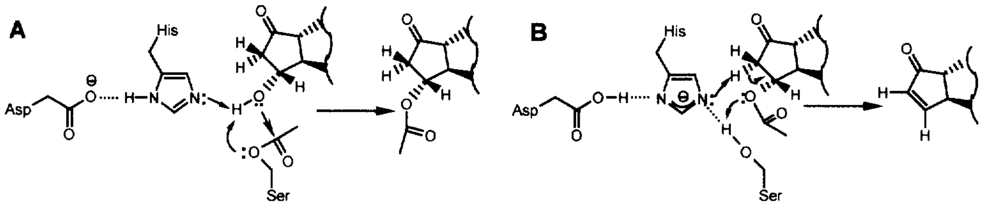
16. The reference samples were taken in all cases from the reaction mixture prior to introduction of the enzyme. The reference sample was stored under the same conditions as the reaction mixture. Only negligible amounts of the degradation products were formed from reference samples during the reaction time (Table 1). The enzymatic reactions described were stopped by filtering off the enzyme.
17. Evidently PGA_2 and its derivatives identified in soft corals may have been formed upon hydrolase-catalysed acylation-deacylation of PGE_2. See also: Brash, A.R.; Baertchi, S.W.; Ingram, C.D.; Harris, T.M. In: Advances in Prostaglandin, Thromboxane and Leukotriene Research; Raven Press: New York,1989; Vol. 19, pp.70.
18. **Figure 4.** A simplified mechanism of the lipase-catalysed acylation (**A**) and elimination (**B**) reactions of PGE_2 (**7**) and PGE_2 11-acetates (**8**) and (**9**), respectively.



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21. Conformational space of PG-s E_1, E_2 and $F_{2\alpha}$ was scanned by using the DGEOM95 package (Chiron Corp.©95).
22. The geometry of the hydrogen bonding between carboxylic acid and hydroxyl groups calculated by AM 1 method was confirmed by the *ab initio* calculations at the HF/6-31+G** level by using acetic acid and methanol as the model system.
23. The melting points of PGs are: $F_{2\alpha}$ 41-43°C; E_2 67-69°C; E_1 115-116°C. (Cayman Chemical Company Product Catalogue. Vol. VIII, 1996, pp. 45-49).
24. The reaction rates were estimated approximately.
25. The accessibility of the 15-OH group of PGs to lipases is probably controlled by distance (we expect that the carboxyl group of PG is "fixed" by coordination with a basic functional group of lipase) as well as conformation being in mutual dependence.
26. Our unpublished results.