Enantioselective Synthesis and Biological Activity of (3*S*,4*R*)- and (3*S*,4*S*)-3-Hydroxy-4-hydroxymethyl-4-butanolides in Relation to PGE_2

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Received October 7, 2003

Abstract: Compounds **9** and **13** were synthesized, and their structures and stereochemistry were elucidated by spectroscopic methods. In competition binding experiments, specific [^{3}H]-PGE_2 binding was significantly displaced by compound **9** and, to a lesser extent, by **13**, in a dose-dependent manner. The biological properties of compound **9** were studied on HL-60 cells, and several effects were found related to those of PGE_2. Compound **9** increases *c-fos* mRNA level as does PGE_2 and antagonizes TPA-induced terminal differentiation.

Introduction. Prostaglandins are small lipid molecules that regulate numerous processes in the body, including kidney function, platelet aggregation, neurotransmitter release, and modulation of immune function.[1,2] One of the best known and most studied prostaglandins is PGE_2. This mediator, which promotes tumor-cell survival, has been found at higher concentrations in tumor tissues than in normal tissues.[3] PGE_2 mediates tumor survival by several mechanisms. It inhibits tumor-cell apoptosis and induces tumor-cell proliferation.[4] It also conduces to tumor progression by altering cell morphology, and increasing cell motility and migration.[5] In addition to the direct effects of PGE_2 on tumor cells, this lipid mediator induces the production of metastasis-promoting matrix metalloproteinases, stimulates angiogenesis,[5] and induces c-*fos* mRNA expression in different cell types.[6,7]

On the other hand, hydroxy-γ-butyrolactones have been widely used as chiral synthons in the synthesis of biologically active molecules.[8] There are several different approaches to the synthesis of this kind of lactone, specifically (3*S*,4*R*)-and (3*S*,4*S*)-3-hydroxy-4-hydroxymethyl-4-butanolides.[9] We isolated butanolide **9**[10] from the fern *Polypodium decumanum* collected in Honduras. Spectroscopical data and circular dichroism established its structure and absolute configuration. Structural similarities are observed between our lactone and PGE_2. Both compounds have a five-membered ring containing a carbonyl group (C-9 for PGE_2 and C-1 for the lactone) and a hydroxyl group (C-11 of PGE_2 and C-3 of the lactone). Differences existed, however, in regard to the chains (R_α and R_β) of PGE_2, that contrasted with the presence of the endocyclic oxygen of the lactone (that occupies the "equivalent position" of the R_α chain) and of a hydroxymethyl group (that occupies "the equivalent position" of the R_β chain). This paper reports on the enantioselective synthesis of **9** and **13** and on several of their biological activities, based on the close structural similarities of these compounds to PGE_2 (Figure 1).

Chemistry. An enantioselective synthesis of (3*S*,4*R*) and (3*S*,4*S*)-3-hydroxy-4-hydroxymethyl-4-butanolides **9** and **13** was developed**.** Compound **9** was synthesized using 1,3-propanediol as starting material, applying the methodology developed by Martin et al.[11] (Scheme 1). This consists of a modified[12] Knoevenagel condensation over aldehyde **3**. This reaction did not afford the desired product **4**, because the protected (TBDPS) hydroxymethyl group was not eliminated under these conditions, so we used an alternative route dispensing with the modified Knoevenagel reaction. In this new route, first 1,3-propanediol was monoprotected with *tert*-butyl diphenylsilane, followed by oxidation to obtain the aldehyde, using Swern's reaction.[13] A Wittig reaction over this aldehyde was then used to form an α,β unsaturated ester **4** which led to the formation of allylic alcohol **5** by treatment with Dibal-H (Scheme 1).

An asymmetric Sharpless epoxidation[14] of alcohol **5** gave the epoxy alcohol **6**, which was submitted to a regioselective opening reaction by treatment with benzylic alcohol and Ti(^{i}OPr)$_4$[15] (Scheme 2). Afterwards, protection of the diol as an acetonide[16] and deprotection of the TBDPS group with TBAF[17] afforded **7**. Treatment of **7** with $HCrO_4$ gave an acid (ketal deprotection and oxidation to acid can be achieved in a single step), which when treated without purification under mild acid conditions (CSA/MeOH), yielded butyrolactone **8**,[18] which was debenzylated to afford (3*S*,4*R*)-3-hydroxy-4-hydroxymethyl-4-butanolide **9** through catalytic hydrogenation.

The synthesis of the related diastereoisomer (3*S*,4*S*)-3-hydroxy-4-hydroxymethyl-4-butanolide (**13**) followed the same route (Scheme 2), with the exception of the asymmetric induction step, using an asymmetric dihydroxylation[19] to afford compound **10**, which was submitted to the same reactions described above. The enantiomeric excess was determined using Mosher's method

Competition for [^{3}H]-PGE_2 Binding. Prostaglandin E_2 is an autocrine lipid mediator that produces a broad range of biological actions in diverse tissues through binding to specific receptors on plasma membranes of target cells.[20] PGE_2 exerts diverse pharmacological actions on tissues. Comparative structural analysis shows that the oxa-cyclopentane ring of **9** is similar to that of the lipid mediator PGE_2 while the OH group at C-3 in **13** is in the β position (see Figure 1).

To investigate further if the structural similarity present in compound **9**, and to a lesser extent in compound **13**, could have physiological consequences, we first performed competition binding experiments with the radioligand [^{3}H]-PGE_2, using a specific anti-

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10.1021/jm034216y CCC: $27.50
Published on Web 12/16/2003

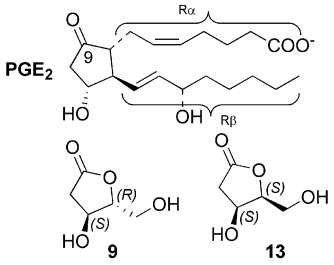
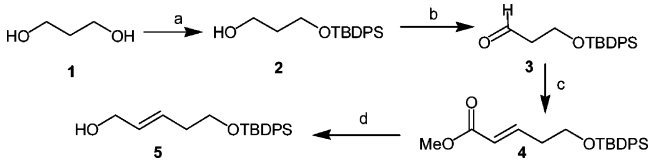


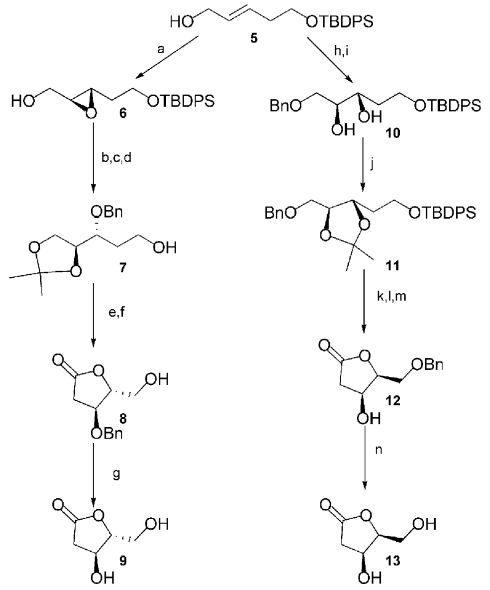
Figure 1. Chemical structures of prostaglandin E_2 and lactones **9** and **13**.

Scheme 1[a]



[a] Reagents: (a) TBDPSiCl, imidazole, THF, 35%; (b) $(COCl)_2$, NEt_3, DMSO, CH_2Cl_2, 90%; (c) $(MeO)_2POCH_2CO_2Me$, BuLi, C_6H_6, 80%; (d) Dibal-H, CH_2Cl_2, 90%.

Scheme 2[a]



[a] Reagents: (a) Ti (^{i}OPr)$_4$, D-(−)-DET, ^{t}BuOOH, CH_2Cl_2, 80%; (b) BnOH, Ti(^{i}OPr)$_4$, CH_2Cl_2; (c) 2-methoxypropene, *p*-TsOH·H_2O, CH_2Cl_2; (d) TBAF, THF, 50%, three steps; (e) $HCrO_4$/acetone; (f) CSA, CH_2Cl_2/MeOH, 80%, two steps; (g) H_2, Pd−C, MeOH, 96%; (h) BnBr, NaH, IN(Bu)$_4$, THF; (i) AD-mix α, $CH_3SO_2NH_2$, ^{t}BuOH:H_2O (1:1), 87%, two steps; (j) 2-methoxypropene, *p*-TsOH·H_2O, CH_2Cl_2, 93%; (k) TBAF, THF; (l) $HCrO_4$/acetone; (m) CSA, CH_2Cl_2/MeOH, 83%, three steps; (n) H_2, Pd−C, MeOH, 95%.

prostaglandin E_2 antibody. As shown in Figure 2, specific [^{3}H]-PGE_2 binding was significantly displaced by compound **9** and, to a lesser extent, by **13**, in a dose-dependent manner. Lactone **9** was more efficient than lactone **13** at all the concentrations tested. The percentage of [^{3}H]PGE_2 binding decreased to 33 ± 8% (3-fold) in the presence of 1000 μM of compound **9**, the highest concentration assayed. A moderate decrease in the percentage of specific [^{3}H]-PGE_2 binding was observed with 125 μM of this compound (78 ± 10%), in a manner similar to 1000 μM of lactone **13** (73 ± 13%).

This result indicates that lactone **9** is more efficient than lactone **13** in competing for PGE_2 binding in this assay system and suggests that it could compete with

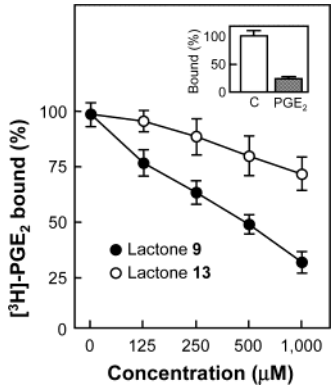


Figure 2. Effects of natural and synthetic lactones on [^{3}H]-PGE_2 binding. An anti-PGE_2 antibody was incubated with 1 nM [^{3}H]-PGE_2 in the absence or presence of the indicated concentration of lactones **9** or **13**, and the bound cpm were determined. The insert shows [^{3}H]-PGE_2 bound in absence (C, control) or presence of 2.5 nM PGE_2 as competitor. The results shown are the mean ± SE of triplicate determinations from a single representative experiment and are expressed as a percentage of specific radioligand binding.

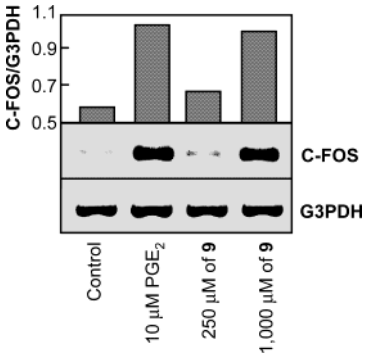


Figure 3. Effects of the natural lactone **9** on c-*fos* mRNA expression in HL-60 cells. Cells were cultured without (control) or with the indicated doses of PGE_2 and lactone **9**. Total RNA was isolated and RT-PCR was performed using gene specific primers for c-*fos* or G3PDH as an internal control, as described in the Experimental Section. The products were analyzed by electrophoresis on agarose gels (lower panel), the bands were quantified by densitometry analysis software and the results were expressed as the ratio c-*fos*/G3PDH in relative arbitrary units (upper panel).

PGE_2 binding sites in target cells. This result also indicates that compound **9** could display effects related to the PGE_2 actions in target cells, and we therefore decided to study the physiological effects of this lactone on HL-60 cells.

Induction of c-*fos* mRNA by Compound 9. It is well documented that PGE_2 rapidly (within minutes) and transiently induces protooncogene c-*fos* mRNA expression in HL-60 and other cell types.[8,9,21,22] Moreover, c-*fos* induction accompanies a myriad of additional ligand-induced cellular responses.[21] Therefore, we next examined whether compound **9** can reproduce PGE_2 action on the c-*fos* mRNA level in HL-60 cells. The results (Figure 3) show that **9** at the highest concentration used (1000 μM) significantly increase c-*fos* mRNA (2-fold compared with the control) as measured by RT-PCR in a short incubation period (30 min). These results demonstrate that **9** mimics the PGE_2 effects, at least at the c-*fos* mRNA level.

Effects of lactone 9 on Morphological Changes and Cell Viability Induced by TPA. When treated

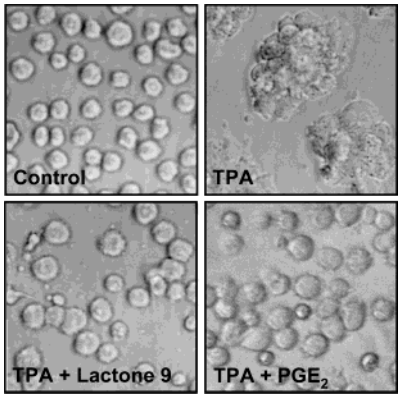


Figure 4. Effects of **9** on the TPA-induced morphology of HL-60 cells as visualized by phase contrast microscopy. The cells were treated with 100 nM TPA alone or in the presence of 1000 μM lactone **9** or 10 μM PGE_2 for 24 h, and control cells were cultured under the same conditions and received vehicle (DMSO) in the same proportion used in the other groups.

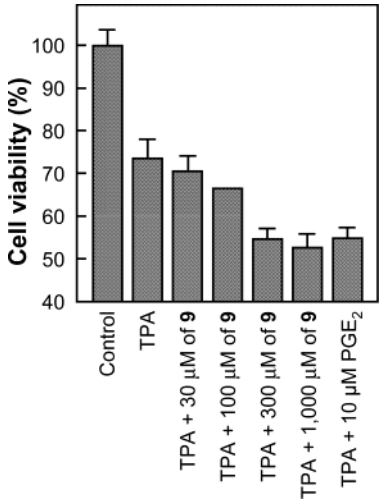


Figure 5. Effects of lactone **9** and TPA on HL-60 cell viability. The cells were cultured with 100 nM TPA alone or in the presence of the indicated concentrations of lactone **9** or PGE_2 for 24 h. Control cells were cultured under the same conditions and received vehicle (DMSO) in the same proportion used in the other groups. Cell viability was determined following the MTT procedure. The results shown are the mean ± SE of triplicate determinations from a single representative experiment and are expressed as percentage of viability with respect to the control.

with tetradecanoyl phorbol acetate (TPA), a protein kinase C activator and a tumor promoter, HL-60 cells undergo terminal differentiation evidenced by a transition from a nonphagocytic suspension culture to an attached fibroblast-like culture with high phagocytic activity.[24] Since it has already been reported that PGE_2 enhances TPA-induced differentiation of HL-60 cells,[25] we wanted to investigate if lactone **9** also mimics the effects of prostaglandin E_2 on the cell morphology. To this end, HL-60 cells were cultured for 24 h with 100 nM TPA alone or in combination with different concentrations of **9** (250 μM, 500 μM, or 1000 μM), and the morphology of the cells was analyzed under phase contrast microscopy. As shown in Figure 4, compound **9** does not enhance TPA-induced differentiation. Moreover, this substance antagonizes TPA-induced terminal differentiation at the highest dose used (1000 μM). Under our experimental conditions PGE_2 was unable to enhance TPA-induced differentiation, as described by Dertinger et al.[25] PGE_2, however, did display similar effects to those of lactone **9** on HL-60 cell morphology (Figure 4). This discrepancy may be explained by differences in the experimental designs (significantly lower doses of TPA and PGE_2, and preincubation of the cells with PGE_2 for several hours before adding TPA[25]).

The effect on HL-60 cells morphology of **9** by itself was studied using a wide range of concentrations (1−1000 μM). Analysis by phase contrast microscopy at 24 h revealed no evidence of morphological changes (results not shown) and the cells appeared to be healthy. In agreement with this observation, **9** showed no cytotoxic effects in the MTT assay (data not shown), at the same doses and incubation periods as above. Similar results to those obtained for **9** were observed when cells were incubated with PGE_2 (10 μM) alone, in both morphological and cytotoxic studies.

However, when HL-60 cells were cultured for 24 h with 100 nM TPA a decrease in the percentage of cell viability (i.e. metabolic activity), measured by the MTT procedure, was observed for **9** in a dose-dependent manner (Figure 5) as compared with untreated cells (control). The effect of TPA on the percentage of viability is a well-known phenomenon and is related to cell differentiation. However, the effect of **9** on cell viability in the presence of TPA is more difficult to explain since this compound is nontoxic, does not increase cell differentiation but does prevent TPA-induced cell differentiation. In this scenario, **9** could be interfering with the normal rate of the cell cycle and/or increasing cell death by apoptosis. Thus, the results shown in Figure 5 are in consonance with those in Figure 4. A more detailed observation of the cells treated with TPA plus 1000 μM of compound **9** (see Figure 4) reveals a significantly lower number of cells with respect to control cells.

In conclusion, the results presented herein show evidence that lactone **9** could compete with PGE_2 for binding to PGE_2 receptors in target cells. There are four subtypes of PGE_2 receptors encoded by distinct genes and coupled to different signal transduction mechanisms. Three subtypes (EP_2, EP_3, and EP_4) are expressed in HL-60,[26] and this makes it difficult to identify the receptor/s that bind/s **9** and whether the binding is transduced in receptor activation (agonistic activity) or receptor inactivation (antagonistic activity). Although the effects of **9** on c-*fos* mRNA expression are probably mediated by the EP_2 receptor as demonstrated for PGE_2,[6,7] the possible implications of the other subtypes cannot be ruled out and could explain other effects shown by **9**.

Acknowledgment. This research was supported in part by FEDER grant no. 1FD1997-1831 (J.B.B) and ICIC-P.I no. 16-2003. Many thanks are given to Drs. Victor S. Martín and Tomás Martín for useful discussions and suggestions.

Supporting Information Available: Experimental procedures and spectral and analytical data for compounds **2**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org

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JM034216Y