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Prostaglandins & other Lipid Mediators 62 (2000) 15–21

PROSTAGLANDINS
& other
LIPID MEDIATORS

The nature and composition of 15-deoxy-$\Delta^{12,14}PGJ_2$

Kirk M. Maxey[a]*, Edward Hessler[b], James MacDonald[a], Linda Hitchingham[a]

[a]*Cayman Chemical Company, 1180 E. Ellsworth Road, Ann Arbor, MI 48108, USA*
[b]*Bridge Organics Co., 311 W. Washington Sreet, Vicksburg, MI 49097, USA*

1. Introduction

At present, it is uncertain whether 15-deoxy-$\Delta^{12,14}PGJ_2$ (15-dJ2) is an endogenous eicosanoid mediator in any organism. What is generally conceded is that Prostaglandin D_2 is the primary cyclooxygenase product, and that 15-dJ2 is either a chemical decomposition product or a downstream metabolite. Therefore, it is instructive to first review what is well established about PGD_2 metabolism before entering into an area where there is considerable controversy.

2. History

Two studies at Vanderbilt in the mid-1980s established that in the intact human, most PGD_2 is reduced to the 11β-alcohol and excreted into the urine as a variety of further metabolized F-type prostaglandins [1,2]. Metabolites in which the D-ring survived represented 5 to 15% of the total, and none of these compounds contained the 15-deoxy-$\Delta^{12,14}$ moiety. Because 75% of the infused radioactively labeled PGD_2 was collected in the urine and accounted for, the possibilities for the existence of 15-dJ2 are well constrained. If it exists in humans, 15-dJ2 is a minor (<25%) PGD_2 metabolite, and it must exhibit pharmacokinetics that divert it entirely into a tissue reservoir or into the bile, but not into the urine. Alternately, if present in urine, 15-dJ2 must be present as polar, hydrophilic substances (e.g. glutathione conjugates) that evade the initial XAD-2 resin extraction [3].

The metabolic fate of 15-dJ2 in animals including humans has not yet been reported. The

* Corresponding author. Tel.: +1-734-971-3335; fax: +1-734-971-3420.
E-mail address: kirk@caymanchem.com (K.M. Maxey).

0090-6980/00/$ – see front matter
PII: S0090-6980(00)00072-1

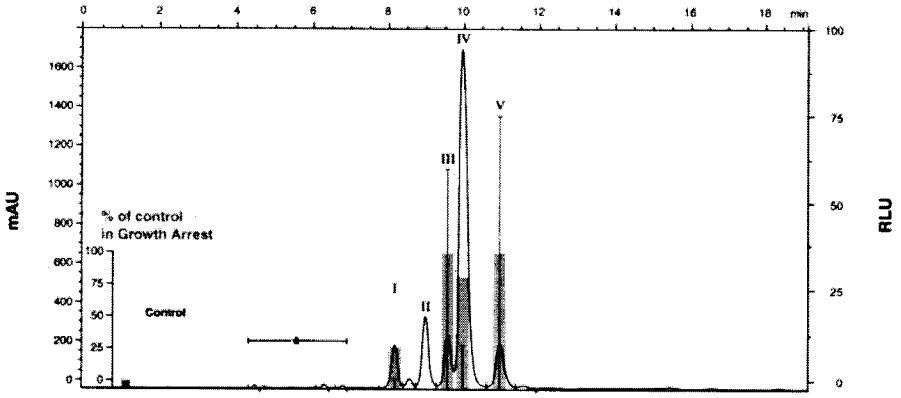

Fig. 1. Separation of 15-deoxy-$\Delta^{12,14}PGJ_2$(15-dJ2) isomers obtained from the base catalyzed decomposition of PGD_2. The crude 15-dJ2 was subjected to normal phase HPLC (column: silica, 5 μ, 4.6 × 250 mm; mobile phase: hexane:isopropanol:acetic acid (965:34:1); detection: 306 nm) to give five pure compounds. Structures of peaks I, III, IV and V were assigned by UV, mass spec and 300 MHz NMR [19] (peaks in the area designated by * have no conjugated dienone component; they include PGJ_2 and other uncharacterized compounds). The peak area shows the relative abundance of each isomer in mAU at 306 nm. The shaded bar represents the relative antiproliferative potency of each pure isomer tested separately on cultured MDA-MB-231 breast carcinoma cells, as percent of control in growth arrest. The vertical bar superimposed on each peak represents the relative PPARγ ligand binding affinity of that isomer in a luciferase reporter assay, expressed as RLU (compounds were at 5 μM in both assays). (Biological data are the preliminary observations of F. Chilton and will be reported in depth elsewhere.) Peak II could not be obtained in >97% purity and was not tested.

closely related compound PGJ_2 has been shown to form glutathione conjugates both with and without the catalytic assistance of glutathione *S*-transferase [4]. When PGJ_2 is added to cultured cells or infused into rats, thiol conjugates of both glutathione and cysteine are formed [4,5]. In rats, more than 90% of infused dose was excreted into the bile in the form of polar substances consistent with thiol conjugates. There is one report of the administration of a cyclopentenone eicosanoid (8-*iso* PGA_2) into a human volunteer [6]. Only 30% of the infused dose was recovered in the urine, and of that virtually all was in the form of polar, hydrophilic metabolites, supporting the hypothesis.

The relevance of the published pharmacokinetic studies of PGJ_2 to 15-dJ2 physiology is uncertain. First, although these substances are close chemical relatives, they are different compounds. Further, 15-dJ2 has been postulated to be synthesized and to act entirely within the nucleus. Thus, the rapid clearance of cyclopentenone eicosanoids, including 15-dJ2, from the circulation and their absence from the urine may be unimportant. It may be more important to document the in vivo synthesis of 15-dJ2.

The in vitro synthesis of 15-dJ2 has been reported [7]. When PGD_2 is exposed to plasma or to human serum albumin in aqueous buffer, dehydration at C-9 and isomerization of the 13,14 double bond are facile reactions due to the relatively acidic protons α to the C-11 ketone (see Dussault & Forman, Fig. 1). It is more difficult to eliminate the C-15 hydroxyl. The relative amounts of PGJ_2, $\Delta^{12}PGJ_2$, and 15-dJ2 from the in vitro synthesis referenced above are 48:49:3, respectively [7]. Both PGJ_2 and $\Delta^{12}PGJ_2$ have been isolated from cultured cells exposed to PGD_2, but 15-dJ2 has never been isolated from these experiments. In

addition, $\Delta^{12}PGJ_2$ was prepared by exposing PGD_2 to dialyzed, ethanol-fractionated human plasma, and no 15-dJ2 was detected [8]. The frequently quoted paper from the group of Hayaishi in Japan reported that $\Delta^{12}PGJ_2$ was recoverable from normal human urine using immunoaffinity chromatography [9]. Using an immunoassay, they reported a 24 h urinary excretion rate of about 150 ng of $\Delta^{12}PGJ_2$ for adult males. In summary, an albumin catalyzed route of decomposition leading from PGD_2 to 15-dJ2 exists. Several authors who studied this pathway identified $\Delta^{12}PGJ_2$ as the end product. Simple chemical decomposition readily leads to the dehydration and isomerization reactions described here, and the biosynthesis of 15-dJ2 in whole animals has not been demonstrated. No enzymatic process leading specifically to 15-dJ2 has been reported.

The first apparent chemical synthesis of 15-dJ2 occurred in the laboratories of Gordon Bundy at the Upjohn Company in 1983, although at the time it went unrecognized. 15-deoxy-$\Delta^{12,14}PGD_2$ was prepared and its UV spectrum recorded during the synthesis of analogs of Prostaglandin D_2, and the authors went on to report that ". . .upon treatment with strong base (15-deoxy-$\Delta^{12,14}PGD_2$) yielded a complex inseparable mixture of tetraenone isomers" [10]. In the same year, Fitzpatrick isolated the compound from albumin-catalyzed decomposition reactions and obtained UV, nmr, and mass spectral data [7]. In a telling precedent that would be passed along for more than 15 years, both authors described and drew a structure with a *trans-trans*-$\Delta^{12,14}$ diene component, although no data were described which supported this assignment of geometrical isomers.

15-dJ2 was again isolated in November of 1989 at Cayman Chemical Company and identified by TLC, nmr, and its characteristic, broad UV absorbance with maxima at 230 and 306 nm (Kirk M. Maxey, Jane Vitkuske, unpublished). Inadvertent decomposition of PGD_2 was once again the source of this material. The apparently homogeneous substance was introduced in 1990 as a commercial product [18]. It languished quietly until nearly simultaneous papers in 1995 identified 15-dJ2 as a potent, endogenous ligand for the orphan PPARγ nuclear receptor [11,12]. Now the subject of much greater scrutiny, the structure and composition of synthetic 15-dJ2 was reinvestigated.

3. Current Investigations

When subjected to HPLC, it becomes apparent that 15-dJ2 obtained by chemical decomposition of PGD_2 consists of a single prominent compound surrounded by a number of isomers with identical molecular weights and very similar spectral characteristics (Fig. 1). These minor compounds are geometric isomers about the three double bonds not contained within the cyclopentenone ring. Isolation of each of these isomers using normal phase HPLC and spectral characterization led to unambiguous structural assignment of the isomers for the first time (Fig. 2). It was something of a surprise to find that the major isomer did not have the structure propagated through the literature over the years, but was in fact the 5-*cis*, 12-*trans*, 14-*cis* compound, Structure IV. Adding to this irony is the fact that the minor isomer I that does have the structure assigned by Bundy and Fitzpatrick is much less active than several other isomers, as seen in Fig. 1.

The purified individual isomers of 15-dJ2 were evaluated in a PPARγ ligand-binding

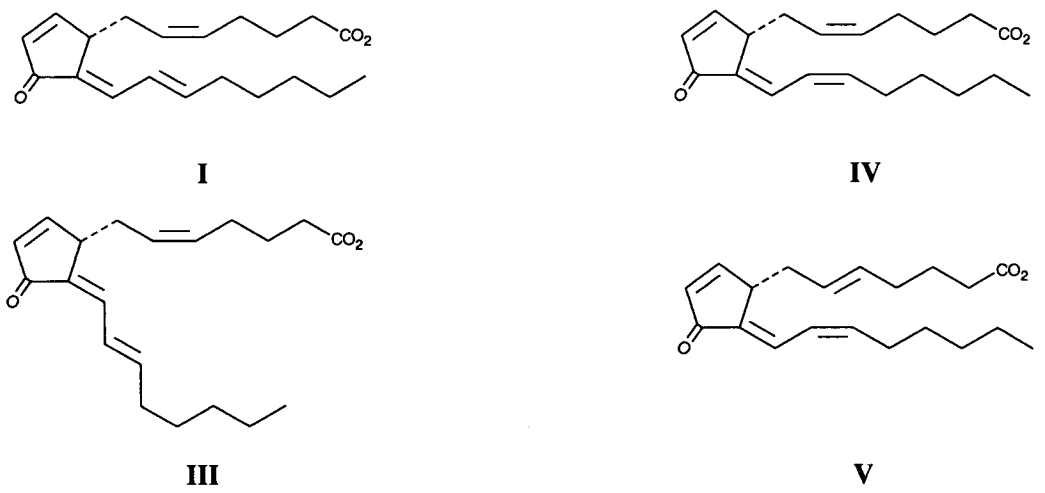

Fig. 2. Structures of 15-deoxy-$\Delta^{12,14}$-PGJ_2 isomers corresponding to the peaks in Fig. 1. Structural assignments were made based on NMR coupling constants of the olefinic protons (Bruker, 300 MHz, 25°C in $CDCl_3$). Selected decoupling experiments were performed and interpreted by E. Hessler [19].

assay with a luciferase reporter. They were also evaluated for their antiproliferative activity in cultures of human mammary adenocarcinoma cells (data of Dr F. Chilton). These studies revealed substantial differences in the relative potency of the isomers. These differences point to a preliminary structure-activity profile for the cyclopentenone nuclear receptor ligands which is distinctly different from that of traditional eicosanoids acting at 7-trans-membrane, G-protein coupled receptors. Most notable is the indifference of the nuclear receptor to the 15-(*S*) hydroxyl group. Although traditional prostanoid receptor agonists lose >90% of their activity upon oxidation or inversion of the 15-(*S*) hydroxyl, the PPARγ ligands are more active if it is eliminated. A free, ionizable C-1 carboxyl group is also essential for G-protein coupled receptor recognition. At the PPARγ nuclear receptor, methyl esterification of C-1 leads to only a modest (<50%) loss of potency (data not shown). Finally, the *trans*-Δ5 isomers of most prostaglandins are usually less potent than the *cis* isomers; as PPARγ ligands, the *trans* compounds are at least twice as potent. Further structure/activity relationships of the 15-deoxy PGJ series are currently being developed and will be reported elsewhere.

The highly electrophilic, chemically reactive nature of 15-dJ2 renders it difficult to study in whole animal experiments. We have administered deuterated 15-dJ2 to a human volunteer, but hemodynamic side effects limited the total dose to less than 250 μg. At this dose no molecular ions corresponding to the unmetabolized compound, its glutathione or cysteine adduct could be detected by LC/MS of the purified 24 hour urine sample. Studies in our own lab have shown that 15-dJ2 reacts readily with cysteine, glutathione and BSA in the presence of glutathione-S-transferase to form covalent adducts. These adducts have been characterized, and are almost exclusively the result of 1,4 addition of the sulfur nucleophile to C-9. The complexity of the mixture of substances comprising 15-dJ2 makes it difficult to study. There are eight theoretically possible geometric isomers, four of which have been isolated.

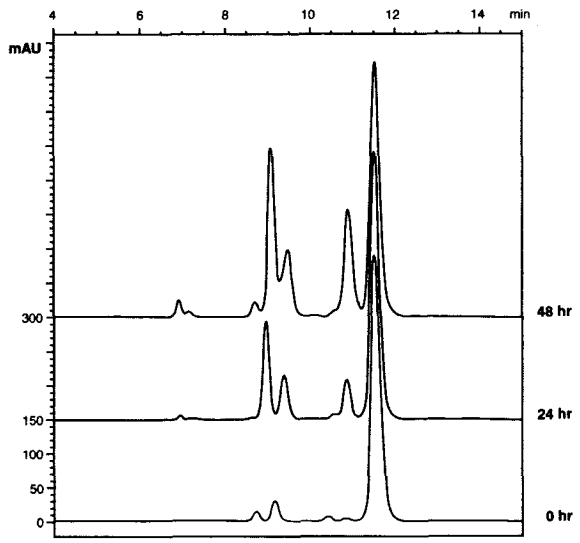

Fig. 3. Purified Isomer V was dissolved in ethanol and placed in a clear borosilicate glass vial near a window. The purity of the material was checked by HPLC at 24 and 48 h.

Instability further complicates the evaluation of 15-dJ2 activity. The less thermodynamically stable isomers at the $\Delta^{12,14}$-conjugated dienone component isomerize under relatively mild conditions to form mixtures. Peak II in Fig. 1 represents an isomer which was only observed when 15-dJ2 was prepared under low temperature conditions. It was too unstable to evaluate by NMR or PPAR-ligand activity assay.

This instability led us to further investigate the chemical integrity and thermal stability of the other isomers. Exposure to a temperature of 37°C in the dark under nitrogen for 48 h had little effect on purified 15-dJ2 isomer V. However, it was rapidly interconverted to a mixture of 15-dJ2 isomers by a 24 h exposure to ambient light, as shown in Fig. 3.

We further reasoned that the basic conditions often employed to decompose PGD_2 into 15-dJ2 could reversibly deprotonate C-8, resulting in the complete racemization of the molecule. Therefore, we obtained an optical rotation on highly purified isomer IV, and found it to be strongly dextrorotary. ($[\alpha]_D^{25} = +254.6$ in methyl acetate) We concluded from these studies that 15-dJ2 is thermally stable. It is optically active by virtue of a single chiral stereocenter of the (*S*) configuration retained at C-8. However, it is photolabile to the extent that purified single isomers can be rendered less than 50% pure by a single day's exposure to ambient light. Finally, our studies and those of others have shown that 15-dJ2 reacts within minutes with any available thiol nucleophile, including cysteine, glutathione, and protein thiols, to form Michael adducts.

The discovery and investigation of 15-dJ2 has followed an anomalous course. From the studies above it must be inferred that biological experiments that employed 15-dJ2 over the last decade have used a heterogeneous mix of nonidentical substances. In the absence of a natural source, biological experiments have relied on commercial compounds with no

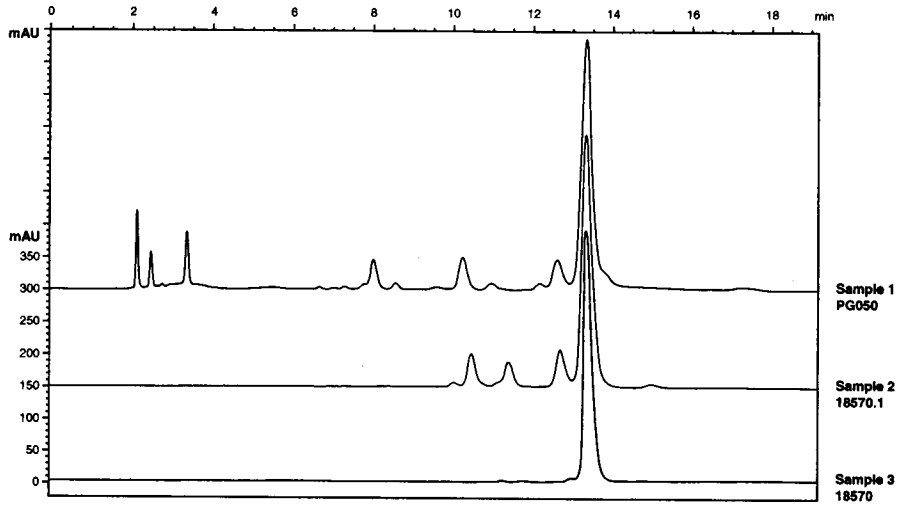

Fig. 4. HPLC chromatogram (see Fig. 1 for conditions) of three commercial samples of 15-deoxy-$\Delta^{12,14}PGJ_2$ obtained from two sources. Sample 2 and sample 3 are two grades of 15-deoxy-$\Delta^{12,14}PGJ_2$ obtained from the same supplier and sold under separate catalog numbers.

established relationship to any endogenous mediator. Experiments conducted before 1995 relied on a single source of material obtained from PGD_2. Thereafter, three distinct types of material were available as illustrated in Fig. 4. A number of investigators have reported inconsistent results with different batches of 15-dJ2, which may have been due to the presence or absence of some of the minor isomers [13,14] (R. Oyasu, R. Ray personal communication). However, it should also be noted that even with the purified isomer preparations, inadvertant loss of chemical integrity can easily occur during the course of experiments.

It is customary to discover a biological activity intrinsic to an exudate of cells, glands or tissues, and then deduce the structure of the mediator through careful isolation, purification, and molecular examination. The obvious advantage of this approach is that isolation from nature carries an imprint of biological relevance. SRS-A, PGX, and RCS (PHD) are the archaic names applied to potent activities of unknown structure, later characterized as LTC_4 [15], PGI_2 [16], and TXA_2 [17], respectively, according to this paradigm. This contrasts with 15-dJ2, which was first discovered through in vitro manipulations of PGD_2. In the years since, biological activity experiments badly outpaced efforts to isolate the substance from nature or to carefully study its chemistry. This led to the unfortunate circumstance where a remarkably large superstructure of biology has been constructed on the wobbliest of foundations. It is possible that the cyclopentenoid prostaglandins are nothing more than interesting synthetic pharmacologic agents with a variety of activities. Or perhaps the cyclopentenoid prostaglandins are actually biosynthesized discretely within the nucleus, reacting rapidly with thiol nucleophiles as they exit into the cytoplasm. In this case, these adducts should still be measurable and detectable by conventional methods. This should be a prime focus of future studies seeking to firmly position these compounds within the natural order.

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