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ORIGINAL ARTICLE

Transformation of Prostaglandin D_2 to 11-Dehydro Thromboxane B_2 by Baeyer-Villiger Oxidation

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Abstract Prostaglandin D_2 is one of five chief prostanoids formed in the cyclooxygenase pathway of arachidonic acid oxidation. Except for a single oxygen atom, PGD_2 is structurally identical to 11-dehydro thromboxane B_2 (11d-TxB_2), a urinary metabolite of the pro-aggregatory platelet activator, thromboxane A_2. The close structural relationship suggested that one might be transformed to the other. Accordingly, we tested whether the cyclopentanone of PGD_2 can be expanded to the δ-lactone of 11d-TxB_2 in a Baeyer-Villiger oxidation. Oxidation of PGD_2 with two standard oxidants showed that 11d-TxB_2 was formed only with H_2O_2 but not with peracetic acid. Byproducts of the H_2O_2-mediated oxidation were hydroperoxide derivatives and isomers of PGD_2. Chemical oxidation of PGD_2 to 11d-TxB_2 may be a model for an equivalent enzymatic transformation, suggesting a possible link in the metabolism of PGD_2 and thromboxane A_2.

Keywords Arachidonic acid · Cyclooxygenase · Eicosanoid · Lactone · Peroxyhemiacetal

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Supporting information Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Abbreviations

COX	cyclooxygenase
FMO	flavin-dependent monooxygenase
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol
NMR	nuclear magnetic resonance
PG	prostaglandin
TPP	triphenylphosphine
Tx	thromboxane
11d-TxB_2	11-dehydro thromboxane B_2

Introduction

Prostaglandins (PG) are formed in the cyclooxygenase (COX)-mediated oxygenation of arachidonic acid (Schneider et al., 2007; Smith et al., 2011; van der Donk et al., 2002). PG, together with other eicosanoids formed in the lipoxygenase, cytochrome P450, and autoxidative pathways, are a large and diverse group of lipid mediators (Brash, 1999; Jahn et al., 2008; Spector, 2009). Additional diversity comes from transformation of related fatty acids, e.g., eicosapentaenoic and docosahexaenoic acids. The resulting products vary in the number and type of oxygen functional groups, cyclizations, and degree of unsaturation (Sud et al., 2007).

The immediate COX product from arachidonic acid, the PG endoperoxide PGH_2, undergoes enzymatic transformation to the chief effector prostanoids, PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (Smith et al., 2011). PG are biosynthesized in a tissue-specific manner and act *via* activation of their cognate G-protein-coupled receptors that are involved in the regulation of physiological and pathophysiological cellular events (Smyth et al., 2009).

The premise for this study is based on the close similarity in structure of PGD_2 and 11-dehydro thromboxane B_2

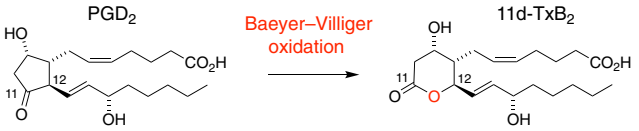


Fig. 1 Proposed conversion of PGD_2 to 11-dehydro thromboxane B_2 (11d-TxB_2) in a Baeyer-Villiger oxidation. The oxygen that expands the cyclopentanone of PGD_2 to the δ-lactone of 11d-TxB_2 is shown in red

(11d-TxB_2), a urinary metabolite of thromboxane A_2 (Roberts II et al., 1981). With identical side chains and hydroxy and oxo functions at the 9- and 11-positions of the prostanoid ring, the only difference is an additional oxygen in 11d-TxB_2, expanding the cyclopentanone of PGD_2 to a δ-lactone. Linear and cyclic ketones can be converted to esters and lactones, respectively, in a Baeyer-Villiger oxidation that inserts a peroxide-derived oxygen next to the carbonyl (March, 1992). Thus, we tested the feasibility of the Baeyer-Villiger oxidation to transform PGD_2 to 11d-TxB_2 as illustrated in (Fig. 1) (Jarving et al., 1988; Jarving et al., 1990). This resulted in the identification of novel reaction products formed in the transformation.

Materials and Methods

Materials

PG were obtained from Cayman Chemical (Ann Arbor, MI, USA). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was obtained from ACROS Organics. Hydrochloric acid, hydrogen peroxide, and triphenylphosphine (TPP) were obtained from Sigma-Aldrich.

Oxidation Reactions

PGD_2 (20 µg) was dissolved in 20 µL of HFIP. Water (1.6 µL), hydrochloric acid (1 N; 0.4 µL), and hydrogen peroxide (10 M; 0.4 µL) were added, and the solution was placed at room temperature. After 1 h, the reaction was evaporated to dryness, and the residue dissolved in 20 µL of methanol. For isolation of products, reactions were scaled 10-fold, and multiple reactions were conducted in parallel.

HPLC

Reactions were analyzed using a Waters Symmetry (Milford, MA, USA) C18 5 µm column (4.6 × 250 mm) eluted with a linear gradient of 20–80% acetonitrile in 0.01% aqueous acetic acid over 20 min. The samples were eluted at a flow rate of 1 mL/min, and UV signals at 205 nm were monitored using an Agilent 1200 diode array detector.

LC–MS

Reactions were analyzed using a Thermo TSQ Vantage triple quadrupole MS instrument (Thermo Fisher Scientific) equipped with a heated electrospray interface operated in negative ion mode. Settings for sheath and auxiliary gas pressures, temperature, and interface voltage were optimized using direct infusion of a solution of PGD_2 in acetonitrile/water 1:1 (v/v). The electrospray needle was maintained at 4.0 kV. The ion transfer tube was operated at 300 °C. A Zorbax Eclipse Plus C18 1.8-µm column (2.1 × 50 mm; Agilent Technologies, Santa Clara, CA, USA) was used for the separation. Water: acetonitrile (80:20, v/v) and acetonitrile: water (20:80, v/v) each containing 0.1% formic acid were the mobile phases (A and B, respectively) used at a flow rate of 0.5 mL/min. A linear gradient was used starting with 100% of solvent A, reaching 100% of solvent B at 5 min, and held for 1 min. The initial conditions were re-established at 6.01 min and held until 7 min. The signal intensities obtained for each ion chromatogram are given in exponential format with the maximum intensity set as 100%. Data acquisition and spectral analysis were performed using the Thermo Finnigan Xcalibur software.

NMR

Nuclear magnetic resonance (NMR) samples were dissolved in 150 µL of $CDCl_3$ in a 3 mm-sample tube and analyzed on a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe. Chemical shifts are reported relative to residual unlabeled solvent ($\delta = 7.25$ ppm).

Results

Oxidation reactions of PGD_2 were carried out in hexafluoroisopropanol (HFIP) in the presence of HCl using peracetic acid or H_2O_2 as oxidant (Berkessel and Andreae, 2001). Reaction progress was monitored by RP-HPLC with diode array detection. Use of peracetic acid resulted in disappearance of PGD_2 without detectable formation of 11d-TxB_2. The majority of products obtained with peracetic acid had diene and triene chromophores, likely representing dehydration products (data not shown). When H_2O_2 was used as oxidant, 11d-TxB_2 (product **2**) was formed as the intended Baeyer-Villiger product together with several byproducts (Fig. 2). Identification of 11d-TxB_2 was accomplished by LC–MS analysis in the MS1 and MS2 modes as well as by co-elution with an authentic standard. The yield of 11d-TxB_2 was 21% based on peak intensities in HPLC

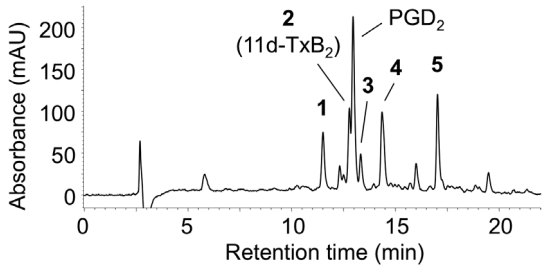


Fig. 2 Analysis of Baeyer-Villiger oxidation of PGD_2. $PGD_2$was reacted with H_2O_2 in acidic hexafluoroisopropanol at room temperature for 1 h. products were analyzed using RP-HPLC with diode array detection. The chromatogram was recorded at 205 nm

analyses, similar in yield to a previous report (Jarving et al., 1988).

LC–MS analyses of crude oxidation reactions (Fig. 3) and isolated peaks were used to determine the molecular weights of the products. The analyses confirmed that 11d-TxB_2 was the main product resulting from insertion of oxygen.

Several byproducts of the H_2O_2-mediated oxidation of PGD_2 were isolated using RP-HPLC. NMR data were collected for some of the products (Table 1 and Supporting Information). Product **1** was tentatively identified as the hydroperoxyhemiacetal of 15-hydroperoxy-PGD_2 (Fig. 4). The structure of **1** was assigned based on LC–MS (*m/z* 401.0 in negative ion LC–MS; Fig. 3) and from the fact that treatment of **1** with TPP to reduce peroxide groups yielded PGD_2. It was unclear whether the hydroperoxy group was located at C-9 or C-15, although comparison to product **4** (see below) suggested the hydroperoxide was more likely to be located at C-15. The structural assignment is tentative since NMR data for **1** could not be obtained.

Product **3** (*m/z* 351.0 in negative ion LC–MS; Fig. 3) was identified as an isomer of PGD_2 with the double bond changed from 5*Z* to 5*E* configuration (J = 15 Hz; Table 1) (Fig. 4). Peak **4** (*m/z* 367.1) showed near-identical NMR data to PGD_2 with the exception of H-15 that was shifted to 4.36 ppm compared with 4.53 ppm in PGD_2. Reduction of **4** with TPP yielded PGD_2, confirming that **4** was 15*S*-hydroperoxy-PGD_2. NMR data of peak **5** suggested a 13-hydro(pero)xy isomer of PGD_2 with the double bond shifted to 14*E* and side chains on the prostanoid ring in *cis* configuration (12-iso). Treatment of **5** with TPP gave four more polar peaks of about equal intensity in LC–MS analyses. Since LC–MS analyses gave inconclusive data for **5** and its reduced products, the structural assignment is considered tentative. None of the products co-eluted with a standard of 15*R*-PGD_2.

The absolute configuration of products **1–5** as shown in Fig. 4 was determined based on the assumption that the stereochemistry of C-9 was unchanged during the reaction. The $^2J_{H,H}$ coupling constants used to determine the relative configuration of substituents on the prostanoid ring of **1–5** were obtained through selective spin decoupling experiments (Table 1 and Supporting Information). Reference values for *cis* and *trans* configured hydrogens at the prostanoid ring were obtained from analysis of commercially available PG with configuration changed relative to PGD_2, namely, 8-iso-PGE_1 and 11β-PGE_2 (Table 2).

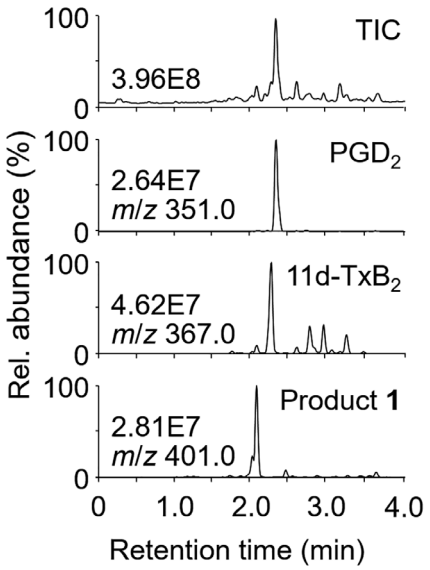


Fig. 3 LC–MS analysis of a crude Baeyer-Villiger oxidation reaction of PGD_2. The chromatograms represent the ion traces for detection of PGD_2 (*m/z* 351) and products 11d-TxB_2 (**2**; *m/z* 367) and **1** (*m/z* 401)

Discussion

Treatment of PGD_2 with H_2O_2 in acidic solvent resulted in Baeyer-Villiger oxidation to 11d-TxB_2. Insertion of oxygen between C-11 and C-12 was compatible with the known mechanism that favors placing oxygen on the side of the higher substituted carbon (Renz and Meunier, 1999). Thus, Baeyer-Villiger oxidation provides convenient synthetic access to corresponding 11-dehydro thromboxanes starting from the respective PGD precursors. Of two standard peroxide reagents used in Baeyer-Villiger oxidations, only H_2O_2 yielded the intended product with low to moderate yield. Peracetic acid did not form 11d-TxB_2.

Quantification of 11d-TxB_2 in urine is used to assess the antiplatelet effect of low-dose aspirin therapy (Patrono, 2014; Patrono et al., 2005). The pharmacodynamic target of low-dose aspirin is COX-1 in platelets (Pedersen and FitzGerald, 1984), supplying PGH_2 as substrate for synthesis of thromboxane A_2 (TxA_2) by thromboxane synthase (Hamberg and Samuelsson, 1974). TxA_2 is the major pro-

Table 1 ^{1}H NMR chemical shifts and correlations for products 3, 4, and 5 formed in the Baeyer-Villiger oxidation of PGD_2

	3			4			5		
Proton no.	^{1}H (ppm)	Multiplicity	Coupling constant (Hz)	^{1}H (ppm)	Multiplicity	Coupling constant (Hz)	^{1}H (ppm)	Multiplicity	Coupling constant (Hz)
2	2.34	m		2.41	t	$J_{2,3} = 3.7$	2.37	t	$J_{2,3} = 7.0$
3	1.73	m		1.74	m		1.72	m	
4	2.13	m		2.19	m		2.14	m	
5	5.49	dd	$J_{5,6} = 15.1$; $J_{5,4} = 8.4$	5.50	dt	$J_{5,6} = 10.5$; $J_{5,4} = 5.6$	5.43	dt	$J_{5,4} = 7.3$; $J_{5,6} = 10.4$
6	5.61	dd	$J_{6,5} = 14.9$; $J_{6,7} = 6.2$	5.58	m		5.34	ddd	$J_{6,5} = 10.8$; $J_{6,7b} = 6.7$
7a	2.19	dd	$J_{7a,7b} = 14.9$; $J_{7a,6} = 7.1$	2.30	ddd	$J_{7a,7b} = 10.6$; $J_{7a,6} = 6.8$; $J_{7a,8} = 3.8$	2.08	m	
7b	2.48	ddd	$J_{7a,7b} = 14.3$; $J_{7b,8} = 3.9$; $J_{7b,6} = 8.0$	2.39	m		2.36	m	
8	1.91	ddd	$J_{8,12} = 11.8$; $J_{7,8} = 3.8$; $J_{8,9} = 3.6$	2.05	dddd	$J_{8,12} = 15.5$; $J_{7a,8} = 4.1$; $J_{7b,8} = 8.1$; $J_{8,9} = 4.1$	2.08	m	
9	4.54	dt	$J_{9,10} = 2.6$; $J_{9,8} = 3.6$	4.54	dd	$J_{9,10} = 3.7$; $J_{9,8} = 3.4$	4.50	dt	$J_{9,10} = 4.8$; $J_{9,8} = 9.2$
10	2.44	d	$J_{10,9} = 2.8$	2.45	d	$J_{10,9} = 4.2$	2.27	dt	$J_{10a,10b} = 14.5$; $J_{10,9} = 5.0$
11	—			—			—		
12	2.80	dd	$J_{8,12} = 12.1$; $J_{12,13} = 8.4$	2.91	dd	$J_{8,12} = 11.9$; $J_{12,13} = 7.3$	2.85	dd	$J_{8,12} = 6.1$; $J_{12,13} = 6.1$
13	5.47	dd	$J_{13,14} = 15.4$; $J_{13,12} = 8.4$	5.70	dd	$J_{13,14} = 15.7$; $J_{13,12} = 7.3$	4.47	dd	$J_{13,14} = 7.7$; $J_{12,13} = 7.6$
14	5.62	dd	$J_{14,13} = 15.3$; $J_{14,15} = 7.3$	5.51	dd	$J_{14,13} = 15.7$; $J_{14,15} = 8.1$	5.37	dd	$J_{14,13} = 8.3$; $J_{14,15} = 15.4$
15	4.21	dt	$J_{15,16} = 6.3$; $J_{14,15} = 6.7$	4.36	dt	$J_{15,16} = 6.9$; $J_{14,15} = 8.0$	5.87	dt	$J_{15,16} = 6.8$; $J_{14,15} = 15.4$
16	1.58	m		1.45, 1.61	m		2.06	m	
17, 18, 19	1.23–1.44	m		1.26–1.41	m		1.38,	1.28	m
20	0.89	t	$J_{19,20} = 6.8$	0.88	t	$J_{19,20} = 7.1$	0.88	t	$J_{19,20} = 7.2$

Spectra were recorded in $CDCl_3$ using a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe. Coupling constants of complex signals were determined using selective spin decoupling experiments. NMR spectra are shown in supporting information.

aggregatory eicosanoid in platelets, and its inhibition is the key mechanism for the cardioprotective effects of aspirin (FitzGerald et al., 1983; Patrignani et al., 1982). TxA_2 is hydrolyzed to inactive TxB_2 and further metabolized to 11d-TxB_2 (Patrono et al., 1985; Roberts II et al., 1981). Thus, if 11d-TxB_2 may also be derived from PGD_2, application of the former as a biomarker for platelet activation in cardiovascular disease will need to be reconsidered, depending on the efficiency of the metabolic pathway.

Enzymatic Baeyer-Villiger oxidations catalyzed by flavin-dependent monooxygenases (FMO) are well described in microorganisms, with many of the reactions and enzymes characterized at the molecular level (Huijbers et al., 2014; Rossner et al., 2017). In microorganisms, the reactions appear to be involved in the mobilization of carbon sources as fuel for cell growth (Tolmie et al., 2019) while the mammalian enzymes play a role in the metabolism of xenobiotics (Phillips and Shephard, 2017). Human flavin-dependent monooxygenase 5 (FMO5) has been shown to catalyze Baeyer-Villiger oxidations of two cancer drugs (Lai et al., 2011; Meng et al., 2015). Although no endogenous substrates for Baeyer-Villiger oxidations have been described, metabolism of the cancer drugs suggests that the corresponding enzymatic machinery is functional

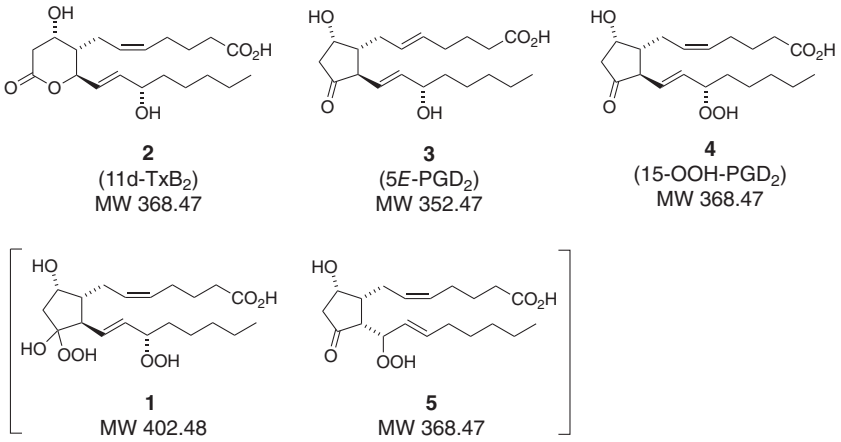
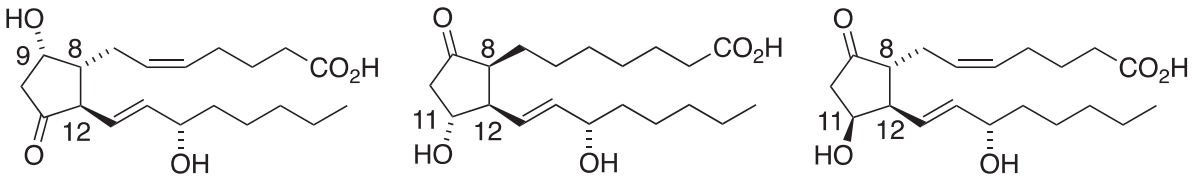


Fig. 4 Products of the H_2O_2-mediated oxidation of PGD_2 conducted in the presence of HCl. Products **1** and **5** shown in square brackets have been identified only tentatively

Table 2 Coupling constants for *cis* and *trans* configured hydrogens in the prostanoid ring of select prostaglandins



Prostanoid	PGD_2	8-iso-PGE_1	11β-PGE_2
		Coupling constant *J* (Hz)	
H8-H12	11.6 (*trans*)	8.2 (*cis*)	11.9 (*trans*)
H8-H9	3.5 (*cis*)	—	—
H11-H12	—	1.6 (*trans*)	3.4 (*cis*)

NMR spectra were recorded in $CDCl_3$ at 600.13 MHz using a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe.

in humans. With regard to transformation of oxylipins Baeyer-Villiger oxidation of a linoleic acid α-ketol, produced by the lipoxygenase-allene oxide synthase pathway in plants (Tijet and Brash, 2002), was catalyzed by a microorganism isolated from soil (Schneider et al., 1997). The predicted ester product of the Baeyer-Villiger oxidation was unstable and underwent hydrolysis to yield the plant hormone, traumatic acid (Schneider et al., 1997; Zimmerman and Coudron, 1979).

Exploring the scope of the Baeyer-Villiger oxidation regarding substrates, products, synthetic application, and its possible occurrence *in vivo* will add novel facets to the wealth of enzymatic and chemical reactions involved in the formations and metabolism of bioactive lipids.

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Conflict of Interest The authors declare that they have no conflict of interest.

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