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Δ^7-Prostaglandin C_1: A Primary Metabolite of Antitumor Δ^7-Prostaglandin A_1 in the Sera

Masaaki Suzuki,*† Toshihiro Kiho,‡ Kyoji Furuta,† Shoji Fukushima,¶ Yoshikazu Takeuchi,¶ and Ryoji Noyori*‡

†*Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-11, Japan*

‡ *Department of Chemistry and Molecular Chirality Research Unit, Nagoya University, Chikusa, Nagoya 464-01, Japan*

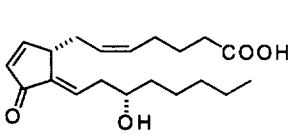
¶*Department of Pharamceutics, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Arise 518, Igawadani-cho, Nishi-ku, Kobe 651-21, Japan*

Abstract: Δ^7-PGA_1, an artificial antitumor prostaglandin, is metabolized rapidly in the rat serum to give Δ^7-PGC_1 as a primary metabolite. This is further degradated to produce a complex mixture. The structure of Δ^7-PGC_1 has been determined by comparison with a sample chemically synthesized by triethylamine treatment of Δ^7-PGA_1.

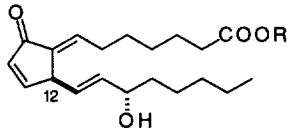
INTRODUCTION

Intense attention has been directed to the antineoplastic and antiviral prostaglandins (PGs) and related compounds.[1] PGs such as Δ^{12}-PGJ_2 (**1**) and Δ^7-PGA_1 methyl ester (**2**) that possess a cross-conjugated dienone unit exhibit unique antitumor activity independent of intracellular cAMP levels. Their activities are characterized by cell cycle arrest at the G_1 phase accompanied with suppression of *c-myc* gene expression at noncytotoxic doses, providing possible new therapeutic strategies.[2] Actually, the unnatural dienone PG **2**, easily prepared by the three-component synthesis,[3] is now under a preclinical study for the treatment of chemotherapeutically resistant ovarian cancer by intraperitoneal administration.[2]

Recently, Fukushima found that **2** and Δ^7-PGA_1 (**3**) are metabolized facilely in the sera in the course of the study on drug delivery.[4] This phenomenon urged us to investigate the structure of the metabolites, because understanding of the metabolic pathway helps rational molecular designing of more cultivated anticancer agents. Described herein is the identification of the primary metabolite of Δ^7-PGA_1 in the serum.



1, Δ^{12}-PGJ_2

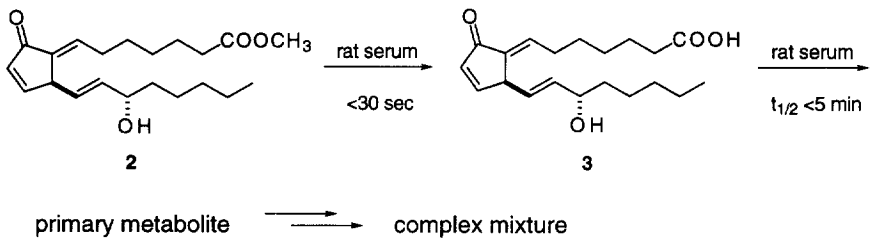


2: R = CH_3, Δ^7-PGA_1 methyl ester
3: R = H, Δ^7-PGA_1

RESULTS AND DISCUSSION

HPLC analysis of the time-course of the substrate disappearance indicated that Δ^7-PGA$_1$ methyl ester (**2**) was hydrolyzed in the rat serum at 37 °C within a half minute to form the corresponding acid **3** and that the latter is further metabolized via a single primary metabolite to give a complex mixture (Scheme 1).

Scheme 1



After 30 min incubation, the HPLC peak of the primary metabolite decreased to the extent of less than half of the height observed after 5 min. Direct incubation of **3** turned out to trace a similar metabolic pathway observed for **2** via **3** under given biological conditions. Thus, **3** was very labile in the serum and underwent rapid metabolism with a half-life of less than 5 min. Reversed-phase HPLC analysis after incubation of **3** in the rat serum for 7 min indicated the formation of a primary metabolite as a major component (t_R = 9.9 min) (Figure 1).

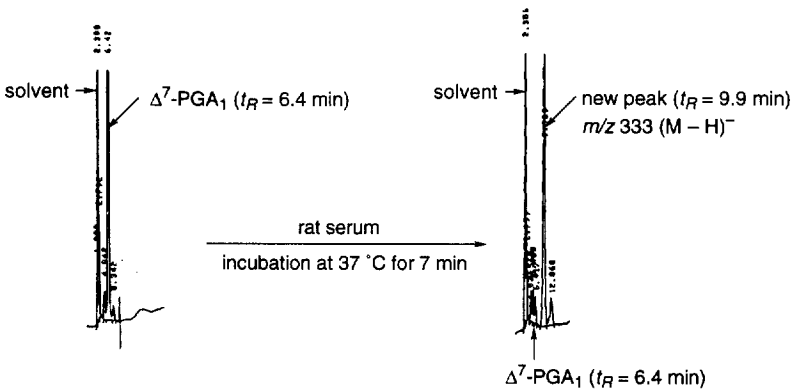
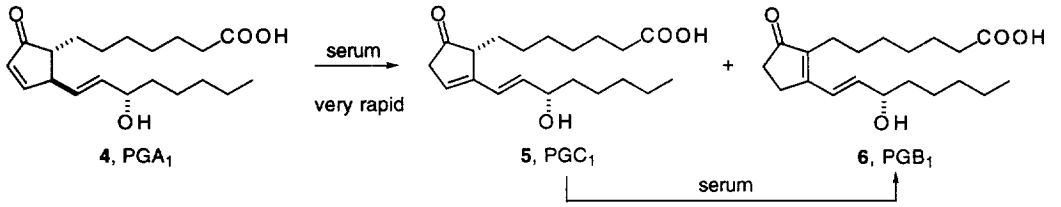
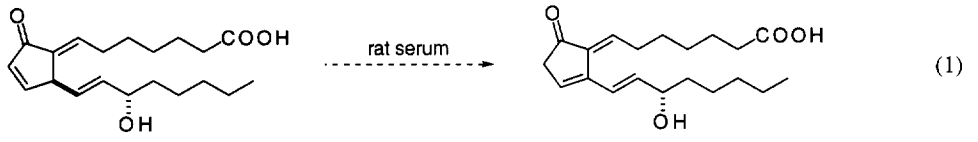


Figure 1. Reversed-phase HPLC analysis of Δ^7-PGA$_1$ (**3**) and its metabolites after incubation at 37 °C for 7 min in the rat serum.

Because the incubation required highly diluted conditions and the metabolite was fairly labile under concentration, isolation of a sufficient amount of the primary metabolite for NMR analysis was difficult. Therefore, we decided the use of LC-MS analysis that is directly applicable to the metabolite mixture in the medium. Thus electrospray MS analysis of the metabolic mixture indicated that the primary metabolite from **3** revealed a prominent ion peak at *m/z* 333 assignable to an $[M - H]^-$ ion, which corresponds to the anion derived from **3**. This suggested the formation of a structural isomer of **3**.

Scheme 2. Metabolic pathway of PGA_1 in the serum.

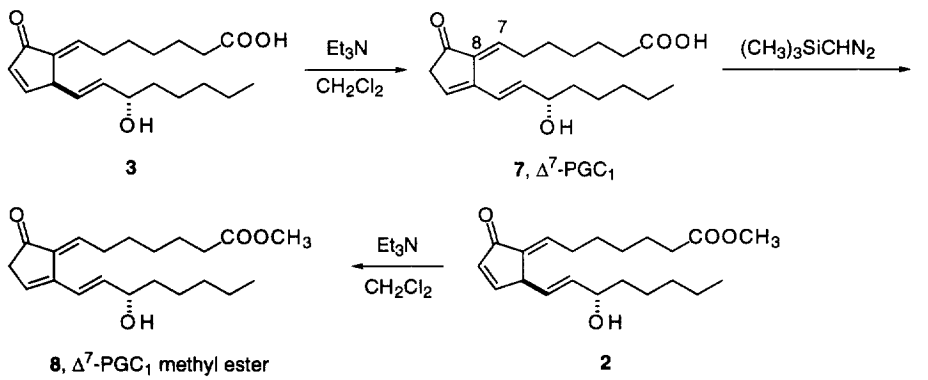




(1)

It is known that PGA_1 (**4**) is converted to PGB_1 (**6**) via short-lived PGC_1 (**5**) by the action of PG isomerases in the sera of various species.[5] Actually, **4** was metabolized in the rat serum under similar conditions to those applied to **3**, giving **5** and **6** with a half-life of <1 min (Scheme 2). Thus, we suspected that **3** comprising the structure analogous to PGA_1 may undergo the similar double-bond isomerization by the enzyme (eq 1). From this view, we next investigated the isomerization of **3** by chemical methods. The cross-conjugated dienone **3** was stable under physiological or weakly basic conditions (pH 7.4–9.3, phosphate buffer) over 24 h. In aqueous THF containing NaOH (pH >10), however, **3** was unstable, giving a complex mixture including double-bond isomers. While, treatment of **3** with excess triethylamine in dichloromethane at ambient temperature induced the isomerization of the A-type structure into the C-type structure, Δ^7-PGC_1 (**7**) selectively (Scheme 3).[6] The structure of **7** was confirmed by NMR analysis and the comparison of TLC and HPLC behavior of the corresponding methyl ester **8**,[7] prepared by treatment with diazomethane. The methyl ester obtained by triethylamine treatment of **2** showed the same chromatographic characteristics.

Scheme 3. Chemical synthesis of authentic PGC_1 and its methyl ester.



Unlike natural PGC_1, **7** resists isomerization to the B-type structure because of the presence of the C_7–C_8 double bond in difference from the structure **5**.[8] Then, we compared the HPLC behavior and the MS spectrum of **7** with those of the primary metabolite in the rat serum to identify the structure of the metabolite, indicating that synthetic Δ^7-PGC_1 exhibited the same retention time in HPLC (Figure 2) and the superimposing fragmentation pattern in the MS spectrum as that of the primary metabolite. Thus we elucidated definitively the structure of the primary metabolite of Δ^7-PGA_1 in the rat serum to be Δ^7-PGC_1 (**7**).

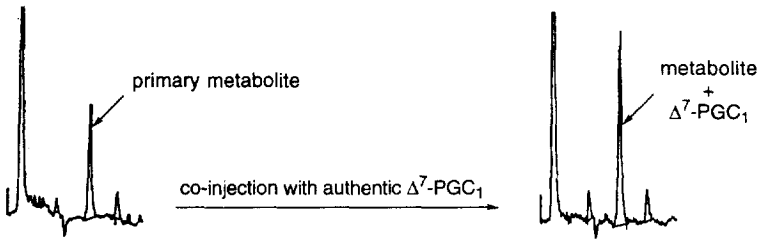


Figure 2. HPLC co-injection of a metabolic mixture and authentic Δ^7-PGC_1 (**7**) with the increase of the central peak.

In conclusion, Δ^7-PGA_1 (**3**), a readily accessible artificial antitumor prostaglandin, suffers from rapid metabolism in the rat serum via Δ^7-PGC_1 (**7**) as a primary metabolite. This phenomenon, in combination with our recent study on the mechanism of the anticancer effect of PGs at the molecular structural level,[1,2f,2i] will provide valuable information for designing an ideal anticancer PG tolerant for the *in vivo* system.

EXPERIMENTAL

General. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a JEOL GSX-270 or JMN-GX-500 spectrometer. Chemical shifts are reported in parts per million (δ) with tetramethylsilane or the deuterium lock signal of $CDCl_3$ as an internal standard. Electron impact mass spectra (EIMS) were obtained on a JEOL JMS-DX 300 (EI) spectrometer. Electrospray mass spectra (ESMS) were measured on a JASCO PLATFORM equipped with a JASCO PU-980 HPLC insturument (LC-MS) using a Crest Pak C18S column (JASCO, 4.6 mm × 150 mm). High-performance liquid chromatography (HPLC) was conducted on a Crest Pak C18S column (reversed-phase, JASCO, 4.6 mm × 150 mm) or a ZORBAX SIL column (DuPont Instruments, 4.6 mm × 250 mm) with a JASCO PU-880 instrument equipped with a JASCO UV-870 detector. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (silica gel 60 F_{254}, 0.25 mm, Merck 5715) and preparative TLC was performed on silica gel 60 (0.5 mm, Merck 5744). Column chromatography was performed on Merck 7734 (70–230 mesh) or Fuji Devison BW-300 (230–400 mesh).

Chemicals. Unless otherwise noted, reagents were used in commercial grade. Dichloromethane and triethylamine were freshly distilled over CaH_2 prior to use. Porcine liver esterase (suspension in 3.2 M ammonium sulfate solution, pH 8.0) was purchased from Sigma.

Δ^7-PGA_1 methyl ester (**2**) was synthesized by the three-component process according to the reported procedure.[3,4] Hydrolysis of **2** to the acid form **3** was conducted in a mixture of a phosphate buffer and a minimum amount of acetone with porcine liver esterase. All chemical reactions were performed under a positive pressure of argon in the dark.

HPLC Analysis of the Metabolite of Δ^7-PGA_1 (3) in the Rat Serum. An ethanolic solution of **3** (1 mg/mL, 40 μL) was incubated with the rat serum (760 μL) at 37 °C and the time-course (40 μL sampling) of the substrate disappearance was monitored by HPLC analysis of the supernatant after deproteination with methanol (200 μL) followed by centrifugation. HPLC analyses were performed on a reversed-phase column eluting with a mixture of methanol and an acetic acid buffer (0.02 M, pH 5.0).

Synthesis of Authentic Δ^7-PGC_1 (7) by Base-Catalyzed Isomerization of 3. To a solution of **3** (3.0 mg, 9.0 μmol) in dichloromethane (1 mL) was added triethylamine (0.5 mL) at ambient temperature. The solution was stirred for 15 h and the solvent was removed under reduced pressure. Then the residue was subjected to preparative TLC using a mixture of dichloromethane and methanol (1:10) as eluant to give **7**: TLC $R_f = 0.47$ (10:1 dichloromethane/methanol); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.9 Hz, 3H, CH_3), 1.2–1.7 (m, 14H, CH_2), 2.33 (t, J = 7.4 Hz, 2H, C(2)H_2), 2.44 (m, 2H, C(6)H_2), 2.93 (br s, 2H, C(10)H_2), 4.24 (q, J = 6.4 Hz, 1H, C(15)H), 5.98 (dd, J = 6.4, 15.8 Hz, C(14)H), 6.22 (s, 1H, C(11)H), 6.35 (dt, J = 1.5, 7.9 Hz, C(7)H), 6.51 (d, J = 15.8 Hz, 1H, C(13)H); MS (ESMS) m/z 333 $(M - H)^-$. HPLC behavior and MS spectrum of **7** were totally identical with those of the primary metabolite of **3** in the rat serum.

Esterification of 7 Leading to 8. A few drops of trimethylsilyldiazomethane (10% in hexane) was added to a solution of **7** (ca 0.1 mg) in a mixture of ethanol (0.1 mL) and methanol (0.1 mL) at 0 °C. The solution was stirred for 2 min at the same temperature, then the mixture was directly subjected to TLC and HPLC analyses (see below).

Synthesis of Δ^7-PGC_1 Methyl Ester (8). To a solution of **2** (9.0 mg, 26 μmol) in dichloromethane (2 mL) was added triethylamine (0.2 mL) at ambient temperature. The mixture was stirred for 12 h, and the solvent was removed under reduced pressure. The residue was subjected to preparative TLC on silica gel (hexane–ether, 1:3) to afford Δ^7-PGC_1 methyl ester (6.9 mg, 77%): TLC $R_f = 0.51$ (1:3 hexane/ether); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 6.9 Hz, 3H, CH_3), 1.2–1.7 (m, 14H, CH_2), 1.92 (br, 1H, OH), 2.30 (t, J = 7.4 Hz, 2H, C(2)H_2), 2.44 (q, J = 7.9 Hz, 2H, C(6)H_2), 2.93 (br s, 2H, C(10)H_2), 3.66 (s, 3H, OCH_3), 4.23 (q, J = 6.4 Hz, 1H, C(15)H), 5.99 (dd, J = 6.4, 15.8 Hz, 1H, C(14)H), 6.23 (br s, 1H, C(11)H), 6.34 (dt, J = 1.5, 7.9 Hz, 1H, C(7)H), 6.51 (d, J = 15.8 Hz, 1H, C(13)H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.5, 25.1, 28.0, 28.6, 28.9, 31.7, 33.8, 37.3, 41.0, 51.6, 72.4, 124.9, 127.3, 134.2, 135.5, 136.9, 143.0, 174.2, 204.5; MS (EI) m/z 349 (M^+). TLC and HPLC behaviors of Δ^7-PGC_1 methyl ester (**8**) were completely identical with those of the esterified product **8** derived from **3** via **7**.

ACKNOWLEDGMENT

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7. The acid **7** was fairly labile and decomposed by concentration.
8. In contrast, PGC_2 is facilely converted to PGB_2 by a trace of base in methanol at 25 °C.[6]

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