*Proc. Natl. Acad. Sci. USA*
Vol. 85, pp. 304–308, January 1988
Biochemistry

# Isomeric prostaglandin $F_2$ compounds arising from prostaglandin $D_2$: A family of icosanoids produced *in vivo* in humans

(9α,11β-prostaglandin $F_2$/isomeric prostaglandin $D_2$/mast cells/mastocytosis)

DANIEL F. WENDELBORN, KAREN SEIBERT, AND L. JACKSON ROBERTS II*

Departments of Pharmacology and Medicine, Vanderbilt University, Nashville, TN 37232

*Communicated by Osamu Hayaishi, June 22, 1987 (received for review March 13, 1987)*

ABSTRACT **Prostaglandin (PG) $D_2$ has been shown to be transformed by human 11-ketoreductase to 9α,11β-$PGF_2$, a biologically active metabolite that is produced *in vivo*. During the course of developing a mass spectrometric assay for 9α,11β-$PGF_2$, several compounds with characteristics similar to $PGF_2$ were detected in both plasma and urine of normal humans by selected ion monitoring. Analysis of pooled plasma obtained from patients with mastocytosis during severe episodes of systemic mast cell activation associated with the release of markedly increased quantities of $PGD_2$ was revealing in that all of these compounds were present in approximately 800-fold greater abundance compared to levels found in normal plasma, suggesting that these compounds arose from $PGD_2$ metabolism. Complete electron impact mass spectra were obtained of these compounds in both plasma and urine; these spectra established that they were all isometric forms of $PGF_2$. Approximately 16 isomeric $PGF_2$ compounds were identified. Treatment with butylboronic acid indicated that the C-9 and C-11 hydroxyls were *trans* in approximately one-third of the compounds and *cis* in approximately two-thirds. Preliminary experiments suggest that $PGD_2$ is a very labile compound *in vivo* and undergoes extensive isomerization, after which reduction by 11-ketoreductase yields a family of more stable isomeric $PGF_2$ compounds. Elucidating the profile of biological activity of these compounds and their mechanism of formation will contribute importantly to our understanding of the biological consequences of $PGD_2$ release *in vivo*. These results also bring into question the reliability of assays for $PGF_{2\alpha}$ and its metabolites in human biological fluids as a specific index of endogenous $PGF_{2\alpha}$ biosynthesis, as these assays may also measure in part isomeric $PGF_2$ compounds arising from $PGD_2$ metabolism.**

Prostaglandin (PG) $D_2$ is the principal cyclooxygenase product produced by mast cells (1, 2). $PGD_2$ is released in markedly increased quantities in patients during episodes of systemic mast cell activation and is a major mediator of the humoral manifestations of such episodes (3, 4). $PGD_2$ has also been considered as a potential mediator in the human pulmonary allergic response (5) and recently has been shown to be released into the lower respiratory tract during acute antigen challenge in patients with allergic asthma (6).

$PGD_2$ is metabolized *in vivo* predominantly via a 11-ketoreductase pathway to PGF-ring metabolites (7–9). *In vitro* studies demonstrated that human liver 11-ketoreductase stereospecifically transforms $PGD_2$ to 9α,11β-$PGF_2$ (10), a finding that subsequently has been generalized to 11-ketoreductase metabolism of $PGD_2$ in other human and animal tissues and organs (11–14). Importantly, 9α,11β-$PGF_2$ has been shown to be produced *in vivo* and to be a biologically active metabolite that is a pressor substance in the rat (10), inhibits platelet aggregation (11, 15), and causes both human coronary and human bronchial smooth muscle to contract (13, 14).

Because of the potential importance of 9α,11β-$PGF_2$ as a biological mediator in human disease, the development of a mass spectrometric assay for 9α,11β-$PGF_2$ was undertaken. This led to the discovery, described in this report, that multiple isomeric forms of $PGF_2$ arising from $PGD_2$ metabolism are present in both plasma and urine of humans.

## EXPERIMENTAL PROCEDURES

**GC/MS Analysis.** Negative-ion chemical ionization (NICI) selected ion monitoring (SIM) analysis and electron impact (EI) mass spectrometry were performed with a Nermag R10-10C GC/MS instrument (14) with a 15-m OV-17 fused silica capillary column (J&W Scientific, Rancho Cordova, CA). NICI-SIM analysis of the pentafluorobenzyl ($F_5Bzl$) ester and trimethylsilyl ($Me_3Si$) ether derivative of $PGF_2$ compounds was accomplished by monitoring the M − 181 ions (M − $CH_2C_6F_5$) $m/z$ = 569 for $PGF_2$, $m/z$ = 573 for [$^2H_4$]$PGF_{2\alpha}$, and $m/z$ = 576 for 9α,11β-[$^2H_7$]$PGF_2$. The M − 181 ions $m/z$ = 491 and 495 were monitored for the $F_5Bzl$ butylboronate $Me_3Si$ derivative of $PGF_2$ and [$^2H_4$]$PGF_{2\alpha}$, respectively.

**Isolation and Purification of 9α,11β-$PGF_2$ and Isomeric $PGF_2$ Compounds from Plasma and Urine.** Initially, 275 pg of 9α,11β-[$^2H_7$]$PGF_2$ (10) was added to samples prepared for NICI-SIM analysis (3 ml of plasma or urine). Urine and plasma were then acidified to pH 3 with 1 M HCl and applied to preconditioned $C_{18}$ Sep-Pak columns (Waters Associates). Sep-Paks were then washed sequentially with 10 ml of water, 10 ml of acetonitrile/water (15:85, vol/vol) (urine only), and 10 ml of heptane, and then eluted with 10 ml of ethyl acetate/heptane (50:50, vol/vol). The ethyl acetate/heptane eluate was dried over anhydrous $Na_2SO_4$ and then applied to a silica Sep-Pak. The Sep-Pak was then washed with 5 ml of ethyl acetate and subsequently eluted with 5 ml of ethyl acetate/methanol (50:50, vol/vol). The ethyl acetate/methanol eluate was evaporated under a stream of $N_2$ and the residue was subjected to TLC using the solvent chloroform/methanol/acetic acid/water (86:14:1:0.8, vol/vol). Compounds migrating in the region of $PGF_{2\alpha}$ ($R_f$ = 0.33) were scraped and extracted from the silica gel with methanol. This TLC step was performed with urine samples but omitted with plasma, which was subjected to TLC only after esterification. Plasma and urine samples were then converted to $F_5Bzl$ esters and subjected to TLC using the solvent chloroform/ethanol (93:7, vol/vol). The solvent chloroform/ethanol (92:8, vol/vol) was used for TLC of methyl (Me) esters. Compounds migrating in the region of the $F_5Bzl$ or Me ester of $PGF_{2\alpha}$ ($R_f$ = 0.15) and the adjacent area 1.5 cm nearer to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PG, prostaglandin; $Me_3Si$, trimethylsilyl; $F_5Bzl$, pentafluorobenzyl; NICI, negative ion chemical ionization; SIM, selected ion monitoring; EI, electron impact.

*To whom reprint requests should be addressed.

the origin were scraped and extracted from the silica gel with methanol. Compounds were then converted to $Me_3Si$ ether derivatives and subsequently analyzed by GC/MS.

## RESULTS

**SIM Analysis of Normal Urine.** After addition of 9α,11β-[$^2H_7$]$PGF_2$, urine obtained from a normal individual was extracted, purified by TLC, and subsequently analyzed by NICI-SIM GC/MS as the $F_5Bzl$ $Me_3Si$ derivative. The selected ion current chromatograms obtained for $m/z$ = 569 (endogenous $PGF_2$) and $m/z$ = 576 (9α,11β-[$^2H_7$]$PGF_2$) are shown in Fig. 1. A single $m/z$ = 576 peak representing the internal standard was present. In contrast, there were several $m/z$ = 569 peaks, some of which were incompletely resolved from each other near the retention time of 9α,11β-$PGF_2$. Levels of these individual compounds ranged from approximately 48 to 460 pg/ml. Similar results were obtained in urine from additional normal volunteers. Normal and reverse-phase HPLC purification prior to analysis removed some but not all of these compounds (data not shown). Interestingly, analysis of different silyl ether derivatives (ethyldimethylsilyl and *n*-propyldimethylsilyl yielded an identical pattern of endogenous peaks. This strongly suggested that all of these compounds had three hydroxyl groups.

**SIM Analysis of Normal Plasma and Plasma Obtained from a Patient with Systemic Mastocytosis.** Selected ion current chromatograms obtained from NICI-GC/MS analysis of plasma obtained from a normal individual are shown in Fig. 2. Again, similar to results obtained from analysis of urine, a single $m/z$ = 576 peak representing the internal standard was present and several partially resolved $m/z$ = 569 peaks representing endogenous compounds were present with retention times similar to the retention time of 9α,11β-$PGF_2$. Levels of these compounds ranged from approximately 1 to 12 pg/ml. Similar results were also obtained in plasma from additional normal volunteers.

Collectively, these data for plasma and urine suggested that these endogenous compounds were similar to $PGF_2$ and may not be irrelevant impurities and raised the question of whether they arise from $PGD_2$ metabolism. To assess this possibility, we then analyzed pooled plasma that had been obtained from patients with systemic mastocytosis during episodes of systemic mast cell activation, which is associated with release of markedly increased quantities of $PGD_2$. The selected ion current chromatograms obtained are shown in Fig. 3. As before, a single $m/z$ = 576 peak representing the internal standard was present. Again, a cluster of $m/z$ = 569 peaks were present in a pattern strikingly similar to that seen in normal plasma. What was revealing, however, was that from comparison of the intensity of the $m/z$ = 569 peaks to that of the internal standard, these compounds in the plasma from the patients with mastocytosis were present in approximately 800-fold greater abundance (1–9 ng/ml) than the levels found in normal plasma. This provided evidence that the formation of these compounds was linked to the metabolism of $PGD_2$.

**Analysis of Plasma and Urine from Patients with Systemic Mastocytosis by EI Mass Spectrometry.** The NICI mass spectrum of the $F_5Bzl$ ester $Me_3Si$ ether derivative of $PGF_2$ is characterized by a single intense M − 181 ion at $m/z$ = 569 formed by the loss of $\cdot CH_2C_6F_5$. Thus, the NICI mass

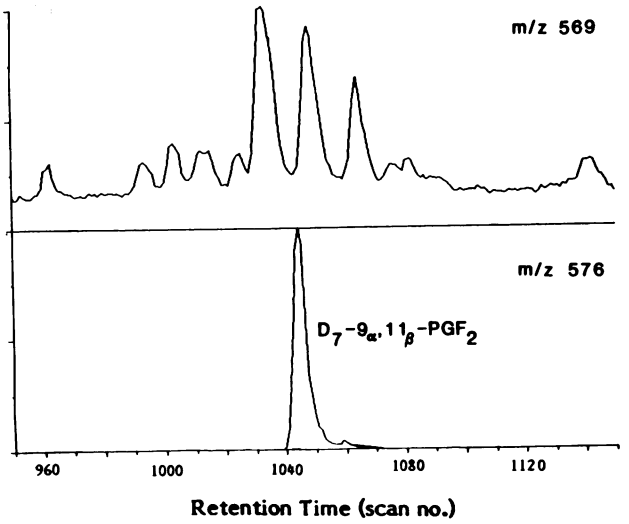


FIG. 2. Selected ion current chromatograms obtained of $m/z$ = 569 (endogenous $PGF_2$) and $m/z$ = 576 (9α,11β-[$^2H_7$]$PGF_2$) from analysis of normal human plasma. Levels of the endogenous compounds range from approximately 1 to 12 pg/ml.

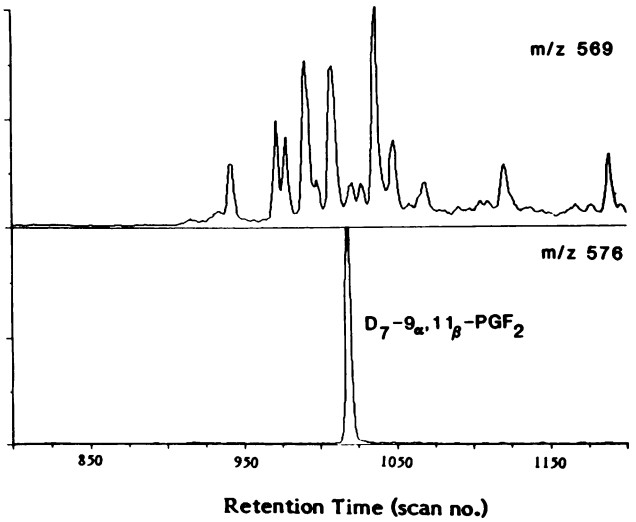


FIG. 1. Selected ion current chromatograms obtained of $m/z$ = 569 (endogenous $PGF_2$) and $m/z$ = 576 (9α,11β-[$^2H_7$]$PGF_2$) from analysis of normal human urine. Levels of the endogenous compounds range from approximately 48 to 460 pg/ml. In Figs. 1–3, D = $^2H$.

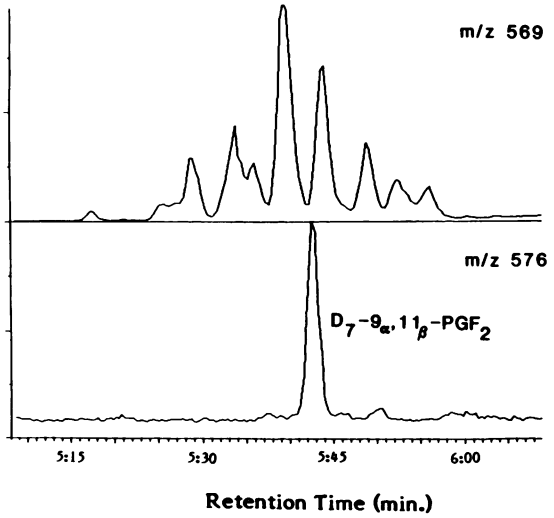


FIG. 3. Selected ion current chromatograms obtained of $m/z$ = 569 (endogenous $PGF_2$) and $m/z$ = 576 (9α,11β-[$^2H_7$]$PGF_2$) from analysis of pooled plasma obtained from patients with mastocytosis during severe episodes of systemic mast cell activation. Levels of the endogenous compounds range from approximately 1 to 9 ng/ml, approximately 800-fold higher than levels in normal plasma (Fig. 2).

spectrum does not provide a great deal of structural information about the molecule, in contrast to EI mass spectra, in which multiple ions are formed as a result of fragmentation at numerous sites on the molecule. Therefore, to obtain more information regarding the structures of the endogenous compounds in plasma and urine detected by NICI SIM, EI mass spectra of these compounds in plasma and urine from the patients with mastocytosis were obtained.

After extraction of plasma (50 ml) and urine (500 ml) by using $C_{18}$ Sep-Paks followed by purification by TLC, the compounds were analyzed by EI mass spectrometry as a Me ester $Me_3Si$ ether derivative. A mass spectrum characteristic for $PGF_2$ was obtained for each peak, indicating that these were all isomeric forms of $PGF_2$. Most gave essentially identical mass spectra except for some minor variation in relative ion abundance. A mass spectrum obtained for one of these compounds in plasma is shown in Fig. 4*A*. However, two mass spectra obtained in the urine exhibited unique characteristics. One of these mass spectra is shown in Fig. 4*B*. One of the unique characteristics is that the base ion is $m/z$ = 173 [$Me_3SiO^+$=$CH(CH_2)_4CH_3$] instead of $m/z$ 191. The other unusual aspect of this mass spectrum is that there is a prominent ion at $m/z$ = 484 (M − 100) formed by rearrangement and the loss of O=$CH(CH_2)_4CH_3$. These findings suggest that these compounds may be isomeric forms of $\Delta^{12}$-$PGF_2$, as these same unique features are also charac-

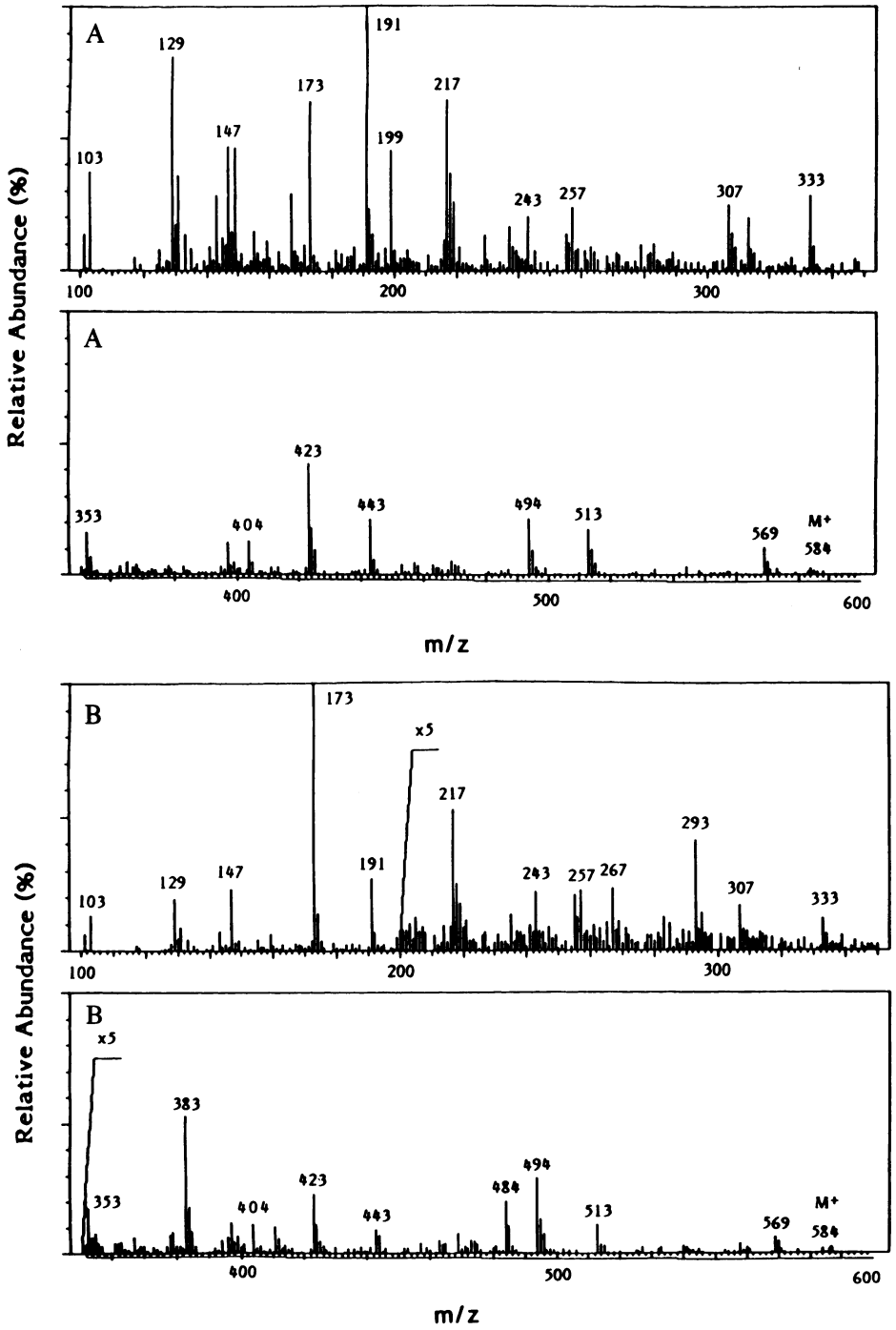


FIG. 4. EI mass spectra of $PGF_2$ isomers. (*A*) Isomer in plasma obtained from patients with mastocytosis during severe episodes of systemic mast cell activation. (*B*) Isomer in urine obtained from a patient with mastocytosis. Compounds were analyzed as Me ester $Me_3Si$ ether derivatives.

teristic of dehydration products of $PGD_2$ in which the $\Delta^{13}$ double bond has shifted to the $\Delta^{12}$ position (16, 17).

**SIM Analysis of Isomeric $PGF_2$ Compounds after Treatment with Butylboronic Acid.** Our previous *in vitro* studies indicated that human 11-ketoreductase stereospecifically reduces $PGD_2$ to $9\alpha,11\beta$-$PGF_2$ (10, 13, 14). We therefore examined whether these isomeric $PGF_2$ compounds in plasma were isomeric forms of $9\alpha,11\beta$-$PGF_2$. This was assessed by determining whether these compounds would form a butylboronate derivative when treated with *n*-butylboronic acid. $PGF_2$ compounds will form a boronate derivative only if the C-9 and C-11 hydroxyls are *cis* in relation to the cyclopentane ring (18).

$PGF_2$ isomers were purified from plasma and urine of the mastocytosis patients by TLC as the $F_5$Bzl ester as previously described. One half of each sample was then converted to a $Me_3Si$ ether derivative and not treated with butylboronic acid and the other half was first treated with butylboronic acid and then converted to a $Me_3Si$ ether derivative.

The samples were then analyzed by NICI SIM. The NICI mass spectrum of the $F_5$Bzl butylboronate $Me_3Si$ derivative of $PGF_2$ is characterized by essentially a single intense M − 181 ion ($m/z$ = 491), loss of $\cdot CH_2C_6F_5$. The ion $m/z$ = 569 was monitored for $PGF_2$ compounds as the $F_5$Bzl $Me_3Si$ derivative and the ion $m/z$ = 491 was monitored for $PGF_2$ compounds as the $F_5$Bzl butylboronate $Me_3Si$ derivative. Fig. 5 shows the results obtained in plasma. Fig. 5*A* depicts

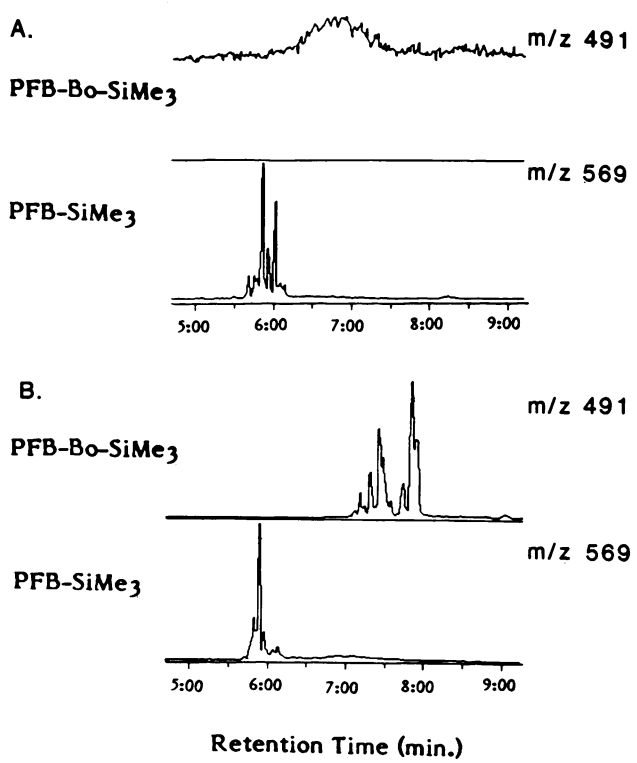


FIG. 5. Selected ion current chromatograms obtained for $m/z$ = 491 and $m/z$ = 569 from analysis of plasma obtained from patients with mastocytosis. PFB, pentafluorobenzyl ester; and Bo, butylboronate. Peaks in the $m/z$ = 491 ion current chromatograms are indicative of $PGF_2$ compounds as $F_5$Bzl butylboronate $Me_3Si$ derivatives and peaks in the $m/z$ = 569 ion current chromatograms are indicative of $PGF_2$ compounds as $F_5$Bzl $Me_3Si$ derivatives. (*A*) Selected ion current chromatograms of $m/z$ = 491 and 569 from analysis of plasma that had not been treated with butylboronic acid. The $m/z$ = 491 chromatogram is plotted at a sensitivity that is approximately 18-fold greater than the $m/z$ = 569 chromatogram. (*B*) Selected ion current chromatograms of $m/z$ = 491 and 569 from analysis of the same plasma as in *A*, but this plasma was treated with butylboronic acid. The $m/z$ = 491 and 569 chromatograms are plotted with approximately equivalent sensitivities.

the selected ion current chromatograms obtained from analysis of the half of the sample that was not treated with butylboronic acid. Again are seen a cluster of $m/z$ = 569 $PGF_2$ isomer peaks. The range of the GC retention time shown in this figure is longer than shown in previous figures, so the cluster of $m/z$ = 569 peaks appears compressed. The GC retention time range is expanded in this figure because the $F_5$Bzl butylboronate $Me_3Si$ derivative of $PGF_2$ has a longer retention time than the $F_5$Bzl $Me_3Si$ derivative. Of importance to note in the plasma sample that was not treated with butylboronic acid (Fig. 5*A*) is that there are no $m/z$ = 491 peaks. It is also important to mention that the $m/z$ = 491 chromatogram in Fig. 5*A* is plotted with a relative sensitivity approximately 18-fold greater than that of the $m/z$ = 569 chromatograms. Fig. 5*B* shows the selected ion current chromatograms obtained from analysis of the half of the plasma sample that was treated with butylboronic acid. Many of the $m/z$ = 569 peaks have now either partially or totally disappeared (cf. Fig. 5*A*) and intense $m/z$ = 491 peaks have now appeared. These chromatograms are plotted with approximately equivalent sensitivities. There appeared to be approximately 10 compounds that formed a butylboronate derivative and 6 that did not. It is difficult to determine the precise number of compounds because of incomplete resolution of GC peaks. $[^2H_4]PGF_{2\alpha}$ was coderivatized with this sample and was quantitatively converted to a butylboronate derivative, which was assessed by monitoring the ions $m/z$ = 573 and $m/z$ = 495. The butylboronate derivative of $[^2H_4]$-$PGF_{2\alpha}$ was eluted essentially in the middle of the cluster of $m/z$ = 491 plasma peaks. These data suggest that the compounds that did not form a butylboronate derivative (represented by the $m/z$ = 569 peaks) cannot be attributed to incomplete reaction of these compounds with butylboronic acid.

The above results indicated that in approximately two-thirds of the isomeric $PGF_2$ compounds in plasma the hydroxyls at C-9 and C-11 are *cis* and in approximately one-third they are *trans*. Similar results were also obtained with urine.

## DISCUSSION

This report describes the discovery of multiple isomeric forms of $PGF_2$ in both plasma and urine of humans. Evidence is also presented that this family of isomeric $PGF_2$ compounds are derived from endogenously produced $PGD_2$ in that their levels increase markedly after systemic mast cell activation with attendant release of large quantities of $PGD_2$.

$PGD_2$, by virtue of the fact that it is a $\beta$-hydroxy-$\beta,\gamma$-unsaturated ketone, is inherently unstable under certain conditions. Because of these structural characteristics, protons at C-12 and C-10 are acidic and easily extractable under relatively mild alkaline conditions. For example, albumin has been shown to catalyze dehydration of $PGD_2$ and a shift in the $\Delta^{13}$ double bond to the $\Delta^{12}$ position, an effect that has been attributed to the alkaline microenvironment of albumin binding sites (16, 17). Thus, it is reasonable to propose that the mechanism of formation of these $PGF_2$ compounds initially involves isomerization of $PGD_2$ followed by reduction of the C-11 keto group of $PGD_2$ isomers by 11-ketoreductase to more stable $PGF_2$ compounds.

Preliminary evidence has been obtained to support this proposed mechanism of formation of isomeric $PGF_2$ metabolites of $PGD_2$. We have found that plasma or serum albumin, in addition to catalyzing dehydration of $PGD_2$, also catalyzes isomerization. Addition of 11-ketoreductase after incubation of $PGD_2$ with plasma or albumin results in the formation of isomeric $PGF_2$ compounds. As presented in *Results*, there are approximately 16 isomeric $PGF_2$ compounds in plasma and urine and in approximately two-thirds the C-9 and C-11

hydroxyls are *cis* and in one-third they are *trans*. Under the conditions employed, however, plasma and albumin catalyze the formation of only approximately 6 isomers and in none are the C-9 and C-11 hydroxyls *cis*. Thus, factors in addition to albumin may be involved in the isomerization of $PGD_2$ *in vivo*.

The multiplicity of isomeric $PGF_2$ compounds arising from $PGD_2$ indicates extensive isomerization involving multiple chiral centers and double bond location and geometry. Several possible mechanisms can be envisioned to account for such isomerization of $PGD_2$ at multiple chiral centers, including (*i*) dehydration/rehydration at C-9 and C-15, (*ii*) oxidation/reduction reaction at C-9 and C-15, (*iii*) migration of the $\Delta^{13}$ double bond to the $\Delta^{12}$ position and possibly also subsequent migration to the $\Delta^{8,12}$ position leading to enolization at C-9, and (*iv*) retro-aldol condensation.

It was of interest to find that in over one-half of the isomeric $PGF_2$ compounds in plasma and urine, the C-9 and C-11 hydroxyls are *cis* in relation to the cyclopentane ring. Because of the previous demonstration that 11-ketoreductase stereospecifically reduces $PGD_2$ to 9$\alpha$,11$\beta$-$PGF_2$ (10–14) this suggests that epimerization at C-9 in some compounds has occurred, leading to the formation of 9$\beta$,11$\beta$-$PGF_2$ compounds. However, it is not possible to exclude the possibility that the C-11 keto group of some isomeric forms of $PGD_2$ may be reduced to a C-11 hydroxyl oriented $\alpha$ if steric influences lead to differences in the orientation of the molecular binding to the active site of the enzyme of isomeric forms of $PGD_2$ compared to that of $PGD_2$.

Bundy and colleagues have chemically synthesized a number of isomeric forms of $PGD_2$, all of which exhibited some biological activity (19). As previously discussed, 9$\alpha$, 11$\beta$-$PGF_2$ also exhibits its own spectrum of biological action. Therefore, it seems likely that at least some of these isomeric $PGF_2$ compounds and/or their corresponding isomers of $PGD_2$ possess biological activity. In support of this possibility, preliminary studies indicate that 12-epi-9$\alpha$,11$\beta$-$PGF_2$, a major isomer formed after incubation of $PGD_2$ with serum albumin followed by reduction with 11-ketoreductase, contracts gastrointestinal smooth muscle strips *in vitro*. Elucidating the specific structures of these isomeric $PGF_2$ compounds, the mechanism(s) involved in their formation, and the profile of their biological actions and those of their corresponding $PGD_2$ isomer will contribute importantly to our understanding of the biological consequences of $PGD_2$ release *in vivo*.

These results also bring into question the reliability of assays for $PGF_{2\alpha}$ in human biological fluids. Since many of the $PGF_2$ compounds arising from $PGD_2$ metabolism have similar or identical chromatographic characteristics on HPLC and GC, physical methods of analysis of $PGF_{2\alpha}$ such as GC/MS may also measure at least in part some of these isomeric $PGF_2$ compounds arising from $PGD_2$ metabolism. Antibodies employed for RIA of $PGF_{2\alpha}$ may also have significant cross-reactivity with some of the $PGF_2$ isomers. In support of this possibility is the fact that an anti-$PGF_{2\alpha}$ antibody has been found to have almost complete cross-reactivity with 9$\alpha$,11$\beta$-$PGF_2$ (O. Hayaishi, personal communication). Further metabolism of the isomeric $PGF_2$ compounds arising from $PGD_2$ would yield isomeric $PGF_2$ metabolites, consistent with the previous finding of more than one isomeric form of several urinary PGF-ring metabolites of $PGD_2$ after both intravenous infusion and endogenous release of $PGD_2$ (7, 8). Thus, these same analytical considerations also apply to measurement of $PGF_{2\alpha}$ metabolites.

In summary, this report describes the discovery of multiple isomeric forms of $PGF_2$ arising from metabolism of $PGD_2$ in human plasma and urine. Future studies aimed at elucidating their precise structures, mechanism(s) of formation, biological actions, and potential role in human physiology and pathophysiology will be of importance.

This work was supported by grant GM 15431 from the National Institutes of Health. K.S. was supported by Training Grant GM 07628 from the National Institutes of Health. L.J.R. is a Burroughs–Wellcome Scholar in Clinical Pharmacology.

1. Roberts, L. J., II, Lewis, R. A., Oates, J. A. & Austen, K. F. (1979) *Biochim. Biophys. Acta* **575**, 189–192.
2. Lewis, R. A., Soter, N. A., Diamond, P. T., Austen, K. F., Oates, J. A. & Roberts, L. J., II (1982) *J. Immunol.* **129**, 1627–1631.
3. Roberts, L. J., II, Sweetman, B. J., Lewis, R. A., Austen, K. F. & Oates, J. A. (1980) *N. Engl. J. Med.* **303**, 1400–1404.
4. Roberts, L. J., II, Fields, J. P. & Oates, J. A. (1982) *Trans. Assoc. Am. Physicians* **95**, 36–41.
5. Hardy, C. C., Robinson, C., Tattersfield, A. E. & Holgates, S. T. (1984) *N. Engl. J. Med.* **311**, 209–213.
6. Murray, J. J., Tonnel, A., Brash, A. R., Roberts, L. J., II, Gosset, P., Workman, R., Captron, A. & Oates, J. A. (1986) *N. Engl. J. Med.* **315**, 800–804.
7. Roberts, L. J., II, & Sweetman, B. J. (1985) *Prostaglandins* **30**, 383–401.
8. Liston, T. E. & Roberts, L. J., II (1985) *J. Biol. Chem.* **260**, 13172–13180.
9. Ellis, C. K., Smigel, M. D., Oates, J. A., Oelz, O. & Sweetman, B. J. (1979) *J. Biol. Chem.* **254**, 4152–4163.
10. Liston, T. E. & Roberts, L. J., II (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6030–6034.
11. Pugliese, G., Spokas, E. G., Marcinkiewicz, E. & Wong, P.Y.-K. (1985) *J. Biol. Chem.* **260**, 14621–14625.
12. Watanabe, K., Iguchi, Y., Iguchi, S., Arai, Y., Hayaishi, O. & Roberts, L. J., II (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1583–1587.
13. Roberts, L. J., II, Seibert, K., Liston, T. E., Tantengco, M. V. & Robertson, R. M. (1987) *Adv. Prostaglandin Thromboxane Leukotriene Res.* **17**, 427–429.
14. Seibert, K., Sheller, J. R. & Roberts, L. J., II (1987) *Proc. Natl. Acad. Sci. USA* **84**, 256–260.
15. Roberts, L. J., II, & Liston, T. E. (1985) *Clin. Res.* **33**, 162 (abstr.).
16. Fitzpatrick, F. A. & Wynalda, M. A. (1983) *J. Biol. Chem.* **258**, 11713–11718.
17. Kikawa, Y., Narumiya, S., Fukushima, M., Wakatsuka, H. & Hayaishi, O. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1317–1321.
18. Pace-Asciak, C. & Wolfe, L. S. (1971) *J. Chromatogr.* **56**, 129–135.
19. Bundy, G. L., Morton, D. R., Peterson, D. C., Nishizawa, E. E. & Miller, W. L. (1983) *J. Med. Chem.* **26**, 790–799.