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Metabolism of Prostacyclin in Rat†

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ABSTRACT: Following a single intravenous administration of [11-^{3}H]prostacyclin in rat, 77% of the administered dose was excreted within 3 days with 33% in urine and 44% in feces. Urinary metabolites were accumulated by chronic intravenous infusions of [11-^{3}H]prostacyclin for 14 days. The drug was extensively metabolized and the structures of seven metabolites were elucidated by combined gas chromatography and mass spectrometry. The urinary products include the dinor and 19-hydroxy dinor derivatives of 6-keto-$PGF_{1\alpha}$ and 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$, ω-hydroxy and ω-carboxyl dinor derivatives of dihydro-6,15-diketo-$PGF_{1\alpha}$, and a dihydrodiketotetranordicarboxylic acid. The metabolic pathways of PGI_2 in rat are similar to that of $PGF_{2\alpha}$.

Prostacyclin (PGI_2) is a labile molecule generated enzymatically from prostaglandin endoperoxide in mammalian blood vessel walls (Gryglewski et al., 1976; Moncada et al., 1976). The synthetase enzyme for PGI_2 was found to occur in many tissues and organs (Sun et al., 1977). The compound is a powerful vasodilator in many vesicular beds. It has been suggested (Moncada & Vane, 1977) that PGI_2 plays a crucial role in the hemostasis of the cardiovascular systems.

In neutral or acidic aqueous medium, PGI_2 is rapidly hydrolyzed to 6-keto-$PGF_{1\alpha}$. The half-life of PGI_2 in physiological pH was estimated to be 10.5 min at 25 °C (Cho & Allen, 1978), and, therefore, it is intuitive that PGI_2 must be hydrolyzed to 6-keto-$PGF_{1\alpha}$ after its release. This is true under most in vitro conditions where 6-keto-$PGF_{1\alpha}$ represents the only stable terminal product from PGI_2. However, under in vivo conditions where there are other metabolic processes involved, whether 6-keto-$PGF_{1\alpha}$ represents the initial metabolic products of PGI_2 remained to be proven.

We have previously shown that PGI_2 can be rapidly oxidized in vitro by 15-hydroxyprostaglandin dehydrogenase of the lung (McGuire & Sun, 1978) and blood vessel (Sun et al., 1978) to the corresponding 15-keto product. Under the same condition 6-keto-$PGF_{1\alpha}$, however, showed little reactivity. Pace-Asciak et al. (1977) recently identified two principal urinary excretion products from 6-keto-$PGF_{1\alpha}$ treated rats. Both still possess the allylic alcohol group at C-15 which indicates they have not been oxidized by PGDH. No information is yet available for the metabolism of prostacyclin itself in whole animals.

Therefore, the present study was designed to isolate and identify major urinary metabolites of PGI_2 in the rat. These results should give an indication as to which compounds should be measured in order to monitor endogenous prostacyclin production.

Materials and Methods

[11-^{3}H]$PGF_{2\alpha}$ and [11-^{3}H]$PGF_{2\alpha}$ methyl ester were kindly synthesized by Dr. D. R. Morton and J. P. McGrath. The specific activity was 79 Ci/mol. PGI_2, 6-keto-$PGF_{1\alpha}$, and 5-iodo-PGI_1 methyl ester were provided by members of the Experimental Chemistry unit of The Upjohn Co.

Rats prepared with chronic indwelling venous cannulas (Weeks, 1972) and the special cold solution reservoir device (Weeks, 1978) for long-term IV infusion of PGI_2 were kindly provided by Dr. J. R. Weeks of the Upjohn Co.

[11-^{3}H]PGI_2 Na salt was prepared by a modification of the procedure described by Johnson et al. (1977). One milligram of [11-^{3}H]$PGF_{2\alpha}$ methyl ester was mixed with 0.5 mL of an aqueous solution containing 0.5 mg of Na_2CO_3 and 1.5 mg of KI. Three milligrams of solid iodine was added and the mixture was stirred overnight in an ice bath. The reaction was then quenched with approximately 5 mg of Na_2SO_3, diluted with 1 mL of saturated salt solution, and extracted three times with

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diethyl ether. The solvent was evaporated and the residue was chromatographed on a 5-g silica gel column (Mallinckrodt silicAR CC-7 200–325 mesh), packed in hexane–acetone (90:10). The desired iodoether product was eluted with hexane acetone (60:40). The center cut of the radioactivity peak was taken and its purity was established by thin-layer chromatography with hexane–acetone (1:1) as the solvent system. Only samples with radioactivity purity greater than 95% were used for the next step.

The purified iodoether 0.3–0.5 mg was treated with a mixture of 0.1 mL of 1,5-diazobicyclo[4.3.0]non-5-ene (DBN) and 0.4 mL of toluene at 45 °C for 24 h. The reaction mixture was diluted with toluene, washed three times with brine, and dried over anhydrous Na_2SO_4. The residue was dissolved in dry acetone and kept at −20 °C in a desiccator. The overall yield was 30%.

One day before the drug was to be administered to the animals, an appropriate aliquot of the labeled PGI_2 methyl ester was hydrolyzed to the sodium salt by treatment with a 1:1 mixture of methanol and 0.05 M NaOH at room temperature overnight. The methanol was evaporated by a stream of nitrogen and the PGI_2 Na salt was diluted with unlabeled compound and dissolved in Tris buffer made isotonic with NaCl (Tris saline) (0.05 M Tris-HCl (pH 9.4) plus 7.42 g/L NaCl) prior to administration to the animals.

The identity of the labeled PGI_2 methyl ester or sodium salt was established by TLC of the methyl ester itself (solvent system hexane–acetone, 1:1) or after conversion to 6-keto-$PGF_{1\alpha}$ free acid or methyl ester by acidification [solvent system A1X organic phase of EtOAc:HOAc:2,2,4-trimethylpentane:water (11:2:5:10)]. Authentic compounds were used for comparison. The radiochemical purity was usually better than 90%. PGI_2 methyl ester when stored at −20 °C in dry acetone is stable for at least 3–6 months. The sodium salt in cold aqueous buffer (pH 9.4) is stable for 24–48 h, but freshly made solutions were always used.

Animal Experiments: Single Bolus IV Administration. Four female Sprague-Dawley rats weighing approximately 200 g were used. [11-^{3}H]PGI_2 (0.14 mg per rat; 4.4 μCi) dissolved in Tris–saline (pH 9.4) was injected into the tail vein. The animals were housed in stainless steel metabolism cages with free access to food and water. Urine and feces samples were collected at appropriate intervals for a total of 80 h.

Accumulation of Metabolites. Six rats were prepared with chronic indwelling venous cannulas (Weeks, 1972) and continuously infused with PGI_2 to accumulate urinary metabolites. The modified stainless steel rat metabolism cage and the cannula feed-through device have been previously described (Weeks, 1978). The initial dose was 0.56 mg per kg per day. At this dose, the rats appeared to be mildly depressed initially but within 2–3 days a tolerance to the drug developed and the rats returned to normal. Accordingly the dose was increased in 0.25 log increments, allowing 2–4 days at each level; finally the dose was increased to 3.2 mg per kg per day without apparent ill effect. Tritium-labeled PGI_2 (8.42 μCi) mixed with appropriate amounts of unlabeled carrier was administered to each rat on the first and last day to provide labeled metabolites for isolation. PGI_2 solution was freshly prepared daily and kept in an ice bath until reaching the animals. Urine specimens were collected daily, pooled, and kept frozen until use.

Chromatographic Methods. Extraction of urine was carried out on Amberlite XAD-2 (Rohm and Haas Co., Philadelphia, Pa.) according to a procedure previously described (Sun, 1974). The crude urine extract was partially purified on a silicic acid column (Mallinckrodt Chemical Works, SilicAR CC-4) in chloroform–acetone (1:1). The bulk of the radioactivity was eluted with the solvent front. The overall recovery of radioactivity from these two steps was approximately 95%.

The crude extract was initially separated by gel permeation chromatography. Sephadex LH-20 was swelled in the solvent mixture of chloroform–acetone 3:1 and packed into a 15 × 750 mm column. The crude extract dissolved in 5 mL of the same solvent was loaded on the top. The column was eluted with 100 mL of chloroform–acetone mixture of 3:1, followed by 200 mL each of $CHCl_3$–acetone 2:1 and 1:1. The radioactivity was resolved into three peaks. Each was collected, pooled, and concentrated.

Further separation was carried out via reversed phase column chromatography. C-18 phase bonded silica gel (C_{18} phase bonded HI-FLOSIL, 80/100 mesh, Applied Science Lab., State College, Pa.) was suspended in chloroform, degassed, and packed into a 10 × 800 mm column. The column was washed initially with 2 volumes of chloroform:acetonitrile (5:95) and then with several volumes each of a stepwise gradient of increasing amounts (in the order of 20, 40, 60, 80, 90%) of water in acetonitrile. All solvent mixture contained 1% of chloroform. The sample was dissolved in 1 mL of acetonitrile and loaded on top of the column. The column was eluted with 3–4 volumes each of $CHCl_3$–acetonitrile–water, 1:10:90, followed by 1:30:70 and 1:50:50. Essentially all PGI_2 metabolites were eluted within this range. The flow rate was carefully adjusted to 10–20 mL/h and 3-mL fractions were collected. The radioactive material under each peak was combined and evaporated to dryness by lyophilization. The same procedure was applied to both free acid and methyl ester.

Silica gel column chromatography used to purify methyl esters of the metabolites was carried out with 5–10 g of SilicAR CC-7 (Mallinckrodt Chemical Works 200–325 mesh) packed in chloroform–acetone (9:1). The compounds were eluted by increasing concentrations of acetone in chloroform under mild pressure (15–20 psi).

Gas chromatography was performed on a Varian Model 2700 gas chromatograph equipped with a flame ionization detector and a Packard Model 894 gas proportional counter. The mass and radioactivity signals were recorded simultaneously on a strip chart recorder. The column was a 6-ft, 1% SE-30 ultraphase (Pierce Chemical Co.) on Chromosorb W (HP) 80–100 mesh, glass coil operated at 215 °C. The carrier gas flow was 40 mL/min.

Mass spectrometry was performed on a LKB-9000 GC-MS instrument with 6-ft column of the same 1% SE-30 ultraphase as the radiometric gas chromatography at 220 °C. The slightly higher temperature allowed nearly identical retention times obtained between the radiometric gas chromatograph and the GC–MS. The electronic energy was set at 22.5 eV. The data was evaluated on line by an IBM 1800 computer and plotted.

The methods used for preparation of the methyl ester and O-methoxime derivatives of PG metabolites were the same as described elsewhere (Green, 1969). The trimethylsilyl derivatives were prepared by treating sample with bis(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane at 37 °C for 20 min. The reagent was removed by evaporation under a stream of nitrogen and dissolved in a small aliquot of acetone before injection. Ethyl ester and *O*-butyloxime were prepared in the same way as the methyl ester and *O*-methoxime. Acetylation was carried out with 1:1 mixture of acetyl anhydride and pyridine containing a few crystals of dimethylaminopyridine as catalyst. The reaction was allowed to stand at room temperature overnight and then quenched with ice-water. The pH was adjusted to 3.0 and the mixture was ex-

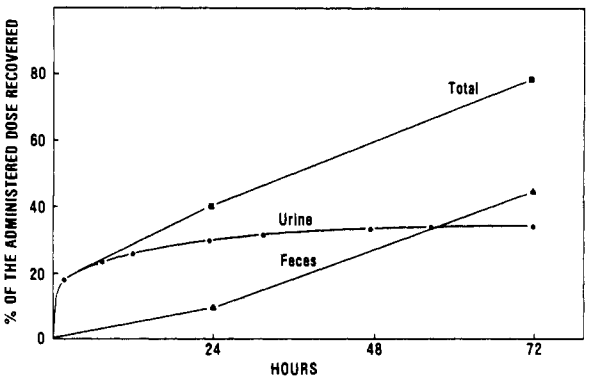


FIGURE 1: Cumulative excretion of 11-[^{3}H]PGI_2 in rat.

tracted three times with ether. The ether extract was evaporated to dryness and the desired acetate derivative was isolated by thin-layer chromatography with hexane–acetone (1:1) as solvent.

Measurement of Radioactivity. Radioactivity was determined with a Packard Tri-Carb 3375 liquid scintillation spectrometer. Quench was corrected by external or internal standard method. Urine and feces sample preparation for counting have been described previously (Sun, 1974).

Results

Excretion of Radioactivity. In the preliminary study, a single bolus dose of prostacyclin was administered intravenously to four rats. Recovery of total radioactivity during a 3-day period accounted for 77% of the administered dose. The distribution of radioactivity in urine and feces routes was about equal, 33% and 44% in urine and feces, respectively. The excretion of radioactivity in the early phase after drug administration was dominated by the urine route, and 77% of the total urinary radioactivity was recovered in 72 h. The pattern of radioactivity excretion is shown in Figure 1.

Extraction and Separation of Urinary Metabolites. The radioactive metabolites were extracted and separated from the bulk of the urinary components as described in the experimental sections. The recovery of radioactivity after the Amberlite XAD and silicic acid column was 95%. The separation of radioactive components was performed in two steps. Sephadex LH-20 column chromatography yielded three fractions (Figure 2), peak 1 (27% of the total radioactivity applied to the column), peak 2 (18.4%), and peak 3 (49.4%). Each peak was then separated by reversed phase column chromatography. Peaks 1 and 2 each yielded two radioactivity components, designated 1a, 1b, 2a, and 2b, respectively. Peak 3 yielded five components (Figure 3). Each subfraction was then esterified with diazomethane and repurified on a reversed phase column. At this point, we started monitoring the components in each fraction by radiometric GC and GC–MS. Some fractions which still contained urinary contaminants were purified by silica gel column chromatography. Most major fractions would give satisfactory mass spectra after the silica gel column. Minor fractions which still contaminated with large amounts of nonradioactive impurities were not studied further.

Identification of the Metabolites. Seven compounds were isolated from the rat urine and identified as PGI_2 metabolites. They can be divided into two series of compounds, each bearing characteristic mass spectral features. The diketo metabolites 1–5 appear to be derived from 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$. They all have a keto group at their original C-15 position, an indication that these compounds have been oxidized by 15-hydroxyprostaglandin dehydrogenase. On the other

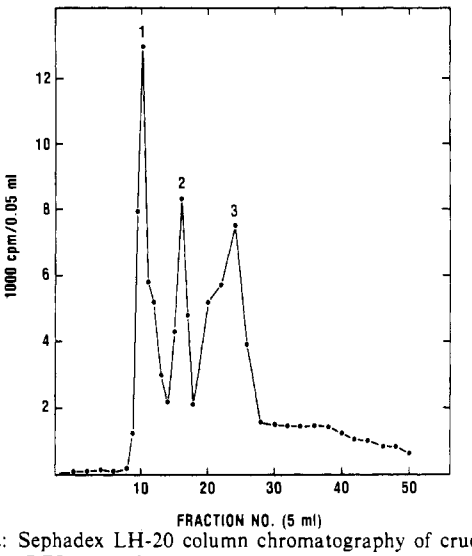


FIGURE 2: Sephadex LH-20 column chromatography of crude urine extract from PGI_2-treated rats.

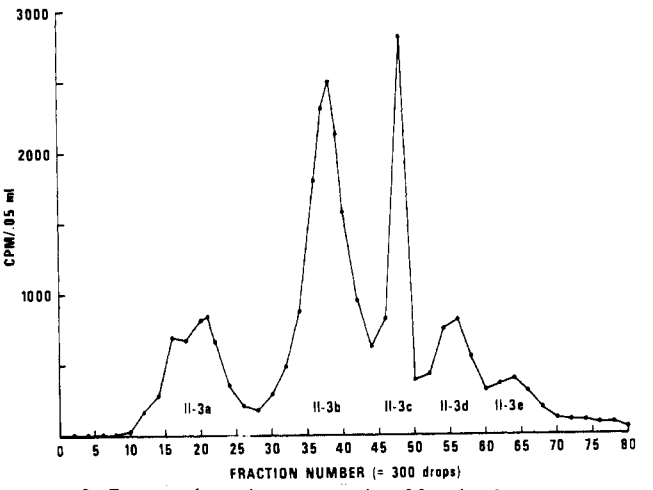


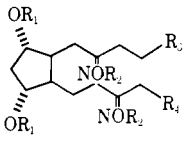
FIGURE 3: Reverse phase chromatography of fraction 3.

hand, compounds 6 and 7 appear to be derived from 6-keto-$PGF_{2\alpha}$. They survived the 15-hydroxyl-PGDH and still retain the allylic hydroxyl group at the original C-15 position.

The identification of the seven metabolites is described in the following sections. The mass spectral data are summarized in Table I and the structures are presented in Figure 4.

The Diketo Metabolites. This series of compounds is derived from 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$. The mass spectra of all five exhibit the same basic pattern for each of the derivatives made. The spectra are so analogous to each other that the variation in functional group can be easily visualized (Table I). The methyl ester, *O*-methoxime, and Me_3Si ether derivative usually give very weak or undetectable molecular ion. The conspicuous ions with the highest mass are M − 31 (OCH_3). Successive elimination of two trimethylsilanol yielded two of the most prominent ions [M − (TMSOH + OCH_3)] and [M − (2 × TMSOH + OCH_3)] in the spectrum. Rupture of the bond between C7 and C8 plus the loss of one trimethylsilanol gave another intense ion [M − (top chain + 90)]. Low intensity ions with some diagnostic values include M − 90 (trimethylsilanol) and the loss of the five-carbon fragment at the ω end. The dibutyloxime gave essentially the same pattern except most fragments were found 42 mass units higher. The presence of the two keto groups can be visualized by the M − 90 ion which was found 84 mass units increased toward the high mass end.

TABLE I: GC-MS Characteristics of PGI_2 Metabolites.



R_1	R_2	R_3	R_4	compound[a]	M^+	$M-OR_2$	$M-R_1OH$	$M-(OR_2+R_1OH)$	$M-2\times R_1OH+OR_2$ base peak	$M-R_1OH$ + top chain	other important prominent ions
Me_3Si	CH_3	$(CH_2)_2CO_2CH_3$	$CH_2CH_2CH_3$	13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$	586	555	496	465	375	310	
Ac	CH_3	$(CH_2)_2COOCH_3$	$CH_2CH_2CH_3$	13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$	526	495	ND[b]	435	375	280	187, 115
Me_3Si	CH_3	$COOCH_3$	$CH_2CH_2CH_3$	1	ND	527	468	437	347	310	
Ac	CH_3	$COOCH_3$	$CH_2CH_2CH_3$	1	498	467	438 (w)	407	347	280	159
Me_3SI	CH_3	$COOCH_3$	$(CH_2)_3OR_1$	2	ND	615	556	525	435	398	117
Ac	CH_3	$COOCH_3$	$CH_2CH_2CH_2OR_1$	2	556	525	496	465	405	338	
Me_3Si	CH_3	$COOCH_3$	$CH_2CHOR_1CH_3$	3	ND	615	556	525	435	398	
Ac	CH_3	$COOCH_3$	$CH_2CHOR_1CH_3$	3	556	525	496	465	405	338	
Me_3Si	CH_3	$COOCH_3$	$CH_2CH_2COOCH_3$	4	ND	571	512	481	391	354	
Me_3Si	$(CH_2)_3CH_3$	$COOCH_3$	$CH_2CH_2COOCH_3$	4	ND	613	596	523	433	396	
Ac	CH_3	$COOCH_3$	$CH_2CH_2COOCH_3$	4	542	511	482 (w)	451	391	324	115
Me_3Si	CH_3	$COOCH_3$	$COOCH_3$	5	ND	543	484	453	363	326	
Me_3Si	$(CH_2)_3CH_3$	$COOCH_3$	$COOCH_3$	5	ND	585	568	495	405	368	
Ac	CH_3	$COOCH_3$	$COOCH_3$	5	514	483	454	423	363	296	
Me_3Si	CH_3	$COOC_2H_5$	$COOC_2H_5$	5	ND	571	512	481	391	340	

[a] Mass spectra are available on request. [b] ND, not determined.

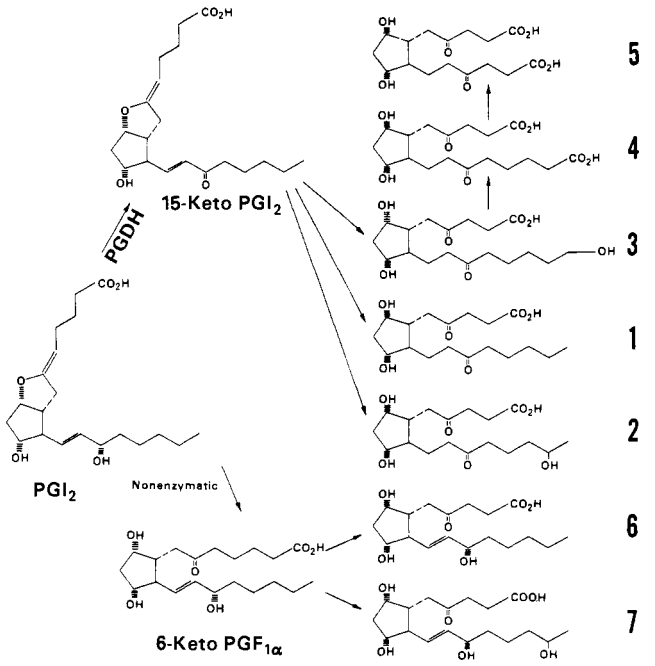


FIGURE 4: Proposed metabolic pathways of PGI_2 in the rat.

The fragmentation patterns of the acetate derivatives of compounds **1–5** are also similar to the Me_3Si derivatives. The dominate ions in these spectra are formed by the losses of OCH_3 (M − 31) from the *O*-methoxime group and acetic acid molecule (M − 60). A relatively weak ion formed by the loss of an acetic acid and the top chain via an apparent McLafferty rearrangement gave key information of the structural features in the bottom chain. In most cases, a weak but significant molecular ion was detectable.

Compound **1** was isolated from fraction 1b. The spectrum of the methyl ester, *O*-methoxime, Me_3Si ether derivative of **1** showed a set of four major fragments, 527 (M − 31), 437 [M − (31 + 90)], 347 [M − (2 × 90 + 31)] and 310 [M − (90 + top chain)]. The pattern is very similar to the corresponding derivative of 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$ except the first three major ions were found 28 mass units less. The 28 mass units correspond to the loss of two methylene groups from dihydrodiketo-$PGF_{1\alpha}$, presumably via β-oxidation. The *m/e* 310 ion indicates the ring as well as the bottom chain is not modified. Weak ion at *m/e* 217 further confirmed the dihydroxyl group on the ring is intact. This result as well as the spectra for the acetate suggest compound **1** is 7,9-dihydroxy-6,15-diketodinorprostan-3-oic acid.

The two hydroxyl compounds **2** and **3** were present in fractions 1a and 2a. The two compounds have almost identical mass spectra in all derivatives, but the difference in retention time suggested they are position isomers. The methyl ester *O*-methoxime Me_3Si derivatives displayed a set of four major fragments at *m/e* 615 (M − 31), 525 [M − (90 + 31)], 435 [M − (2 × 90 + 31)], and 398 (loss of the top chain + 90). Comparing with the analogous ions of **1**, these fragments are found 89 mass units greater which corresponds to the addition of an OTMS group on the molecule. The *m/e* 398 ion indicated the hydroxyl group is at the bottom chain. The spectra of the two isomers showed some differences in the low mass end. The fast emerging isomer displayed a strong *m/e* 117 ion which is absent in the spectra of the slow emerging isomer. The 117 ion is interpreted as $Me_3Si\text{-}O\text{-}CH\text{-}CH_3$ which is a diagnostic property of 19-hydroxyl prostanoid. Minor peaks at m − (90 + 177) and m − (90 + 31 + 117) gave additional support to the structure. From a number of previous studies of PG metabolism, it was always observed that the Me_3Si derivative of the 19-hydroxyl prostanoids has a shorter retention time than the 20-hydroxyl prostanoids. Therefore, we concluded the two compounds are 9,11,19-trihydroxy-6,15-diketo-1,2-dinorprostan-3-oic acid and 9,11,20-trihydroxy-6,15-diketo-1,2-dinorprostan-3-oic acid.

Compound **4** was found in fraction 3c. The mass spectrum of the methyl ester *O*-methoxime Me_3Si derivative had a set of four prominent fragments *m/e* 571 (M − 31), 481 [M − (90 + 31)], 391 [M − (2 × 90 + 31)], and 354 (M − top chain + 90). The increase of 44 mass units over the analogous fragments of **1** indicates the introduction of an additional carboxylic acid group into the molecule. The loss of top chain and a trimethylsilanol shows the fragment of *m/e* 310 in **1** was replaced by 354 in **4**, thus indicating the metabolic alteration on

TABLE II: Relative Abundance of PGI_2 Urinary Metabolites in Rat.

		%[a]
1	9,11-dihydroxy-6,15-diketo-1,2-dinorprostan-3-oic acid	8.8
2	9,11,19-trihydroxy-6,15-diketo-1,2-dinorprostan-3-oic acid	14.5
3	9,11,20-trihydroxy-6,15-diketo-1,2-dinorprostan-3-oic acid	
4	9,11-dihydroxy-6,15-diketo-1,2-dinorprosta-3,20-dioic acid	7.4
5	9,11-dihydroxy-6,15-diketo-1,2,19,20-tetranorprosta-3,18-dioic acid	19.0
6	9,11,15-trihydroxy-6-keto-1,2-dinorprosta-13-enoic acid	13.4
7	9,11,15,19-tetrahydroxy-6-keto-1,2-dinorprosta-13-en-3-oic acid	17.1

[a] The percentages were calculated from the recoveries of two column chromatographic separations. They should be treated only as rough estimates.

the bottom chain. The spectrum of methyl ester *O*-butyloxime Me_3Si derivative showed that in addition to the four major ions at *m/e* 613, 523, 433, and 396, the peaks of m − 90 were found at *m/e* 596 which is 84 mass units greater than the corresponding ion 512 of the *O*-methoxime and gives the evidence that the diketo system is not modified. The spectrum of the methyl ester, *O*-methoxime acetate, gave a weak molecular ion at *m/e* 542 and other major ions at 511, 451, and 391. The molecular ion and the m − 31 ion were 60 mass units less than the corresponding ion of the Me_3Si derivative, indicating the presence of two hydroxyl groups. From these data it was concluded that the compound has the structure of 9,11-dihydroxy-6,15-diketo-1,2-dinorprosta-3,20-dionic acid.

Compound **5** was found mainly in fraction 1a but trailed through the entire Sephadex LH-20 separation. So it was also found in fractions 2a and 3a. The four major ion set in the mass spectrum of methyl ester *O*-methoxime Me_3Si derivative was found at *m/e* 543, 453, 363, and 326. This group of fragments was 28 mass units or two methylene less when compared with compound **5**. Since the 28 mass units loss also occurred in the (m − top chain) fragment, the metabolic alteration must take place in the bottom chain. With the aid of the mass spectra of the methyl ester *O*-butyloxime Me_3Si ester and methyl ester *O*-methoxime acetate derivatives, this metabolite was assigned the structure of 9,11-dihydroxy-6,15-diketo-1,2,19,20-tetranorprosta-3,18-dioic acid.

The Monoketo Metabolite. The two monoketo metabolites **6** and **7** had been identified previously by Pace-Asciak & coworkers (1977) after isolation from urine of 6-keto-$PGF_{1\alpha}$-treated rats. Metabolite **6** was found to be 6-ketodinor-$PGF_{1\alpha}$ or 9,11,15-trihydroxy-6-keto-1,2-dinorprosta-Δ^5-3-enoic acid. It was found exclusively in fraction 2b. Metabolite **7**, found in fraction 3b, was identified as 6-keto-19-hydroxydinor-$PGF_{1\alpha}$ or 9,11,15,19-tetrahydroxy-6-keto-1,2-dinorprosta-Δ^5-3-enoic acid. The fragmentation patterns of the Me_3Si derivatives of **8** and **9** were identical with those published and discussed, although the previously reported spectra were obtained at 70 eV. In addition, the spectra of methyl ester *O*-methoxime acetate derivatives of these two metabolites were compatible with the structures assigned.

Relative Abundance of PGI_2 Metabolites. The relative abundances of compounds **1**–**7** were estimated from the recoveries of radioactivity in each column fraction and are summarized in Table II. These numbers represent only the percentages of each compound in a pooled urine sample. The proposed metabolic pathways of PGI_2 in rat are depicted in Figure 4.

Discussion

The experiment described here indicates that the metabolic fate of PGI_2 in rat is similar to $PGF_{2\alpha}$ (Sun, 1974). The metabolic pathways involved are: (1) the oxidation of hydroxyl group at C-15; (2) reduction of the C-13 double bond; (3) β oxidation; and (4) 19-hydroxylation and 20-hydroxylation and oxidation to the dicarboxylic acid. An important feature observed was that the top chain β oxidation stops at C-3; apparently, the keto group at C-6 prevented further chain shortening. In addition, we found no compounds with a hydroxyl group at C-15 but without the Δ^{13} double bond. This indicates the reduction of 15-ketodihydro metabolites of PGI_2 does not occur in rat, although metabolites of this type have been found in both PGE_2 and $PGF_{2\alpha}$ series.

The finding that the majority of the metabolites possess a keto group at C-15 suggests that a substantial part of the administered dose of PGI_2 is metabolized via the 15-hydroxyprostaglandin dehydrogenase pathway. However, a portion of the compounds (metabolites **6** and **7**) escaped the dehydrogenase activity and appeared in the urine with the allylic alcohol group intact. Since 6-keto-$PGF_{1\alpha}$ is a relatively poor substrate for 15-hydroxyprostaglandin dehydrogenase, metabolites **6** and **7** must be the survivors of first pass metabolism and derived from 6-keto-$PGF_{1\alpha}$.

Pace-Asciak et al. (1977) reported the urinary metabolites of 6-keto-$PGF_{1\alpha}$ in rat included the unchanged drug and compounds **6** and **7**. All three compounds have unchanged allylic alcohol group at C-15 as if they had never been exposed to the 15-hydroxyprostaglandin dehydrogenase. Although their results are partly due to the poor substrate activity of 6-keto-$PGF_{1\alpha}$ for PGDH, the most probable cause is that the high dose administered in a short time span produced sharply elevated blood and tissue levels of the drug, which saturated the capacity of PGDH. A large portion of the drug thus escaped the dehydrogenase action and was taken up in the liver where β oxidation and hydroxylation activities were high and PGDH activity was low. The compounds may not be exposed to the dehydrogenase again before excretion.

It should then be noted that when radiolabeled 6-keto-$PGF_{1\alpha}$ was given to rats by chronic intravenous infusion, a fair amount of 6,15-diketo metabolites were also found in the urine. The result indicates 6-keto-$PGF_{1\alpha}$ could still be metabolized via the 15-PGDH pathway and the slow continuous intravenous infusion gave the drug plenty of exposure to the degradative enzyme. Furthermore, if PGI_2 escaped the first pass dehydrogenase action and hydrolyzed to 6-keto-$PGF_{1\alpha}$, it can still be oxidized to give 6,15-diketo metabolites.

Recently investigators studying the cardiovascular effect of PGI_2 have noted that duration of action was exceedingly short. The low blood pressure maintained by continuous intravenous infusion of PGI_2 in rat began to rise immediately after termination of the infusion (Weeks, 1978). These results indicate prostacyclin is rapidly inactivated in the body. However, Armstrong et al. (1978) have described that the vasodepressor response of PGI_2 in rat and rabbit is not diminished by passage through the lung. We have also demonstrated

(Taylor & Sun, 1978) that the isolated perfused lungs from rabbits or guinea pigs converted less than 15% of the injected PGI_2 radioactivity into 6,15-diketo metabolites, in contrast to the greater than 90% conversion of PGE_1. This evidence indicates that although prostacyclin is a good substrate for lung prostaglandin dehydrogenase, it is not a substrate for the pulmonary prostaglandin transport system and therefore clears the lung without significant metabolism. One of the most likely sites of prostacyclin inactivation is vascular tissue. 15-Hydroxyprostaglandin dehydrogenase in the cytoplasmic fractions of blood vessel wall rapidly transformed PGI_2 to 6,15-diketo metabolites in the presence of NAD. Furthermore, when radiolabelled PGI_2 was incubated in a bovine aortic sac, suspended in Kreb's buffer, the drug was rapidly transformed to a product with identical TLC mobility to 6,15-diketo-$PGF_{1\alpha}$. We feel that the rapid disappearance of PGI_2 depressor activity in the intact animal is largely due to the metabolic capacity of the blood vessels themselves. Further studies are on going to prove this hypothesis.

In connection with this study, we have measured the bioactivities of 15-keto-PGI_2 methyl ester which is presumably the immediate dehydrogenase product of PGI_2. This compound when compared with PGI_2 methyl ester is 2000 times less active in blocking human platelet aggregation. It also lacks any detectable smooth muscle stimulation activity.

In the development of methods for separation of PGI_2 metabolites, we have found that a recently introduced C_{18} bonded silica gel reversed phase absorbant (C_{18} bonded HIFLOSIL) is far superior to the previously utilized liquid partition chromatography on hydrophobic Celite (Hamberg, 1973) or the lipophilic dextran gels of the Lipidex series (Brash & Jones, 1974). This material has a high loading factor, good efficiency, reproducibility, and quantitative recoveries. The powder is free flowing and easy to handle and the packed column can be used repeatedly. In this study, we have successfully separated the PGI_2 metabolites both as free acids and methyl esters with this method. It should be easy to adapt this technique to the separation of other types of prostanoids.

During the course of this study, the formation of artifacts from these metabolites was most annoying. 6-Keto-$PGF_{1\alpha}$ and related compounds are notorious for their easy conversion to cyclic 6,9-acetals under conditions such as acidic alcohol or alcoholic diazoalkanes. Once formed, the keto group at carbon 6 is no longer available for oxime formation. For this reason, we have restricted the use of alcoholic solvent to a minimum. Another disturbing artifact was the formation of Me_3Si ester of the carboxyl group of the top chain. Even though the compound is repeatedly esterified with diazomethane or methyl iodide a significant amount still ends up as the Me_3Si esters. The formation of cyclic 1,9-lactone or the ester exchange between the methyl ester and the Me_3Si reagent were thought to be the probable reasons. Therefore, a single purified metabolite often yielded multiple GC peaks upon derivatization and attempts to alleviate the source of artifacts have been unsuccessful.

In conclusion, we have found seven metabolites in the urine of PGI_2 treated rats. The metabolic pattern would provide important background information for more detailed studies concerning the endogenous production of PGI_2 in this species.

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