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Received April 30, 1969.

# Structures of Urinary Metabolites of Prostaglandin $F_{2\alpha}$ in the Rat

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One of the urinary metabolites of prostaglandin $F_{1\alpha}$ in the rat has been found to be dinor-$PGF_{1\alpha}$.[1] Recently the main urinary metabolites of prostaglandin $E_2$ in guinea pig and man have been identified as 5$\beta$,7$\alpha$-dihydroxy-11-keto-tetranorprostanoic acid and 7$\alpha$-hydroxy-5,11-diketo-16-carboxy-tetranorprostanoic acid, respectively.[2,3]

9$\beta$-$^3$H- and/or 17,18-$^3$H-prostaglandin $F_{2\alpha}$ (3.2 $\mu$C/$\mu$mole; 1.4 $\mu$mole per rat) was administered intravenously to female rats. The urine was collected during 24 h, acidified to pH 3 and extracted with butanol (50 % of administered radioactivity was recovered). Separation of the extract by reversed phase partition chromatography [4] gave four peaks of radioactivity at retention volumes 150 ml (peak I), 300 ml (peak II), 700 ml (peak III), and 1300 ml (peak IV), respectively (system C-38, 45 g of hydrophobic Hyflo-Supercel).

After treatment with diazomethane the materials in peak I and III were each separated on a silicic acid column. In both chromatograms two peaks appeared, compound Ia and Ib eluted with methanol/chloroform 2/98 and 8/92, respectively, and compound IIIa and IIIb eluted with ether/hexane 60/40 and 80/20, respectively.

*Compound IIIa.* The *O*-methyloxime acetyl (MO-AC) derivative was prepared [5] and chromatographed on a Barber-Colman Gas Chromatograph model 500 with simultaneous registration of mass and radioactivity using a 1 % Se-30 column at 220°C. One peak appeared at 21.9-C.[6] This C-value is consistent with a $C_{16}$ prostaglandin with two acetoxy groups and one methoxime group.

Mass spectra of the MO-Ac and acetyl derivatives were obtained on an LKB 9000 A gas chromatograph-mass spectrometer. Some relevant ions are listed in Table 1 (acetyl derivative) and Fig. 1 (MO-Ac derivative). The molecular ions were seen in both spectra (*m/e* 398 and *m/e* 427) and eliminations characteristic for methyl ester acetyl derivatives (15, 31, 32, 59, 60, and 73) are easily identified. These data strongly support the structure shown in Fig. 2. The loss of 56 in both spectra is due to $\beta$-cleavage at the keto or methoxime group and transfer of hydrogen to the charge retaining ion, while $\alpha$-cleavage on each side of the keto group of the acetyl derivative causes elimination of 71 and 99 mass units. The ion at *m/e* 156 is due to the ionized side chain attached to C-8. The mass spectrum of the MO-Ac derivative deuterated in the methoxime group supported the eliminations proposed above.

*Compound Ia.* The MO-Ac derivative had a C-value of 24.9 on GLC and some mass spectral data are listed in Table 1 and Fig. 1. The eliminations involving 100 mass units are due to $\beta$-cleavage at the methoxime group and it is shown in Fig. 1 that this gives rise to ions common for metabolites IIIa and Ia. Therefore the higher molecular weight of Ia must be due to a carbomethoxy group in the eliminated fragment. The GLC and mass spectral data strongly suggest the structure in Fig. 2. Mass spectra of MO-Ac derivatives deuterated in the methoxime group or the acetyl groups confirmed the structure and proposed eliminations.

*Compound Ib.* On GLC the MO-Ac and MO-TMS (*O*-methyloxime trimethyl silyl ether) derivatives gave C-values of 25.7 and 24.1,

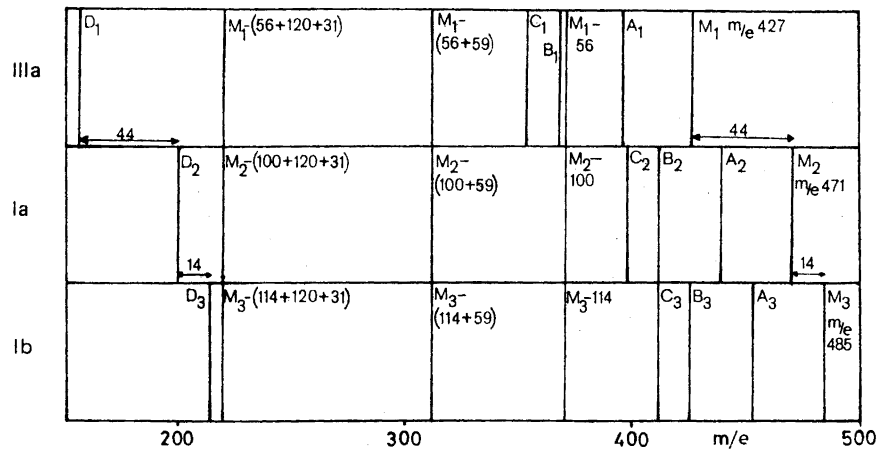


*Fig. 1.* Schematic presentation of some mass spectral data for the MO-Ac derivatives of the methyl esters of metabolites IIIa, Ia, and Ib. A, B, and C are ions due to eliminations of 31, 59, and 73, respectively, from the molecular ions. The loss of 56, 100, and 114 are due to $\beta$-cleavage at the methoxime group in the side chain and transfer of hydrogen to the charge retaining ion. The ion designated D is due to the complete side chain attached to C—8.

*Table 1.* Mass spectral data.

| Derivative | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ia MO-Ac | | Ib MO-Ac | | II TMS | | IIIa Ac | |
| *m/e* | ion | *m/e* | ion | *m/e* | ion | *m/e* | ion |
| 471 | M | 485 | M | 530 | M | 398 | M |
| 440 | M−31 | 454 | M−31 | 515 | M−15 | 383 | M−15 |
| 412 | M−59 | 426 | M−59 | 499 | M−31 | 367 | M−31 |
| 398 | M−73 | 412 | M−73 | 459 | M−71 | 342 | M−56 |
| 380 | M−(60+31) | 394 | M−(60+31) | 440 | M−90 | 338 | M−60 |
| 371 | M−100 | 371 | M−114 | 369 | M−(90+71) | 325 | M−73 |
| 356 | M−115 | 366 | M−(60+59) | 350 | M−2×90 | 315 | M−(56+59) |
| 352 | M−(60+59) | 334 | M−(2×60+31) | 279 | M−(2×90+71) | 278 | M−2×60 |
| 320 | M−(2×60+31) | 312 | M−(114+59) | | | 246 | M−(2×60+32) |
| 312 | M−(100+59) | 251 | M−(114+2×60) | | | 222 | M−(2×60+56) |
| 220 | M−(100+2×60+31) | 220 | M−(114+2×60+31) | | | 207 | M−(2×60+71) |
| 200 | | 214 | | | | 191 | M−(2×60+56+31 |
| | | | | | | 179 | M−(2×60+99) |

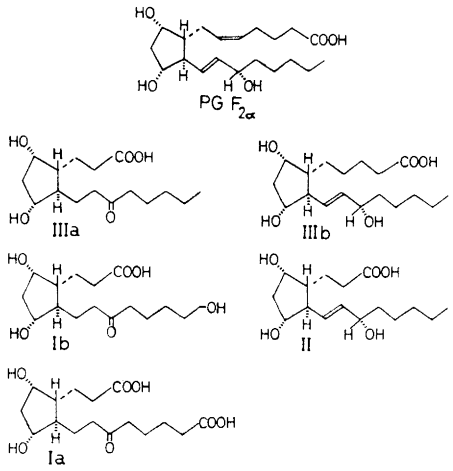


*Fig. 2.* The structures of five metabolites of prostaglandin $F_{2\alpha}$ in rat urine.

respectively, indicating a trihydroxy compound as one acetyl group adds about 2.5 and one TMS group adds about 1.95 to the C-value. Mass spectral data of the MO-Ac derivative are shown in Fig. 1 and Table 1. The elimination of 114, (114+59) and (114+120+31) gives fragments common for metabolites IIIa, Ia, and Ib. These data strongly indicate the structure shown in Fig. 2. The difference in C-value between the MO-Ac derivative of Ib and IIIa (C-3.8) indicates that the additional hydroxyl group in Ib is located in $\omega$ 1 position. The structure and eliminations proposed above were further supported by mass spectral data from MO-Ac derivatives deuterated in the methoxime or acetyl groups.

*Compound II.* The methyl ester of this compound had a retention time of C-22.4 as acetate and C-21 as TMS derivatives indicating a $C_{16}$ trihydroxy compound. The methyl ester triacetate of dinor-$PGF_{1\alpha}$ appears at 24.4-C under the same conditions. The mass spectral data of the TMS (Table 1), acetyl and methyl ether derivatives supported the structure shown in Fig. 2. A reference compound was prepared from $PGF_{2\alpha}$ using the $\beta$-oxidizing system of rat liver mitochondria.[7] The mass spectrum of the methyl ester triacetate of this compound was identical with that of the corresponding derivative of metabolite II. The acetylated methyl ester of metabolite II, obtained after injection of $9\beta$-$^3$H- and 17,18-$^3$H-$PGF_{2\alpha}$ was subjected to oxidative ozonolysis and the resulting mixture was treated with diazoethane. Upon GLC two radioactive peaks appeared at 10.2 C and 17.2 C, respectively (1 % SE-30) demonstrating that the double bond is located in the $\Delta^9$-position (*cf.* Ref. 6).

*Compound IIIb.* The acetyl and TMS derivatives of this material gave on GLC one peak at 24.4 C and 22.7 C, respectively. These data indicated that metabolite IIIb was a trihydroxy $C_{18}$ prostaglandin. The mass spectrum of the acetyl derivative was identical with that of dinor-$PGF_{1\alpha}$ earlier found in rat urine.[1]

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Received May 6, 1969.