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Metabolism of Prostaglandin E_2 in the Rat*

Krister Gréen

ABSTRACT: Tritium-labeled prostaglandin E_2 was injected intravenously into female rats in doses ranging from 1.4 mmoles to less than 0.5 nmole per rat. About 55% of the administered radioactivity was excreted into the urine. After extraction with butanol, nine metabolites were isolated and identified.

All metabolites could be isolated after injection of different amounts of prostaglandin E_2 indicating that the identified metabolites can be normally excreted. The identified metabolites are 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid (tetranorprostaglandin E_1), 11-hydroxy-5-ketotetranorprosta-4(8),9-dienoic acid (tetranorprostaglandin B_1), 11,16-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid, 11,15-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid, 11-hydroxy-5-ketotetranorprosta-4(8),9-diene-1,16-dioic acid, 5β,7α-dihydroxy-11-ketotetranorprostanoic acid, 7α-hydroxy-5,11-diketotetranorprostanoic acid, 7,16-dihydroxy-5,11-diketotetranorprostanoic acid, and 7-hydroxy-5,11-diketotetranorprosta-1,16-dioic acid. The structures were established by comparison with reference compounds (in some cases), by gas–liquid chromatographic and mass spectrometric analyses of different derivatives of the metabolites, deuterated or not, and by chemical degradations. To study the metabolism of tetranorprostaglandin E_1, tetranorprostaglandin B_1, 5β,7α-dihydroxy-11-ketotetranorprostanoic acid, and 7α-hydroxy-5,11-diketotetranorprostanoic acid, the purified metabolites were injected into rats and the radioactive urinary products were isolated. The results indicate that prostaglandin E_2 probably is degraded to three groups of metabolites *via* three separate pathways.

Early studies of the metabolism of prostaglandin E compounds demonstrated that they are subject to oxidation of the alcohol group at C-15 and to saturation of the Δ^{13} double bond in preparations of lung tissue (Änggård *et al.*, 1965; Änggård and Samuelsson, 1964, 1965). The dehydrogenase, catalyzing the oxidation at C-15, has been purified and it was found to be active on a large number of different prostaglandins (Änggård and Samuelsson, 1966). It has also been shown that prostaglandins incubated with rat liver mitochondria undergo one or two steps of β oxidation forming dinor and tetranor compounds, respectively (Hamberg, 1968). Recently the formation of 19- and 20-hydroxylated derivatives from prostaglandin A_1 and B_1 in incubations with guinea pig liver microsomes has been demonstrated (Israelsson *et al.*, 1969).

The major urinary metabolite of PGE_2[1] in man and guinea pig has been identified as 7α-hydroxy-5,11-diketotetranorprosta-1,16-dioic acid and 5β,7α-dihydroxy-11-ketotetranorprostanoic acid, respectively (Hamberg and Samuelsson, 1969a,b). The human metabolite is formed from PGE_2 after four types of reactions have taken place: (1) oxidation of the alcohol group at C-15 (Änggård *et al.*, 1965), (2) reduction of the trans double bond (Änggård *et al.*, 1965), (3) two steps

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[1] The abbreviations used are: PGE_1, prostaglandin E_1, 11α,15-dihydroxy-9-ketoprost-13-*trans*-enoic acid; PGE_2, prostaglandin E_2, 11α,15-dihydroxy-9-ketoprosta-5-*cis*,13*trans*-dienoic acid; PGE_3, prostaglandin E_3, 11α,15-dihydroxy-9-ketoprosta-5-*cis*,13-*trans*,17-*cis*-trienoic acid; PGA_1, prostaglandin A_1, 15-hydroxy-9-ketoprosta-10,13-dienoic acid; PGB_1, prostaglandin B_1, 15-hydroxy-9-ketoprosta-8(12),13-dienoic acid; $PGF_1\alpha$, prostaglandin $F_1\alpha$, 9α,11α,15-trihydroxyprost-13-*trans*-enoic acid; $PGF_2\alpha$, prostaglandin $F_2\alpha$, 9α,11α,15-trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid; MO, *O*-methyloxime; TMSi, trimethylsilyl ether.

of β oxidation (Hamberg, 1968), and (4) ω oxidation. The formation of the metabolite in guinea pig urine involves the first three reactions mentioned above and in addition reduction of the keto group in the five-membered ring to a 5β-hydroxy compound.

One of the main urinary metabolites of $PGF_{1\alpha}$ in the rat was previously identified as dinor-$PGF_{1\alpha}$ (Granström *et al.*, 1965). Recently the structures of one metabolite of $PGF_{2\alpha}$ in human urine (Granström and Samuelsson, 1969a) and one in guinea pig urine (Granström and Samuelsson, 1969b) have been determined. The identification of five metabolites of $PGF_{2\alpha}$ in rat urine has been published in preliminary form (Gréen, 1969a). The present work, which is concerned with the metabolism of PGE_2 in the rat, has led to the identification of nine metabolites, four of which have not been previously identified.

Experimental Section

Preparation of [17,18-^{3}H]Prostaglandin E_2. [17,18-^{3}H]Prostaglandin E_2 was prepared by catalytic hydrogenation of PGE_3, using tritium gas, as described earlier (Änggård *et al.*, 1965). Part of the obtained material was added to 2 mg of unlabeled crystalline PGE_2 (a gift from Dr. John Pike, The Upjohn Co., Kalamazoo, Mich.). This material was purified on reversed phase partition chromatography (Norman and Sjövall, 1958) using system C-45 (Table I). The fractions containing PGE_2 were rechromatographed with the same solvent system, and the purity of the obtained [17,18-^{3}H]PGE_2 as judged by thin-layer chromatography using solvent system AII (Gréen and Samuelsson, 1964) was higher than 97%. Gas–liquid chromatography of the MO–TMSi derivative of this material on a 1% Se-30 column demonstrated radioactive purity of at least 98%. Unlabeled PGE_2 was usually added to this material to make a specific activity of about 0.32 μCi/μmole before injection into rats.

Preparation of 5,6,8,11,12,14,15-Heptatritioprostaglandin E_2. 5,8,11,14-Eicosatetraynoic acid (5 mg) (donated by Dr. U. Gloor, Hoffman-La Roche & Co, Basle, Switzerland) was dissolved in 0.5 ml of methanol and then 4 mg of Lindlar catalyst and 1 mg of chinolin were added (*cf.* Hamberg *et al.*, 1968; Hamberg, 1969; Van Dorp *et al.*, 1964; Kunau, 1968). This mixture was stirred under tritium atmosphere (approximately 100 mm, 98% $^3H_2$ gas, Radiochemical Centre, Amersham) for 105 min (*cf.* Änggård *et al.*, 1965) and then the solution was filtered. Unlabeled arachidonic acid (2 mg) was added, and after treatment with diazomethane, this material was chromatographed on a silicic acid column containing 25% silver nitrate. Methyl 5,6,8,9,11,12,14,15-octatritioarachidonate (7 mCi) was obtained, and no impurities could be detected on gas–liquid chromatography using a 10% EGSS-X column. Part of this material (0.5 mCi) was refluxed with 10% KOH in methanol–water (1:1, for 2 hr under argon. The octatritioarachidonic acid was extracted and incubated with microsomes (the pellet obtained by centrifuging the 8000*g* supernatant at 100,000*g* for 60 min) from 8 g of sheep vesicular gland in 24 ml of Bücher medium (Hamberg and Samuelsson, 1967b). Before the incubation, 20 mg of reduced glutathione was added to the microsome suspension.

The obtained 5,6,8,11,12,14,15-heptatritioprostaglandin E_2 (0.1 mCi, 20% yield) was purified by silicic acid chromatography (eluted with ethyl acetate–benzene, 6:4, v/v). A small part of this material was run with a carrier on thin-layer chromatography using system AII and on gas–liquid chromatography as the MO–TMSi derivative (1% Se-30 column). The purity, based on radioactivity, was higher than 98%. The 5,6,8,11,12,14,15-heptatritioprostaglandin E_2 was diluted with unlabeled material to a specific activity of 5 μCi/μmole before injection into rats.

Preparation of 7α,11-Dihydroxy-5-ketotetranorprost-9-enoic Acid (Tetranorprostaglandin E_1). Tetranor-PGE_1 has been tentatively identified from incubations of tracer amounts of PGE_1 with rat liver mitochondria (Hamberg, 1968) although the amounts obtained were too small to permit gas chromatographic–mass spectrometric analysis. Therefore 1 mg of [17,18-^{3}H]PGE_2 (0.64 μCi/μmole) was incubated with mitochondria, obtained from 20 g of rat liver, according to the method of Hamberg. The extract of the incubation mixture was subjected to reversed-phase partition chromatography, system C-45, using a 4.5-g Hyflo Super Cel column. Three radioactive peaks appeared at 40-, 75-, and 105-ml retention volume, respectively. The material in the first peak (about 5% of incubated radioactivity) was identified as 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid (see Results).

Preparation of 7α-Hydroxy-5,11-diketotetranorprostanoic Acid. This compound was prepared from [5,6-^{3}H]PGE_1 (0.32 μCi/μmole) by oxidation with active MnO_2 in chloroform followed by catalytic hydrogenation and incubation with rat liver mitochondria (Änggård and Samuelsson, 1964; Hamberg, 1968).

Preparation of 5β,7α-Dihydroxy-11-ketotetranorprostanoic Acid and 5α,7α-Dihydroxy-11-ketotetranorprostanoic Acid. These compounds were synthesized from $PGF_{1\beta}$ and $PGF_{1\alpha}$ (Hamberg and Samuelsson, 1969b; Granström and Samuelsson, 1969b).

Animal Experiments. [17,18-^{3}H]PGE_2 (0.5 mg/rat, 0.32 μCi/μmole) was injected into the tail vein of female rats weighing about 200 g (Sprague–Dawley strain). The rats were immobilized in cages and the urine was collected into test tubes kept in ethanol and solid carbon dioxide, through small funnels that covered the urethral opening and that were applied under ether anesthesia. The urine was collected during a 24-hr period, thawed, acidified to pH 3, and extracted three times with water-saturated 1-butanol. The combined organic phases were washed with distilled water to pH 5 and evaporated immediately. About 45% of injected activity appeared in the butanol while about 5% stayed in the water phase and 5% in the wash water.

In one experiment [17,18-^{3}H]PGE_2 with higher specific activity (0.5 mg/rat, 1.5 μCi/μmole) was injected. Some of the metabolites obtained in this experiment were injected into rats.

In another experiment [17,18-^{3}H]PGE_2 with a very high specific activity (about 7 mCi/μmole, less than 0.5 nmole/rat) was injected into two rats in order to see whether the proportions between the different urinary metabolites were changed.

5,6,8,11,12,14,15-Heptatritioprostaglandin E_2 (3 mg; 5 μCi/μmole) was injected into six rats in order to obtain metabolites suitable for degradation by oxidative ozonolysis.

Radioactivity Assay. A Frieseke–Hoepfner FH 90A gas-flow counter, operated in the proportional range, was used for measuring radioactivity in chromatography fractions. A liquid scintillation spectrometer (Packard TriCarb scintillation spectrometer, Model 3375) was used for determinations of total activities in extracts and chromatographic peaks. Internal standards were used to correct for quenching.

Chromatographic Methods. Reversed-phase partition chromatography was carried out as described by Norman (Norman

and Sjövall, 1958; Norman, 1953). The solvent systems used are shown in Table I. Silicic acid chromatography was performed using activated silicic acid, (Mallinckrodt, analytical reagent). The columns were first eluted with different mixtures of ethyl acetate and benzene or ether and hexane and then with methanol.

A Barber-Colman gas chromatograph Model 5000 with simultaneous registration of mass and radioactivity was used for gas–liquid chromatography. The length of the columns was 1.8 m and the inner diameter was 6 mm. The column packings were either 1% Se-30 (F&M Scientific Corp., Avondale, Pa.), 15% silicone (Dow Corning, high-vacuum crease), 1% or 10% EGSS-X (Wilkins Instr. and Research Inc., Walnut Creek, Calif.) applied on silanized Gas Chrom P (100–120 mesh) as described by Horning (Horning *et al.*, 1963), or 2% Epon 1001 purchased ready for use (Applied Science Laboratories Inc., State College, Pa.) applied on Gas Chrom P (100–120 mesh).

Mixtures of normal saturated fatty acid methyl esters were used as standards and diagrams were constructed by plotting retention times on a logarithmic scale *vs.* the number of carbon atoms of the acid (*C* value) on the linear scale. The retention times of the derivatives were converted into *C* values using these diagrams (Bergström *et al.*, 1963).

Mass Spectrometry. Mass spectra were obtained with the combined gas chromatograph–mass spectrometer LKB 9000, equipped with a 1.5% Se-30 column. The electron energy was set to 22.5 eV and the trap current was 60 μA.

Preparation of Derivatives for Gas–Liquid Chromatography and Mass Spectrometry. The methyl esters of the compounds to be analyzed were prepared by treatment with diazomethane. The *O*-methyloxime, acetyl, and trimethylsilyl ether derivatives, deuterated (D_3MO, D_3Ac, D_9TMSi) or not, were prepared as described earlier (Gréen, 1969b). Derivatives labeled with three deuterium atoms in the methyl ester group were obtained through transmethylation in 0.5 N $NaOCD_3$ (Gréen, 1969b). When a ketone reacts with methoxyamine, the syn and anti isomers of the *O*-methyloxime are usually formed, and if the keto group is attached to the five-membered ring of a prostaglandin, the isomers separate on a 1% Se-30 column. The mass spectra recorded from the two isomers are very similar showing only intensity differences (Gréen, 1969b). To avoid confusion I will discuss the quantitatively more important isomer (always with the highest *C* value) in this paper.

Degradation by Oxidative Ozonolysis. Oxidative ozonolysis was performed essentially as described before (Hamberg and Samuelsson, 1966). The compounds (10–50 μg) were dissolved in 0.3 ml of chloroform cooled to −15°; ozone was passed through the solution for 1 min and the solvent evaporated 15 min later. The residue was dissolved in 0.2 ml of glacial acetic acid and 0.05 ml of 30% hydrogen peroxide (Perhydrol, E. Merck, A.G.). The mixture was left in a stoppered test tube overnight at 45° and then evaporated almost to dryness under reduced pressure. The reaction products were dissolved in methanol and treated with diazomethane before gas–liquid chromatographic and mass spectrometric analyses.

Ultraviolet Spectrometry. Ultraviolet spectra were obtained using a Zeiss DMR 21 ultraviolet spectrometer.

Solvents. The solvents used for reversed-phase partition chromatography and extractions were all analytical grade. The ethyl acetate, benzene, and hexane used for silicic acid chromatography were analytical grade but redistilled before use. The ether (anhydrous ether, analytical reagent, Mallinckrodt), methanol, and chloroform (analytical grade, Merck, Darmstadt) were used without further purification.

Results

The product from PGE_2 incubation with rat liver mitochondria which eluted from the reversed-phase partition chromatography at 40 ml was treated with diazomethane and its MO–Ac and MO–TMSi derivatives were prepared. Gas–liquid chromatography data for these compounds are listed in Table III. The difference in *C* value between the two derivatives on 1% Se-30 (1.0 *C*) indicate a dihydroxyprostaglandin. Since the MO–Ac derivative of the methyl ester of PGE_2 appears at 25.5 *C* on 1% Se-30 under the same conditions, these data indicate a C_{16}-prostaglandin with two hydroxy and one keto group.

The mass spectrum of the MO–TMSi derivative is shown in Figure 4. The molecular ion at *m/e* 485 and ions formed by eliminations of 15 (*m/e* 470), 31 (*m/e* 454), 71 (*m/e* 414), 90 − 15 (*m/e* 470), 31 (*m/e* 395), 90 + 15 (*m/e* 380), 90 + 31 (*m/e* 364), and 90 + 71 (*m/e* 324) are easily identified. The eliminations of 15, 31, and 90 mass units are due to loss of CH_3, CH_3O, and trimethylsilanol, respectively. The fragment at *m/e* 414 is formed by α cleavage at the trimethylsilyloxy group of the C-8 side chain eliminating the pentyl group. The ion appearing at *m/e* 312 (M − 173) might be formed by loss of the three secondary ring carbons together with the methoxime and trimethylsilyl ether groups (*cf.* Gréen, 1969b). The mass spectra of the MO–TMSi derivatives of the methyl esters of PGE_1 and PGE_2 (Gréen, 1969b) show prominent ions at *m/e* 225, probably formed by elimination of 173 and the C-8 side chain (143 and 141 mass units, respectively). Therefore the formation of the ion at *m/e* 225 in this spectrum could involve elimination of 173 + 87 (87 = the C-4 side chain). The ion at *m/e* 241 is probably due to loss of 173 + 71. These data strongly indicate that the compound isolated from the incubation mixture is 7α,11-dihydroxy-5-keto-tetranorprost-9-enoic acid.

Further support for this structure was obtained from the mass spectrum of the MO–Ac derivative of the methyl ester. The molecular ion was seen at *m/e* 425 (M) and prominent ions at *m/e* 365 (M − 60), *m/e* 354 (M − 71), *m/e* 334 [M − (60 + 31)], *m/e* 305 (M − 2 × 60), and *m/e* 274 [M − (2 × 60 + 31)], the base peak. The eliminations of 60 and 31 result from loss of acetic acid and a methoxy group from either the methyl ester or methoxime groups, respectively (Gréen, 1969b).

To obtain unequivocal evidence for the location of the double bond, the MO–Ac derivative of the methyl ester was degraded by oxidative ozonolysis. The reaction products were treated with diazomethane and subjected to gas–liquid chromatography giving one radioactive peak at 9.6 *C* (1% Se-30). This *C* value is consistent with that of methyl 2-acetoxyheptanoate (Hamberg and Samuelsson, 1967b). The mass spectrum of the material in this peak was identical with that obtained from methyl 2-acetoxyheptanoate. Therefore this compound, isolated from the incubation of PGE_2 with rat liver mitochondria, must be 7α,11-dihydroxy-5-keto-tetranorprost-9-enoic acid.

Separation of the Urinary Metabolites. The butanol extract of the urine was immediately evaporated and separated on a reversed-phase partition chromatography column using the butanol–water system (Table I). Four radioactive peaks appeared in the chromatogram at about 20, 27, 50, and 140 ml (Figure 1). The retention volumes refer to a 4.5-g Hyflo

TABLE I: Solvent Systems Used in Reversed-Phase Partition Chromatography.

System	Moving Phase (v/v)	Stationary Phase (v/v)
C-38	Methanol–water (114:186)	Chloroform–isooctyl alcohol (15:15)
C-45	Methanol–water (135:165)	Chloroform–isooctyl alcohol (15:15)
D	Water (300)	1-Butanol (100)

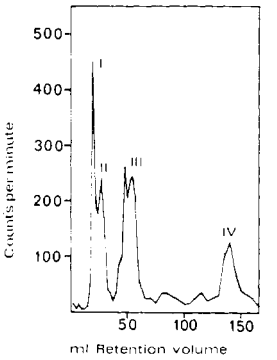

FIGURE 1: Reversed-phase partition chromatography of the butanol extract of urine obtained from rats injected with [17,18-^{3}H]prostaglandin E_2. System D was used (Table I).

Super Cel column. The peaks were numbered from I to IV. Peak I constituted 7–9% of the injected radioactivity, peak II 5–8%, peak III 13–15%, and peak IV 4–6%. Since between 14 and 18% of the injected radioactivity remained on the column this material was rechromatographed on reversed-phase partition chromatography using system C-38 (Figure 2). Three peaks appeared at about 50-, 95-, and 170-ml retention volumes, respectively, based on a 4.5-g column, and were designated number V, VI, and VII. Compound V constituted 7–8%, compound VI 3–5%, and compound VII 3–8% of injected activity.

When the methyl ester *O*-methyloxime derivative of the material in peak II was chromatographed on a silicic acid column, two peaks appeared which were eluted with ethyl acetate–benzene, 4:6 and 8:2 (v/v), respectively. These peaks were present in about equal amounts and were called IIa and IIb. In the same way the methyl ester of the material in peak III separated into two peaks when subjected to silicic acid chromatography. These compounds were also present in about equal amounts and were eluted with ethyl acetate–benzene, 6:4 and 10:0 (v/v), respectively. They were designated IIIa and IIIb. The trimethylsilyl ether and acetyl derivatives of the methyl ester of compound IIIb separated on gas–liquid chromatography into two peaks called IIIb:1 and IIIb:2; the proportions between them were about 1:4. The radioactivity in peak I is due to more than one metabolite; however the structural work on these compounds has not yet been completed.

The urine obtained from two rats injected with [17,18-^{3}H]-PGE_2 with a very high specific activity (about 7 mCi/μmole, less than 175 ng/rat) were treated separately. About 60 and 50% of the administered radioactivity were extracted from the urine with 1-butanol. When [17,18-^{3}H]PGE_2 with a lower specific activity (0.32 μCi/μmole, 0.5 mg/rat) was injected about 10% was found in the water phases and 7–9% in peak I, but in this experiment 15 and 13% of the administered amount were left in the two water phases and only 2 and 3% in peak I indicating that these very polar metabolites and perhaps a small amount of metabolite II probably were left in the aqueous phase. The other metabolites were found in the following amounts in the urine from the two rats: metabolite IIa + IIb, 20 and 16%; metabolite IIIa + IIIb:1 + IIIb:2, 7 and 4%; metabolite IV, 3 and 3%; metabolite V, 14 and 12%; metabolite VI, 8 and 6%; and metabolite VII, 6 and 5%, respectively.

The Structure of Metabolite VI. The material in peak VI (Figure 2) was subjected to silicic acid chromatography and one radioactive peak was eluted with ethyl acetate–benzene 4:6 (v/v). The methyl ester of this material was used for preparation of MO–Ac and MO–TMSi derivatives and also MO–Ac derivatives labeled with deuterium in the methoxime or acetyl groups.

The gas–liquid chromatography data of the MO–Ac and MO–TMSi derivatives of the methyl ester of metabolite VI are listed in Table II. The difference between the acetyl and trimethylsilyl ether derivatives (0.5 *C*) obtained on a 1% Se-30 column indicates a monohydroxy compound. That the mass spectra obtained from the MO–Ac, D_3MO–Ac, and MO–D_3Ac derivatives of the methyl ester of metabolite VI showed molecular ions at *m/e* 412, 418, and 415, respec-

TABLE II: *C* Values of the MO–Ac and MO–TMSi Derivatives of the Methyl Esters of Metabolite VI and 7α-Hydroxy-5,11-diketotetranorprostanoic Acid Obtained on 1% Se-30 (225°) and 2% Epon 1001 (220°) Columns.[a]

Compound	Column	Derivative	*C* Value
Metabolite VI	1% Se-30	MO–Ac	21.5
	1% Se-30	MO–TMSi	21.0
	2% Epon 1001	MO–TMSi	22.6
7α-Hydroxy-5,11-diketotetranor-prostanoic acid	1% Se-30	MO–Ac	21.5
	1% Se-30	MO–TMSi	21.0
	2% Epon 1001	MO–TMSi	22.6

[a] For definition of *C* values, see Chromatographic Methods.

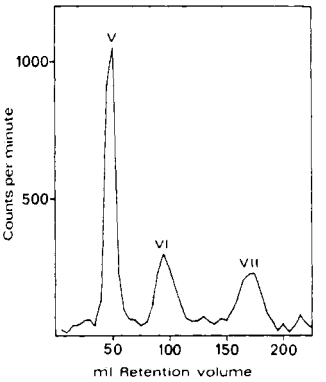

FIGURE 2: Reversed-phase partition chromatogram of the radioactive material that stayed on the column after elution with butanol-saturated water (Figure 1). System C-38 was used (Table II).

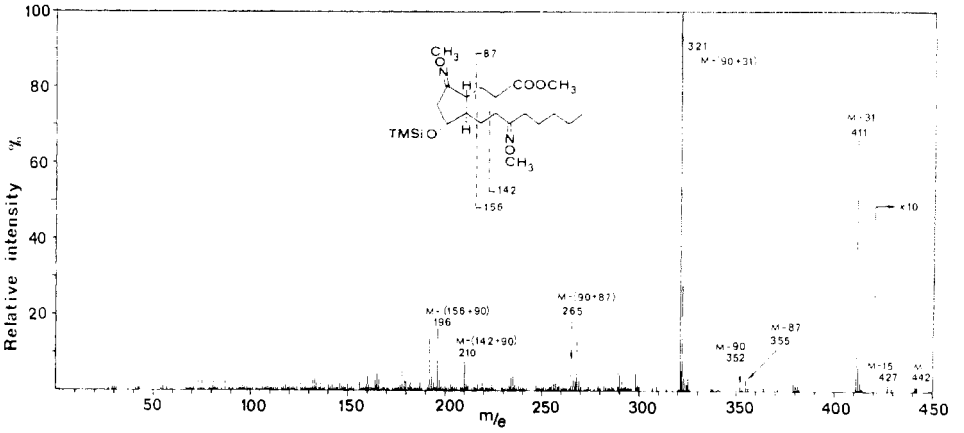

FIGURE 3: Mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite VI. The *m/e* values above *m/e* 420 have been increased ten times.

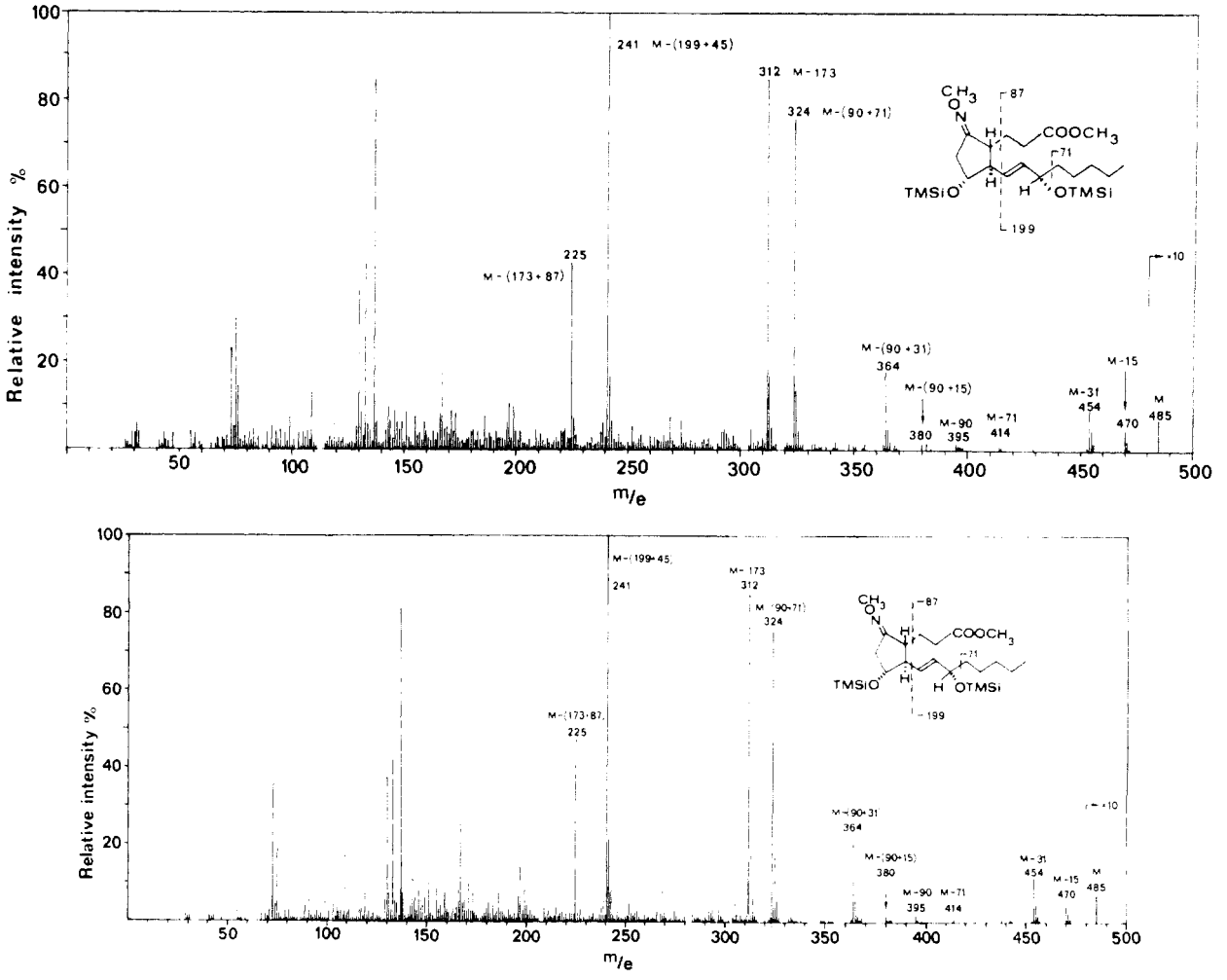

FIGURE 4: Mass spectra of the MO–TMSi derivatives of the methyl esters of the material isolated after incubation of PGE_2 with rat liver mitochondria (upper spectrum) and of the urinary metabolite V (lower spectrum). The *m/e* values above *m/e* 480 have been increased ten times.

tively demonstrated that this metabolite has two keto groups and one hydroxy group. These data strongly indicate a C_{16}-prostaglandin, as the *C* values of the MO-Ac and MO-TMSi derivatives of the methyl esters of metabolite VI and 7α-hydroxy-5,11-diketotetranorprostanoic acid are the same on 1% Se-30 and 2% Epon 1001 columns (Table II).

The mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite VI (Figure 3) showed a molecular ion at *m/e* 442 and ions at *m/e* 427 (M − 15), *m/e* 411 (M − 31), *m/e* 352 (M − 90), and *m/e* 321 [M − (90 + 31)]. The ion at *m/e* 355 can be due to loss of one methoxy group from the methyl ester or one of the methoxime groups (31) and a

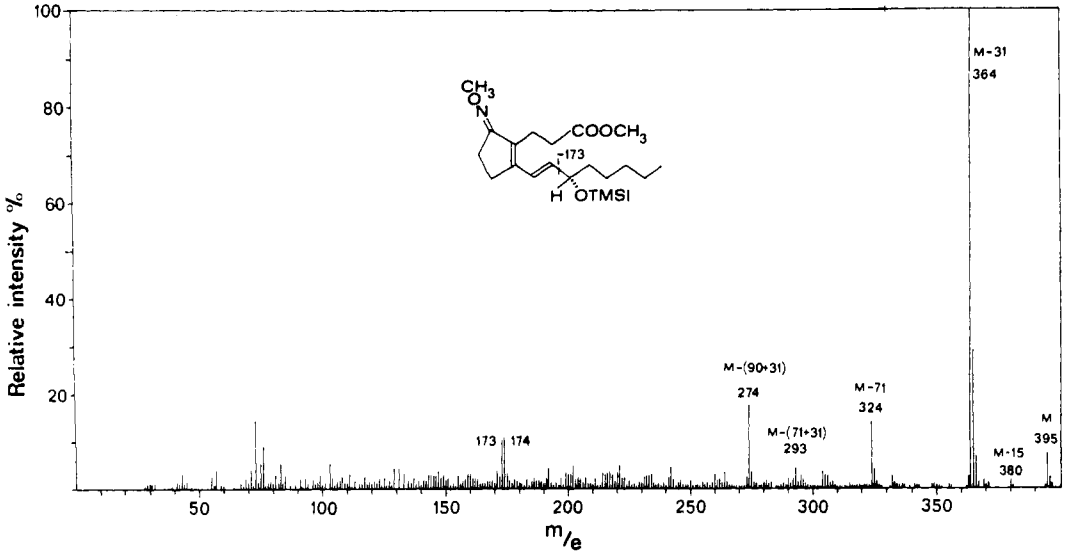

FIGURE 5: Mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite VII.

fragment formed by a McLafferty type of rearrangement at the methoxime group attached to C-11, eliminating the terminal four-carbon fragment (56). These fragmentations are characteristic for prostaglandin derivatives with a methoxime group in the side chain (Gréen, 1969b). However, this ion might also result from loss of the C-4 side chain (87). Additional loss of trimethylsilanol very likely forms the ion at *m/e* 265. The ions at *m/e* 210 and 196 are probably due to loss of trimethylsilanol together with fragments formed by cleavage between C-9 and C-10 (142 mass units) and C-8 and C-9 (156 mass units), respectively. Furthermore the mass spectra of the MO–Ac derivatives of metabolite VI and 7α-hydroxy-5,11-diketotetranorprostanoic acid are identical. The fragmentation of this derivative of 7α-hydroxy-5,11-diketotetranorprostanoic acid on electron impact has been discussed in detail elsewhere (Gréen, 1969b). The data obtained therefore demonstrate that metabolite VI is 7α-hydroxy-5,11-diketotetranorprostanoic acid.

The Structure of Metabolite V. Metabolite V was purified by silicic acid chromatography as such or as the methyl ester *O*-methyloxime derivative and was eluted with ethyl acetate–benzene, 6:4, (v/v) (late) or 4:6, respectively. The gas–liquid chromatography data of the MO–Ac and MO–TMSi derivatives of metabolite V and of 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid were identical as is shown in Table III.

The mass spectrum of the MO–Ac derivative of the methyl ester of metabolite V showed the same prominent ions and ion intensities as the corresponding derivative of 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid. The MO–TMSi derivative of metabolite V was also subjected to gas–liquid chromatography–mass spectrometric analysis. The mass spectrum (Figure 4, lower spectrum) corresponds to that obtained from the same derivative of 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid (Figure 4, upper spectrum) which has been discussed above. The structure and fragmentations were confirmed by mass spectra obtained from the D_3MO–Ac, MO–D_3Ac, and MO–D_9TMSi derivatives of the methyl ester of metabolite V.

The MO–Ac derivative of the methyl ester of metabolite V (obtained from [17,18-^{3}H]PGE_2) was also subjected to oxidative ozonolysis as described in the Experimental Section. The reaction products were treated with diazomethane and run on gas–liquid chromatography giving one radioactive peak at 9.6 *C* (1% Se-30, 117°). That the mass spectrum of the material in this peak was identical with that of methyl 2-acetoxyheptanoate (Hamberg and Samuelsson, 1966) demonstrates that the double bond is present in Δ^9 position. Therefore metabolite V must be 7α,11-dihydroxy-5-ketotetranorprost-9-enoic acid.

The Structure of Metabolite VII. Metabolite VII was purified on silicic acid chromatography, giving one radioactive peak, which was eluted with ethyl acetate–benzene (4:6, v/v). The ultraviolet spectrum of this material showed λ_{max}^{EtOH} at 278 mμ suggesting a PGB compound. No evidence for the presence of material absorbing at 217 mμ was found. The *C* values of the Ac, TMSi, MO–Ac, and MO–TMSi derivatives of the methyl esters, chromatographed on 1% Se-30, were 20.7, 20.2, 21.1, and 20.5 *C*, respectively, indicating a monohydroxyprostaglandin.

The mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite VII is shown in Figure 5. The molecular ion is found at *m/e* 395 and other prominent ions are seen at *m/e* 380 (M − 15), 364 (M − 31), 324 (M − 71), 293 [M − (71 + 31)], 274 [M − (90 + 31)], 174, and 173.

TABLE III: Gas–Liquid Chromatography Data of the MO–Ac and MO–TMSi Derivatives of the Methyl Esters of Metabolite V and 7α,11-Dihydroxy-5-ketotetranorprost-9-enoic Acid, Obtained from Incubation of PGE_2 with Rat Liver Mitochondria, on 1% Se-30 (225°) and 1% EGSS-X (190°) Columns.[a]

Compound	Column	Derivative	*C* Value
Metabolite V	1% Se-30	MO–Ac	22.1
	1% Se-30	MO–TMSi	21.1
	1% EGSS-X	MO–TMSi	23.3
7α,11-Dihydroxy-	1% Se-30	MO–Ac	22.1
5-ketotetranor-	1% Se-30	MO–TMSi	21.1
prost-9-enoic acid	1% EGSS-X	MO–TMSi	23.3

[a] For definition of *C* values, see Chromatographic Methods.

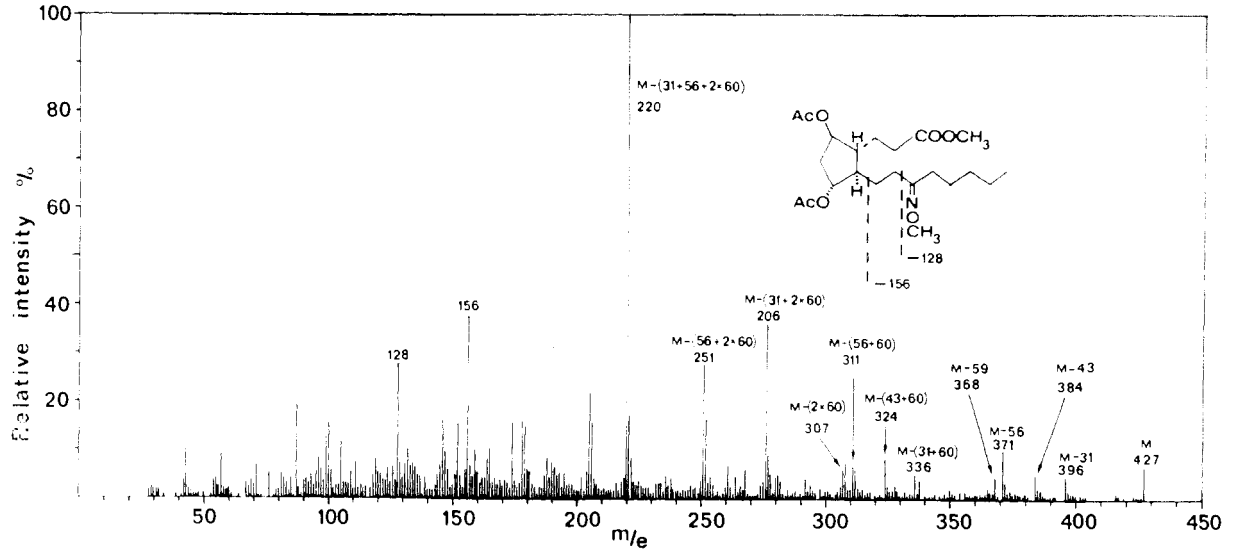

FIGURE 6: Mass spectrum of the MO–Ac derivative of the methyl ester of metabolite IV.

The last ion might be formed by α cleavage at the hydroxy group attached to C-11 with the charge being retained by the six-carbon fragment. These data indicate the structure shown in Figure 5. The mass spectrum of the MO–Ac derivative gave further support to this structure as the principal ions were found at *m/e* 365 (M), 334 (M − 31), 322 (M − 43), 305 (M − 60), 292 [M − (42 + 31)], and 274 [M − (60 + 31)]. The fragmentations were supported by the mass spectrum of the MO–D_3Ac derivative. Furthermore the mass spectrum of the acetyl derivative of the methyl ester of metabolite VII was the same as that obtained from the corresponding derivative of tetranor-PGB_1 published earlier (Hamberg, 1968).

The acetyl derivative of the methyl ester of metabolite VII, obtained after injection of 5,6,8,11,12,14,15-heptatritioprostaglandin E_2, was subjected to oxidative ozonolysis. Two peaks appeared at 7.0 and 9.6 *C* when chromatographed on a 15% silicone column. These *C* values and the mass spectra obtained from the two peaks were identical with those of the dimethyl ester of succinic acid and of methyl 2-acetoxyheptanoate, respectively. Therefore metabolite VII is 11-hydroxy-5-ketotetranorprosta-4(8),9-dienoic acid (tetranor-PGB_1).

The Structure of Metabolite IV. The material in this peak was chromatographed on a silicic acid column as such or as the *O*-methyloxime derivative of the methyl ester and was eluted with ethyl acetate–benzene, 8:2 or 6:4 (v/v), respectively.

Gas–liquid chromatography data for the MO–Ac, Ac, MO–TMSi, and TMSi derivatives of the methyl ester of this metabolite and for the MO–TMSi derivatives of the methyl esters of 5α,7α-dihydroxy-11-ketotetranorprostanoic acid and 5β,7α-dihydroxy-11-ketotetranorprostanoic acid are listed in Table IV. The *C* values of the MO–TMSi derivatives of metabolite IV and the 5β isomer are identical while that of the 5α isomer is different on a 1% EGSS-X column.

The Ac and TMSi derivatives of the methyl esters of metabolite IV were subjected to gas–liquid chromatography–mass spectrometry. The mass spectra were identical with those of the corresponding derivatives of 5β,7α-dihydroxy-11-ketotetranorprostanoic acid which have been published and discussed elsewhere (Hamberg and Samuelsson, 1969b; Granström and Samuelsson, 1969b).

The mass spectrum of the MO–Ac derivative of the methyl ester of metabolite IV (Figure 6) recorded the molecular ion at *m/e* 427, and other ions are seen at *m/e* 396 (M − 31), 384 (M − 43), 371 (M − 56), 368 (M − 59), 336 [M − (31 + 60)], 324 [M − (43 + 60)], 311 [M − (56 + 60)], 307 (M − 2 × 60), 276 [M − (31 + 2 × 60)], 251 [M − (56 + 2 × 60)], and 220 [M − (56 + 31 + 2 × 60)]. The ions at *m/e* 128 and 156 are probably due to $[C(NOCH_3)(CH_2)_4CH_3]^+$ and $[(CH_2)_2C(NOCH_3)(CH_2)_4CH_3]^+$, respectively. The proposed eliminations were supported by the mass spectrum of the D_3MO–Ac derivative. Additional evidence that metabolite IV must be 5β,7α-dihydroxy-11-ketotetranorprostanoic acid was obtained from the fragmentation in the mass spectrometer of the MO–TMSi and MO–D_9TMSi derivatives of the methyl ester of metabolite IV. These data demonstrate that metabolite IV is 5β,7α-dihydroxy-11-ketotetranorprostanoic acid.

TABLE IV: *C* Values of Some Derivatives of the Methyl Ester of Metabolite IV and the MO–TMSi Derivatives of the Methyl Esters of 5β,7α-Dihydroxy-11-ketotetranorprostanoic Acid and 5α,7α-Dihydroxy-11-ketotetranorprostanoic Acid Obtained on 1% Se-30 (210°) and 1% EGSS-X (190°) Columns.[a]

Compound	Column	Derivative	*C* Value
Metabolite IV	1% Se-30	MO–Ac	21.8
	1% Se-30	Ac	21.7
	1% Se-30	MO–TMSi	20.5
	1% Se-30	TMSi	20.5
	1% EGSS-X	MO–TMSi	22.5
5β,7α-Dihydroxy-11-ketotetranorprostanoic acid	1% Se-30	MO–TMSi	20.5
	1% EGSS-X	MO–TMSi	22.5
5α,7α-Dihydroxy-11-ketotetranorprostanoic acid	1% EGSS-X	MO–TMSi	22.8

[a] For definition of *C* values, see Chromatographic Methods.

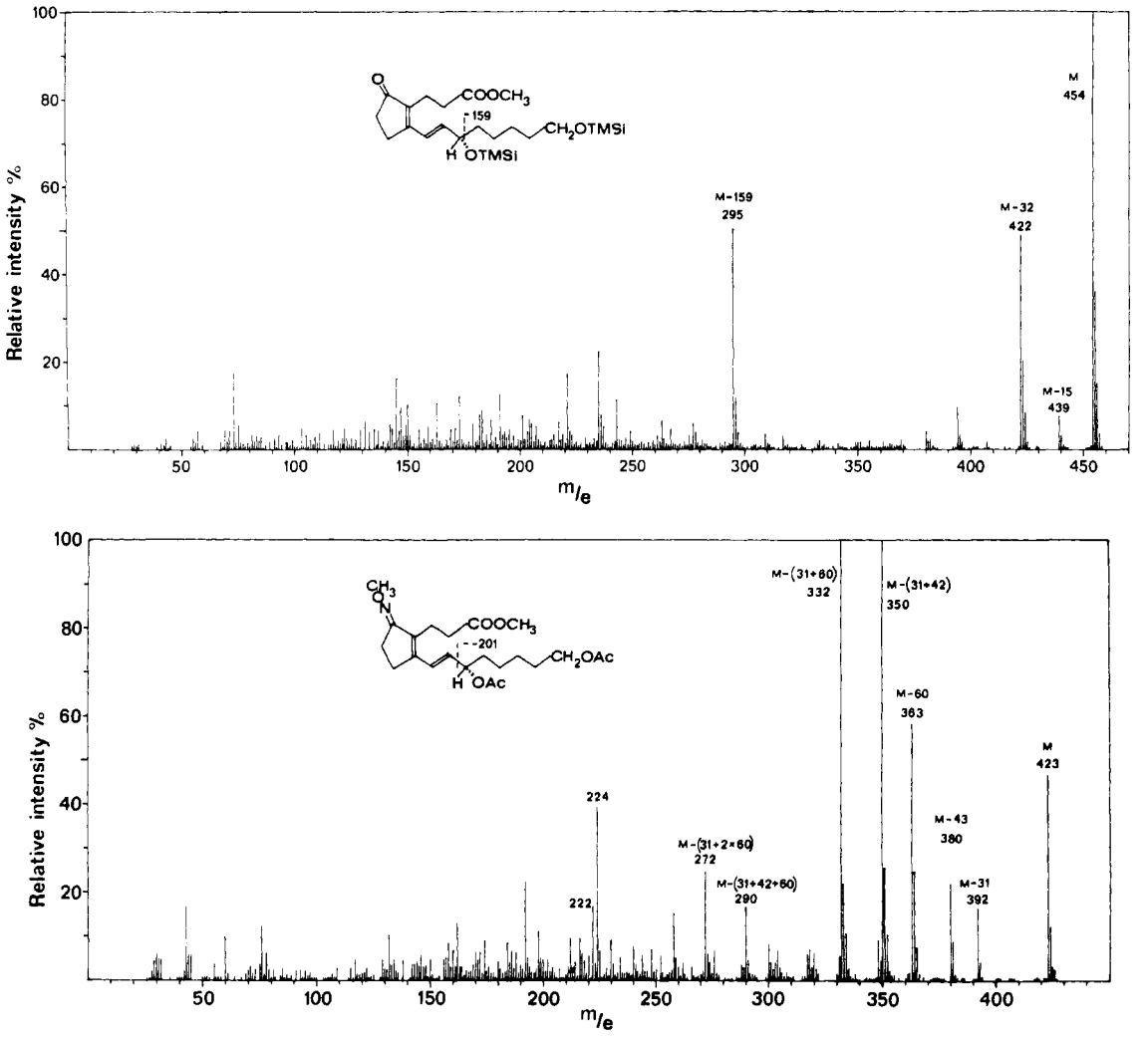

FIGURE 7: Mass spectrum of the TMSi (upper spectrum) and the MO–Ac (lower spectrum) derivatives of the methyl ester of metabolite IIIb:2.

The Structure of Metabolites IIIb:1 and IIIb:2. The Ac and TMSi derivatives were prepared from peak IIIb. On gas–liquid chromatography the acetylated material showed two radioactive compounds at 23.6 and 24.5 *C* when chromatographed on 1% Se-30. The metabolites were called IIIb:1 and IIIb:2, and the proportions between them were about 1:4. The TMSi derivative gave two peaks at 22.6 *C* (IIIb:1) and 23.4 *C* (IIIb:2). The difference between the last two compounds (0.8 *C*) is the same as that found between the corresponding derivatives of 19- and 20-hydroxy-PGB_1 (Israelsson *et al.*, 1969). The MO–Ac and MO–TMSi derivatives of the material in peak IIIb were also run on gas–liquid chromatography. However the methoxime isomer of metabolite IIIb:2 which had the lowest *C* value (the quantitatively less important) did not separate from the main isomer of metabolite IIIb:1 when chromatographed on 1% Se-30. Therefore it was not possible to obtain a pure mass spectrum from the methoximes of metabolite IIIb:1. The *C* values of the MO–TMSi and MO–Ac derivatives of the methyl ester of metabolite IIIb:2 were 23.6 and 24.2 *C* (1% Se-30, 225°).

The mass spectrum, obtained from the TMSi derivative of the methyl ester of metabolite IIIb:2 (Figure 7, upper spectrum), recorded the molecular ion at *m/e* 454; ions due to loss of 15 (*m/e* 439), 32 (*m/e* 422), and 159 (*m/e* 295) are seen. The ion at *m/e* 295 is probably formed by cleavage between C-11 and C-12 with elimination of the five-carbon fragment with one trimethylsilyloxy group. This spectrum is consistent with the structure shown in Figure 7 and the fragmentation is similar to that of the TMSi derivative of the methyl ester of 20-hydroxyprostaglandin B_1 (Israelsson *et al.*, 1969). The more complex mass spectrum of the acetyl derivative of metabolite IIIb:2 showed prominent ions at *m/e* 394 (M), 363 (M − 31), 352 (M − 42), 334 (M − 60), 320, 302, 278, 274 (M − 2 × 60), 260, 195, and 163.

In the mass spectrum of the MO–Ac derivative (Figure 7, lower spectrum) the molecular ion is found at *m/e* 423, and ions due to loss of 31 (*m/e* 392), 43 (*m/e* 380), 60 (*m/e* 363), 42 + 31 (*m/e* 350), 60 + 31 (*m/e* 332), 60 + 42 + 31 (*m/e* 290), and 2 × 60 + 31 (*m/e* 272) are seen. The spectra of the D_3MO–Ac and MO–D_3Ac derivatives not only support the fragmentations proposed above but also show that the fragments at *m/e* 222 and 224 have lost the deuterium label of two acetoxy groups while retaining the deuterium in the methoxime group. The molecular ions of the D_3MO–Ac and MO–D_3Ac derivatives, found at *m/e* 426 and 429, demonstrate the presence of one keto group and two hydroxy groups in metabolite IIIb:2. On electron impact the MO–TMSi derivative of the methyl ester of metabolite IIIb:2 shows ions of high intensities at *m/e* 483

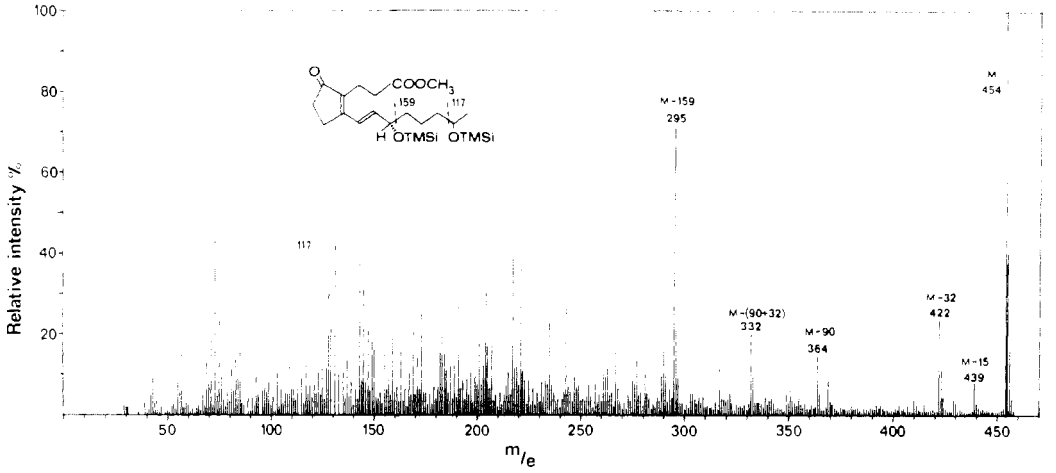

FIGURE 8: Mass spectrum of the TMSi derivative of the methyl ester of metabolite IIIb:1.

(M), 468 (M − 15), 452 (base peak, M − 31), 362 [M − (90 + 31)], 324 (M − 159), and 272 [M − (2 × 90 + 31)]. The elimination of 159 has been discussed under the TMSi derivative.

That the TMSi and Ac derivatives of the methyl ester of metabolite IIIb:2 were stable during gas–liquid chromatography indicated that either the hydroxy group in the five-membered ring had been eliminated or the ring ketone had been reduced to a hydroxy group (*cf.* Gréen, 1969b). The mass spectral data strongly indicate the structure shown in Figure 7.

In Table V the *C* values of the MO–TMSi and MO–Ac derivatives of metabolite VII, IIIb:2, and IIIa are compared. Usually a carbomethoxy group adds about 3.0, a trimethylsilyloxy group about 1.9, and an acetoxy group about 2.5 to the *C* value. The significant difference between the values of the two derivatives of metabolite VII and IIIb:2 (3.1 *C* in both cases) indicates that metabolite IIIb:2 is a ω-1-hydroxy compound since the *C* values and mass spectra rule out a dicarboxylic acid. As can be seen in Table VI this is also supported by the *C* values of the trimethylsilyl ether derivatives of the methyl esters of prostaglandin B_1, 19- and 20-hydroxyprostaglandin B_1 (from Israelsson *et al.*, 1969), and the corresponding derivatives of metabolite IIIb:2, IIIb:1, and VII. Thus the differences between the *C* values of PGB_1 and 20-hydroxy-PGB_1 and between metabolite VII and IIIb:2 are 3.1 and 3.2 *C* respectively, while the difference between PGB_1 and 19-hydroxy-PGB_1 is 2.3 *C*. Moreover the *C* values of 20-hydroxy-PGB_1 and metabolite IIIb:2 show a difference of 3.8 *C* which is in accordance with carbon chains of 20 and 16 carbons, respectively.

The data presented strongly indicate that metabolite IIIb:2 is 11,16-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid. The final identification was obtained by chemical degradation which is described below.

The behavior of metabolite IIIb:1 on reversed-phase partition and silicic acid chromatography indicates a structure similar to IIIb:2. The mass spectrum of the TMSi derivative of the methyl ester of metabolite IIIb:1 (Figure 8) recorded the ion with highest *m/e* value at *m/e* 454 and ions due to elimination of 15 (*m/e* 439), 32 (*m/e* 422), 90 (*m/e* 364), 90 + 32 (*m/e* 332), and 159 (*m/e* 295). The molecular ion (*m/e* 454) and the ions at *m/e* 439, 422, and 295 are similar to those of the corresponding derivative of metabolite IIIb:2 (Figure 7). The ion at *m/e* 117 indicates that one trimethylsilyloxy group probably is situated in ω-2 position (*cf.* Israelsson *et al.*, 1969). The mass spectrum of the acetyl derivative was similar to that of metabolite IIIb:2, *i.e.*, prominent ions were seen at *m/e* 394 (M), 363 (M − 31), 352 (M − 42), 334

TABLE V: Comparison between the *C* Values of the MO–TMSi and MO–Ac Derivatives of the Methyl Esters of Metabolite VII, IIIa, and IIIb:2 Obtained on a 1% Se-30 Column.[a]

Derivative	*C* Values		
	Metabolite VII	Metabolite IIIa	Metabolite IIIb:2
MO–TMSi	20.5	23.3	23.6
MO–Ac	21.1	23.7	24.2

[a] For definition of *C* values, see Chromatographic Methods.

TABLE VI: Comparison between the *C* Values of the TMSi Derivatives of the Methyl Esters of 19-Hydroxy-PGB_1, PGB_1, 20-Hydroxy-PGB_1 (*cf.* Israelsson *et al.*, 1969), Metabolite IIIb:2, Metabolite VII, and Metabolite IIIb:1 Obtained on a 1% Se-30 column.[a]

Compound	Derivative	*C* Value	Difference in *C* Value
19-OH-PGB_1	TMSi	26.4	
			2.3
PGB_1	TMSi	24.1	
			3.1
20-OH-PGB_1	TMSi	27.2	
			3.8
Metabolite IIIb:2	TMSi	23.4	
			3.2
Metabolite VII	TMSi	20.2	
			2.4
Metabolite IIIb:1	TMSi	22.6	

[a] For definition of *C* values, see Chromatographic Methods.

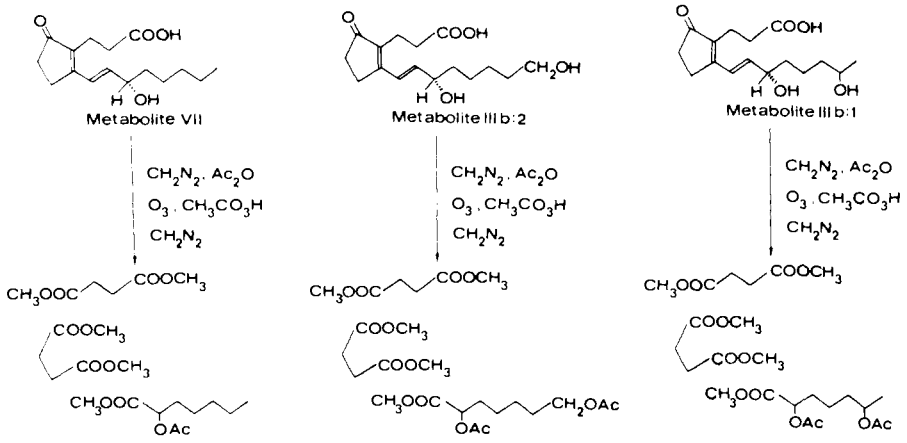

FIGURE 9: Chemical degradations of metabolite VII, IIIb:2, and IIIb:1.

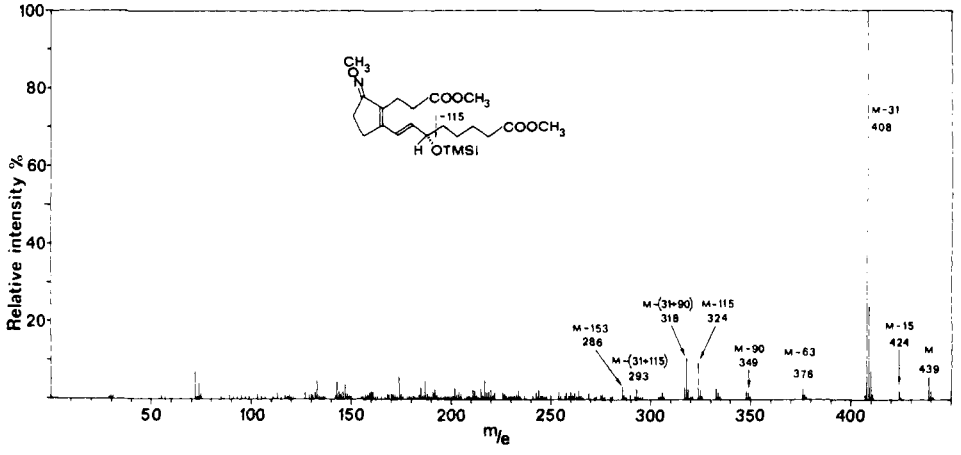

FIGURE 10: Mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite IIIa.

(M − 60), 320, 302, 274 (M − 2 × 60), 260, 195, and 163. The similarity between the mass spectra of the Ac and TMSi derivatives of metabolite IIIb:1 and IIIb:2 indicates a rather small structural difference. The gas–liquid chromatographic data (Table VI) show that metabolite IIIb:1 probably is 11,15-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid.

Since metabolite IIIb:1 and IIIb:2 did not separate on reversed-phase partition or silicic acid chromatography, a mixture of the acetyl derivative of the methyl esters of metabolite IIIb:1 and IIIb:2, obtained after injection of 5,6,8,11,12,-14,15-heptatritioprostaglandin E_2, was subjected to oxidative ozonolysis (Figure 9). Three peaks appeared at 7.0, 12.9, and 13.6 *C* (1% Se-30, 115–130°). The mass spectra of those peaks were the same as those obtained from dimethyl succinate, methyl 2,6-diacetoxyheptanoate, and methyl 2,7-diacetoxyheptanoate (Israelsson *et al.*, 1969). No evidence for the presence of fragments from a metabolite with a Δ^6 double bond was seen. The data, therefore, demonstrate that metabolite IIIb:2 is 11,16-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid and metabolite IIIb:1 is 11,15-dihydroxy-5-ketotetranorprosta-4(8),9-dienoic acid.

The Structure of Metabolite IIIa. The *C* values of the Ac, MO–Ac, TMSi, and MO–TMSi derivatives of the methyl ester of this metabolite (1% Se-30, 225°) were 23.7, 23.7, 23.1, and 23.3 *C* (see also Table V). As pointed out for metabolite IIIb, the stability of the TMSi derivative during gas–liquid chromatography indicates a prostaglandin dehydrated in the five-membered ring or a prostaglandin with two ring hydroxy groups.

The mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite IIIa (Figure 10) located the molecular ion at *m/e* 439 and prominent ions due to elimination of 15 (*m/e* 424), 31 (*m/e* 408), 63 (*m/e* 376), 90 (*m/e* 349), 115 (*m/e* 324), 31 + 90 (*m/e* 318), 31 + 115 (*m/e* 293), and 153 (*m/e* 286). The loss of 63 could be due to elimination of a methoxy radical and methanol from the two carbomethoxy groups. This fragmentation has been demonstrated for dimethyl esters of shorter acids (Howe and Williams, 1968). The additional loss of trimethylsilanol (90 mass units) presumably forms the ion at *m/e* 286, while the ion at *m/e* 324 is probably due to cleavage between C-11 and C-12 eliminating the five-carbon fragment with the carbomethoxy group.

On electron impact, the TMSi derivative of the methyl ester gave a molecular ion at *m/e* 410 as well as ions due to eliminations of 15 (*m/e* 395), 31 (*m/e* 379), 32 (*m/e* 378), 63 (*m/e* 347), 90 + 15 (*m/e* 305), and 115 (*m/e* 295). In addition to the molecular ion at *m/e* 409, the mass spectrum of the MO–Ac derivative of the methyl ester of metabolite IIIa showed prominent ions at *m/e* 378 (M − 31), 366 (M − 43), 349 (M − 60), 336 (M − 73), 318 [M −(31 + 60)], 286 [M − (31 + 32 + 60)], 276 M − (60 + 73), 258, 244, 224, and 192. In order to facilitate the interpretation, mass spectra were obtained from corresponding derivatives labeled with deuterium in the methoxime, acetyl, and methyl ester groups.

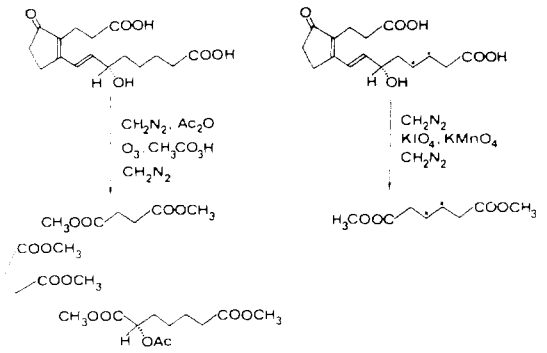

FIGURE 11: Chemical degradations of metabolite IIIa.

The molecular ions in these spectra (*m/e* 412, 412, and 415) demonstrated the presence of one keto, one hydroxyl, and two carboxyl groups in metabolite IIIa. The spectra of the deuterium-labeled compounds showed that when the ion at *m/e* 336 is formed the deuterium atoms in the methoxime group and two deuterium atoms from the acetoxy group are lost. Therefore this ion is probably formed by elimination of CH_3O (31) from the methoxime and CH_2CO (42) from the acetoxy group. The isotope experiments also show that formation of the ion at *m/e* 286 very likely occurs through loss of acetic acid, one methoxy radical, and methanol. The ion at *m/e* 276 probably results from loss of acetic acid and CH_2COOCH_3 (or $CH_2CO + CH_3O$) as indicated by the deuterium experiments. The mass spectrum of the Ac derivative of the methyl ester of metabolite IIIa also supported the structure, showing prominent ions at *m/e* 380 (M), 349 (M − 31), 338 (M − 42), 320 (M − 60), 307 (M − 73), 306, 289 [M − (60 + 31)], 275 (M − 105), 215 [M − (105 + 60)], 195, and 163.

In order to obtain unequivocal evidence for the structure, the acetyl derivative of the methyl ester of metabolite IIIa, obtained after injection of 5,6,8,11,12,14,15-heptatritioprostaglandin E_2, was subjected to oxidative ozonolysis followed by treatment with diazomethane (Figure 11). Gas–liquid chromatography of this material on 1% Se-30 gave two peaks at 7.0 and 12.6 *C*. The *C* values of the first peak on 1% Se-30 and 15% silicone columns were identical with these of the dimethyl ester of succinic acid. The gas–liquid chromatographic and mass spectrometric data of the second peak indicated that this product was the dimethyl ester of monoacetoxypimelic acid. Thus in the mass spectrum prominent ions were found at *m/e* 215 (M − 31), 204 (M − 42), 187 (M − 59), 173 (M − 73), 155 [M − (60 + 31)], 145 (M − 101), 113, 76, and 43. Therefore the methyl ester of metabolite IIIa, obtained by injecting [17,18-^{3}H]PGE_2 into rats, was oxidized with periodate–permanganate (Änggård *et al.*, 1965). After treatment with diazomethane (Figure 11), the products were subjected to gas–liquid chromatography on a 15% silicone column (180°). One peak of radioactivity appeared at 9.1 *C*. Both the *C* values and the mass spectra of this compound and the dimethyl ester of adipic acid were identical. Thus the data clearly demonstrate that metabolite IIIa is 11-hydroxy-5-ketotetranorprosta-4(8),9-diene-1,16-dioic acid (Figure 15).

The Structure of Metabolite IIb. As noted previously the *O*-methyloxime derivative of the methyl ester of metabolite IIb was eluted from a silicic acid column with ethyl acetate–benzene (8:2, v/v). This material was used for preparation of derivatives suitable for gas–liquid chromatography and gas–liquid chromatography–mass spectrometry. The MO–Ac derivatives (deuterated or not) were further purified on silicic acid columns and were eluted with ether–hexane (6:4, v/v). The difference between *C* values (24.1 and 25.1 *C*) of the MO–TMSi and MO–Ac derivatives of the methyl ester of metabolite IIb, obtained on a 1% Se-30 column (225°), indicates a dihydroxy compound. The methyl ester of the metabolite was reduced with excess sodium borohydride in methanol and the acetyl derivative of this product gave a rather broad peak at 26.0 *C* (227°, 1% Se-30). Two keto groups had thus been reduced by the borohydride.

In the mass spectrum of the MO–Ac derivative of the methyl ester of metabolite IIb (Figure 12), the ion with the highest *m/e* value obtained is found at *m/e* 470 (M). Other prominent ions are seen at *m/e* 439 (M − 31), 411 (M − 59), 379 [M − (60 + 31)], 325 [M − (114 + 31)], 320 [M − (60 + 59 + 31)], 265 [M − (114 + 60 + 31)], 210 [M − (200 + 60)], and 196 [M − (214 + 60)]. The mass spectra of the D_3MO–Ac and MO–D_3Ac derivatives both showed molecular ions at *m/e* 476 clearly demonstrating the presence of two keto groups and two hydroxyl groups. The spectra of the deuterated derivatives demonstrate that formation of the ion at *m/e* 325 involves loss of the deuterium label of one acetoxy and one methoxime group. This ion is probably formed by a combination of β cleavage at the methoxime group at C-11, eliminating $CH_2{=}CH(CH_2)_2OCOCH_3$ (114), and loss of CH_3O from one methoxime group. Additional loss of acetic acid forms the ion at *m/e* 265 (see above metabolite VI). The ions at *m/e* 196 and 210 have lost the deuterium label of two acetoxy groups and one methoxime group. Therefore they are probably formed by eliminating acetic acid and by splitting between C-8 and C-9 and between C-9 and C-10, respectively (Figure 12).

In Figure 13 some relevant ions from the mass spectra of the MO–Ac derivatives of the methyl esters of metabolite VI, IIa, and IIb are shown for comparison. As can be seen β cleavage at the methoxime attached to C-11 or cleavage between C-8 and C-9 give rise to ions common to the three compounds.

The mass spectrum of the MO–TMSi derivative of the methyl ester of metabolite IIb showed a molecular ion at *m/e* 350 and prominent ions due to elimination of 15, 31, 90, 90 + 31 (base peak), 114 + 31, 144 + 90 + 31, 230 + 90, and 244 + 90. Formation of the four last ions involves loss of fragments (144, 230, 244) that are 30 mass units heavier, or the difference between an acetoxy and a trimethylsilyloxy group, than the corresponding MO–Ac derivative. Therefore the elimination of 144 mass units probably results from β cleavage at the C-11 methoxime group while loss of 244 and 230 mass units is caused by cleavage between C-8 and C-9 and between C-9 and C-10 as discussed above. These data strongly support the structure shown in Figure 12. That β cleavage with hydrogen transfer to the charge-retaining ion occurs indicates that the hydroxy group of the C-8 side chain is attached to C-15 or -16. In Table VII the *C* values of the MO–TMSi derivatives of metabolite VI, IIa, and IIb are compared. The differences between the MO–TMSi and MO–Ac derivatives of metabolite VI and IIb are 3.1 and 3.6 *C*, respectively. The high *C* values of these derivatives (1.2 and 1.1 *C* more than the theoretical *C* values) of metabolite IIb strongly indicate that the hydroxyl group of the C-8 side chain is situated in the ω-1 position (*cf.* Table VI). These data demonstrate that metabolite IIb is 7,16-dihydroxy-5,11-diketotetranorprostanoic acid.

The Structure of Metabolite IIa. The methoxime derivative

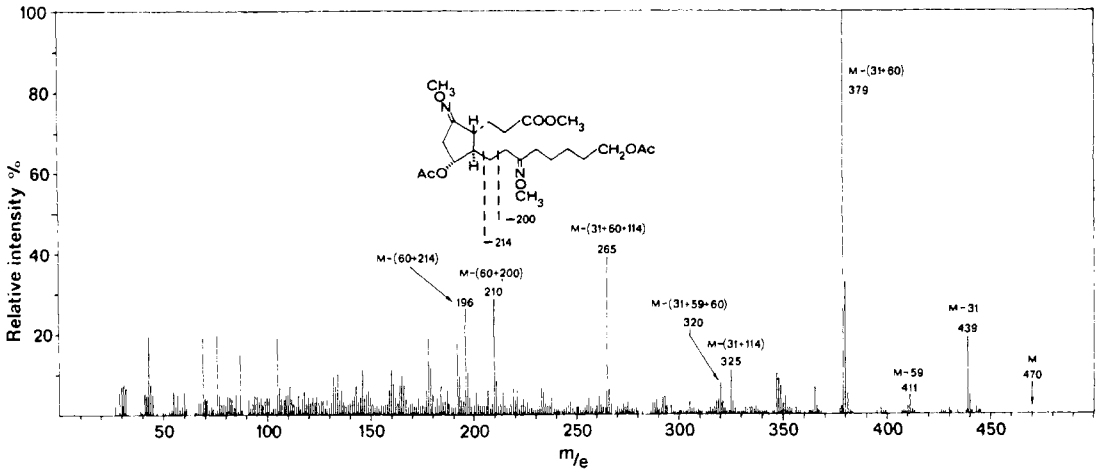

FIGURE 12: Mass spectrum of the MO–Ac derivative of the methyl ester of metabolite IIb.

of the methyl ester of metabolite IIa was eluted from the silicic acid column with ethyl acetate–benzene (4:6, v/v) as described above. The MO–Ac derivatives were further purified on another silicic acid column before gas–liquid chromatography–mass spectrometry and were then eluted with ether–hexane (6:4, v/v).

The *C* values of 24.5 and 24.0 for the MO–Ac and MO–TMSi derivatives of the methyl ester of this metabolite when chromatographed on 1% Se-30 (225°) indicates a monohydroxy compound. Since the molecular ions in the mass spectra of the MO–Ac and D_3MO–Ac derivatives of the methyl ester were seen at *m/e* 456 and 462 and since the molecular ion of the MO–Ac derivative of the methyl ester deuterated in the methyl ester groups was found at *m/e* 462, metabolite IIa is a tetranorprostaglandin with two keto, one hydroxy, and two carboxy groups. The differences between the *C* values of the MO–Ac and MO–TMSi derivatives of metabolite VI and IIa (3.0 *C*) are consistent with this (Table VII). The mass spectrum of the MO–Ac derivative of the methyl ester (Figure 14) also showed ions at *m/e* 425 (M − 31), 397 (M − 59), 396 (M − 60), 365 [M − (60 + 31)], 334 [M − (60 + 2 × 31)], 325 [M − (100 + 31)], 265 [M − (100 + 31 + 60)], 224 [M − (172 + 60)], 210 [M − (186 + 60)], and 196 [M − (200 + 60)]. Fragmentations involving loss of 100 mass units are due to β cleavage at the methoxime group at C-11 (*cf.* metabolites IIb and VI). Eliminations of 172, 186, and 200 mass units are probably caused by cleavages between C-10 and C-11, C-9 and C-10, and C-8 and C-9. The mass spectra of the deuterated derivatives supported the proposed fragmentations (see also Figure 13). The MO–TMSi derivative of the methyl ester of metabolite IIa was also subjected to gas–liquid chromatography–mass spectrometric analysis. The mass spectra of the MO–Ac and MO–TMSi derivatives of metabolite IIa are identical with those obtained from the corresponding derivatives of the urinary metabolite of PGE_2 in man (Hamberg and Samuelsson, 1969a). The methyl ester of metabolite IIa was reduced with excess sodium borohydride in methanol and acetylated. This derivative gave a somewhat broad peak at 25.5 *C* when chromatographed on 1% Se-30 (227°) (*cf.* Hamberg and Samuelsson, 1969a). This *C* value as well as the mass spectrum of this derivative corroborates the structure given in Figure 14. Thus metabolite IIa is identical with the metabolite of PGE_2, isolated from human urine, *i.e.*, 7-hydroxy-5,11-diketotetranorprosta-1,16-dioic acid (Figure 15).

Urinary Products from Metabolite IV. After injection of [17,18-^{3}H]PGE_2 (0.5 mg/rat, 1.5 μCi/μmole) into rats, metabolite IV was isolated and purified on silicic acid chromatography. Part of this material was identified on gas–liquid chromatography as the MO–TMSi derivative (1% Se-30 and 1% EGSS X). Metabolite IV (0.45 μCi) was injected intravenously into one female rat, and the urine was collected during 21 hr, extracted, and chromatographed as described

TABLE VII: Comparison between the *C* Values of the MO–TMSi and MO–Ac Derivatives of the Methyl Esters of Metabolite VI, IIa, and IIb Obtained on a 1% Se-30 Column.[a]

Derivative	*C* Values		
	Metabolite VI	Metabolite IIa	Metabolite IIb
MO–TMSi	21.0	24.0	24.1
MO–Ac	21.5	24.5	25.1

[a] For definition of *C* values, see Chromatographic Methods.

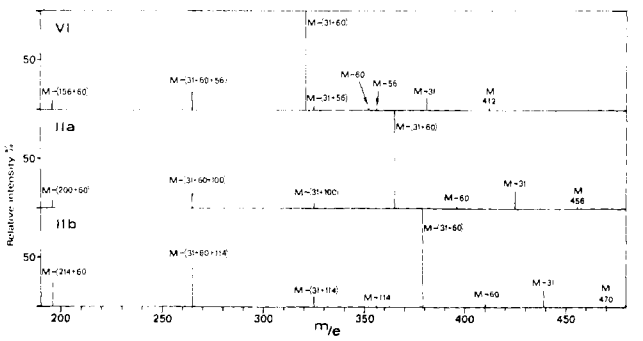

FIGURE 13: Comparison between some relevant ions from the NO-Ac derivatives of the methyl esters of metabolites VI, IIa, and IIb. Fragmentations involving β cleavage at the methoxime group at C-11 (eliminating 56, 100, or 114 mass units, respectively) or cleavage between C-8 and C-9 (eliminating 156, 200, or 214 mass units, respectively) gives rise to ions appearing at the same *m/e* value, *i.e.*, 356, 325, 265, and 196 in the three spectra.

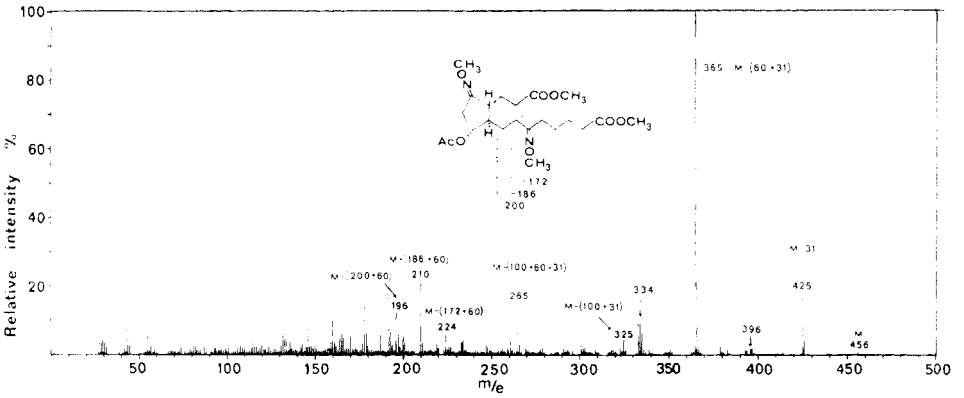

FIGURE 14: Mass spectrum of the MO-Ac derivative of the methyl ester of metabolite IIa.

in the Experimental Section. About 57% of the injected radioactivity was excreted in the urine, 5% stayed in the water phase, while 52% was extracted with 1-butanol. In the reversed-phase partition chromatogram (system D) about 5% of injected radioactivity was found in peak I and 45% in peak IV. Metabolite IV was at this stage further identified by gas–liquid chromatography of the TMSi, MO-TMSi, and MO-Ac derivatives of the methyl ester on 1% Se-30 and of the MO-TMSi derivative on 1% EGSS-X.

Urinary Products from Metabolite V. Metabolite V was isolated in the same experiment as metabolite IV and was injected intravenously into one rat (0.7 μCi) after purification on a silicic acid column and identification on gas–liquid chromatography as the MO-TMSi derivative (1% Se-30, 21.1 *C*). After 4 hr 66% and after 22 hr 95% of injected activity had been excreted. The urine was collected, extracted (about 10% of excreted activity stayed in the water phase), and chromatographed as described above. The metabolites were identified by the retention volumes on reversed-phase partition chromatography and by gas–liquid chromatography data. About 10% of excreted radioactivity was recovered as metabolite I (not subjected to gas–liquid chromatography), 6% as metabolite IIIa, 3% as metabolite IIIb:1, 11% as metabolite IIIb:2, 45% as metabolite V, and 8% as metabolite VII. Metabolites IIIa and IIIb were identified as TMSi derivatives of the methyl esters, metabolite V as MO-TMSi and MO-Ac derivatives, and metabolite VII as TMSi and MO-TMSi derivatives on a 1% Se-30 column. No transformation into metabolite VI could be demonstrated.

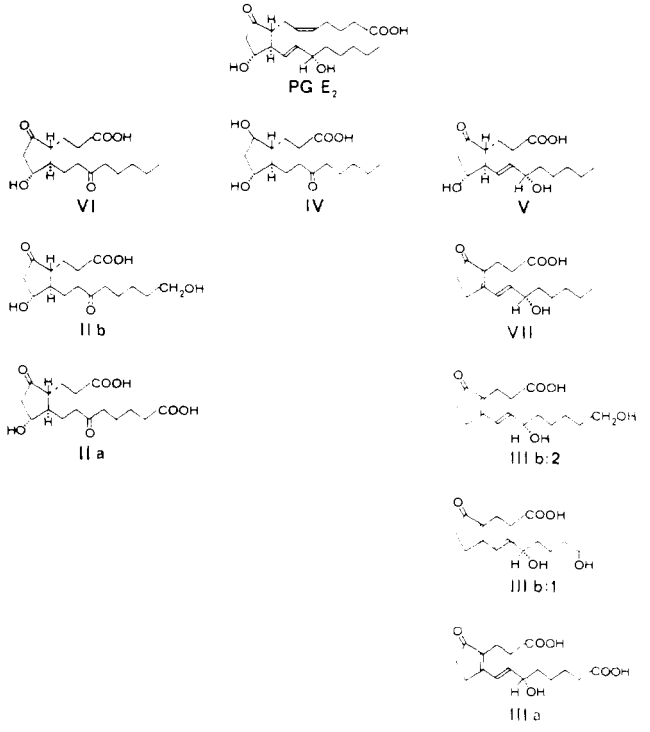

FIGURE 15: The structures of PGE_2 and the urinary metabolites identified in this paper.

Urinary Products from Metabolite VI. Metabolite VI was isolated, purified, and identified by gas–liquid chromatography. After injecting 0.15 μCi intravenously into one female rat, the urine was collected during 22 hr, extracted, and chromatographed. About 75% of the injected radioactivity was excreted of which 55% was extracted with butanol and 20% was left in the water phase. The butanol extract was run on reversed-phase partition chromatography as described above and the metabolites were identified by their retention volumes on reversed phase partition chromatography and by their *C* values on gas–liquid chromatography (except peak I). About 6% of the injected radioactivity appeared in peak I, about 15% in peak II, and about 30% in peak VI. Metabolites IIa and IIb were identified by their *C* values as MO-TMSi derivatives on a 1% Se-30 column and metabolite VI was identified by the *C* values of the MO-TMSi derivative on a 1% Se-30 and on a 2% Epon 1001 column. No formation of metabolite IV could be demonstrated.

Urinary Products from Metabolite VII. Metabolite VII was isolated, purified, and identified as described above. After 0.25 μCi was injected intravenously into one rat, 91% of the injected activity was excreted after 4 hr, and 98% after 22 hr. Extraction (about 5% of the radioactivity stayed in the water phase) and reversed-phase partition chromatography were performed as described above. The products were characterized by the retention volumes on reversed phase partition chromatography and the *C* values as TMSi derivatives of the methyl esters on 1% Se-30. About 18% of the excreted activity was recovered as metabolite IIIa, 55% as metabolite IIIb:1 + IIIb:2 (the proportion was about 1:20), and 18% as unchanged metabolite VII.

Discussion

In this paper the structures of nine metabolites of intravenously administered prostaglandin E_2 in rat urine have been determined and the transformation of four of these

metabolites have been studied. Metabolites IIa and IV were previously found in urine from man and guinea pig while metabolites VI and VII have been isolated after incubation of 11-hydroxy-9,15-diketoprostanoic acid and PGB_1 with rat liver mitochondria (Hamberg, 1968). Tetranor-PGE_1 (metabolite V) has been tentatively identified (Hamberg, 1968).

Some gas–liquid chromatography data for different derivatives of the metabolites identified in this paper are summarized in Table VIII.

The excreted amounts of radioactive products and the proportions between the metabolites were rather constant in the different injection series, when a high dose of PGE_2 (500 μg) was administered to each rat. After injection of [17,18-^{3}H]PGE_2 with a very high specific activity (about 7 mCi/μmole, less than 0.5 nmole/rat) changes in the proportions between the metabolites were seen. Thus metabolite IIa and IIb increased from 5–8 to about 18%, while metabolite IIIa, IIIb:1, and IIIb:2 decreased from 13–15 to about 6%. An increase in the percental amount of metabolite V (7–8 to about 13%) was also seen. All nine metabolites could thus be isolated in this experiment indicating that they might normally be formed from endogenous PGE_2 released into the blood stream.

Since a structural feature common to all metabolites is that the carbon chain consists of 16 carbon atoms (*cf.* Hamberg, 1968), some or all of the PGE_2 metabolites isolated in this paper may be identical with metabolites of PGE_1 for the extra double bond of PGE_2 is in Δ^5 position (*cf.* Samuelsson, 1964). Furthermore metabolites VII, IIIa, IIIb:1, and IIIb:2 might possibly be formed from prostaglandins A and B.

Both ω-1- and ω-2-hydroxylated tetranor-PGB_1 was found (metabolites IIIb:1 and IIIb:2) whereas only ω-1-hydroxylated 7α-hydroxy-5,11-diketotetranorprostanoic acid could be demonstrated. However the major methoxime isomers of the MO–Ac and MO–TMSi derivatives of the methyl ester of metabolite IIb were, on gas–liquid chromatography (1% Se-30), preceded by a rather broad small peak (about one-tenth of the major peak) which, in addition to the minor isomers of metabolite IIb, might contain a small amount of ω-2-hydroxylated 7α-hydroxy-5,11-diketotetranorprostanoic acid. ω Oxidation of fatty acids in different species has been demonstrated by many authors. The formation of ω-hydroxylated and dicarboxylic metabolites from PGE_2 in the rat is consistent with earlier findings. Bergström *et al.* (1954) demonstrated that [1-^{14}C]2,2-dimethylstearic acid is converted to 2,2-dimethyladipic acid in the rat. Later it was shown (Preiss and Bloch, 1964) that rat liver preparations converted [^{14}C]stearic acid into labeled 17-hydroxyoctadecanoic acid, 18-hydroxyoctadecanoic acid, and octadecane-1,18-dioic acid. Evidence for the mechanism has been obtained by Robbins (1968), who demonstrated that decanoic acid is transformed to decanedioic acid in rat liver homogenate *via* 10-hydroxydecanoic acid and 10-oxodecanoic acid (Robbins, 1968).

Depending on the functional groups at C-5 and -11 and the presence of the Δ^9 double bond, the isolated metabolites can be divided into three groups: (1) metabolites IIa, IIb, and VI with keto groups at C-5 and C-11 and with no double bond (Figure 15); (2) metabolite IV with one hydroxy group at C-5 and one keto group at C-11 and no double bond; and (3) metabolites IIIa, IIIb:1, IIIb:2, V, and VII with one keto group at C-5, one hydroxy group at C-11, and with the Δ^9 double bond intact.

Formation of metabolite VI from PGE_2 involves three reactions: (1) oxidation of the alcohol group at C-15 to a keto group, (2) saturation of the Δ^{13} double bond in the PGE_2 molecule, and (3) two steps of β oxidation. These reactions have previously been observed *in vitro* (Änggård *et al.*, 1965; Hamberg, 1968). The β-oxidation steps are probably preceded by the oxidation of the alcohol group at C-15 in the PGE_2 molecule and saturation of the Δ^{13} double bond since metabolite V (tetranor PGE_1) is not transformed into metabolite VI and since tetranor PGE_1 is a poor substrate for the prostaglandin dehydrogenase (Nakano *et al.*, 1969). Injection of metabolite VI into a rat demonstrated that it can be ω hydroxylated leading to metabolite IIb which is probably further oxidized to metabolite IIa. Metabolite VI is also transformed into more polar material.

After the injection of radioactive metabolite V or VI formation of metabolite IV could not be demonstrated. This finding indicates that the reduction of the keto group at C-9 in the PGE_2 molecule to a β-hydroxy group might be an early step in the degradation of PGE_2 leading mainly to metabolite IV and to a small extent also to some more polar material.

Tritium-labeled metabolites VII, IIIb:2, IIIb:1, and IIIa were isolated from the urine after injection of [13,14-^{3}H]-tetranorprostaglandin E_1 (metabolite V). In addition radioactive polar material was found in peak I. No labeled metabolite VI or IV could be demonstrated. Tetranorprostaglandin B_1 (metabolite VII) was converted into metabolite IIIb:2, IIIb:1, and IIIa plus more polar material. These data demon-

TABLE VIII: Some Gas–Liquid Chromatography Data of Different Derivatives of the Methyl Esters of the Identified Metabolites, Obtained on a 1% Se-30 column (210–225°).[a]

Compound		Derivative	*C* Value
Metabolite IIa	7-Hydroxy-5,11-diketo-tetranorprosta-1,16-dioic acid	MO–Ac	24.5
		MO–TMSi	24.0
Metabolite IIb	7,16-Dihydroxy-5,11-diketotetranorprostanoic acid	MO–Ac	25.1
		MO–TMSi	24.1
Metabolite IIIa	11-Hydroxy-5-ketotetranorprosta-4(8),9-diene-1,16-dioic acid	MO–Ac	23.7
		MO–TMSi	23.3
Metabolite IIIb:1	11,15-Dihydroxy-5-keto-tetranorprosta-4(8),9-dienoic acid	Ac	23.6
		TMSi	22.6
Metabolite IIIb:2	11,16-Dihydroxy-5-keto-tetranorprosta-4(8),9-dienoic acid	Ac	24.5
		TMSi	23.4
Metabolite IV	5β,7α-Dihydroxy-11-ketotetranorprostanoic acid	Ac	21.7
		MO–Ac	21.8
		TMSi	20.5
		MO–TMSi	20.5
Metabolite V	7α,11-Dihydroxy-5-keto-tetranorprost-9-enoic acid	MO–Ac	22.1
		MO–TMSi	21.1
Metabolite VI	7α-Hydroxy-5,11-diketo-tetranorprostanoic acid	MO–Ac	21.5
		MO–TMSi	21.0
Metabolite VII	11-Hydroxy-5-ketotetranorprosta-4(8),9-dienoic acid	Ac	20.7
		MO–Ac	21.1
		TMSi	20.2
		MO–TMSi	20.5

[a] For definition of *C* values, see Chromatographic Methods.

strate that in the rat tetranorprostaglandin E_1 is probably dehydrated to tetranorprostaglandin A_1 which is very easily isomerized to tetranorprostaglandin B_1 (*cf.* Israelsson *et al.*, 1969). This latter compound can be further oxidized to metabolites IIIb:1, IIIb:2, and IIIa. It is, however, very likely that tetranorprostaglandin A_1 can also be further oxidized forming ω-1- and ω-2-hydroxylated products (Israelsson *et al.*, 1969) as well as a dicarboxylic acid. These compounds should be easily isomerized to metabolites IIIb:1, IIIb:2, and IIIa, respectively. It is thus most probable that tetranor-PGA_1 is initially formed and then oxidized at C-15 and C-16 comprising products which either in the body or during the processing of the urine are isomerized to metabolites IIIb:1, IIIb:2, and IIIa. It is of interest in this connection that tetranor-PGE_1 very easily is transformed to tetranor-PGB_1 when treated with alkali (M. Hanberg, personal communication).

Thus it seems likely that prostaglandin E_2 is degraded *via* three separate pathways (Figure 15). (1) PGE_2 is attacked by prostaglandin dehydrogenase and prostaglandin reductase (Änggård *et al.*, 1965; Nakano *et al.*, 1969) and then β-oxidized forming metabolite VI which is further oxidized to the ω-hydroxy and ω-carboxy metabolites IIa and IIb, respectively. (2) A pathway apparently leading to the 5β-hydroxy derivative (metabolite IV); however it is not possible to draw any conclusions from the data now available about the reaction sequence leading to this metabolite (Änggård and Samuelsson, 1966; Hamberg, 1968; Hamberg and Samuelsson, 1969b). (3) PGE_2 is β-oxidized forming metabolite V which is possibly dehydrated to metabolite VII or more likely to the corresponding PGA derivative (tetranorprostaglandin A_1) which is then further oxidized to the ω-1- and ω-2-hydroxylated compounds (metabolites IIIb:1 and IIIb:2) and the dicarboxylic acid.

The identification of the urinary metabolites of PGE_2 provides a basis for quantitative determination of endogenously produced and excreted amounts of the metabolites. Such work is in progress in this laboratory.

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