On the Metabolism of Prostaglandins \( \text{E}_1 \) and \( \text{E}_2 \) in the Guinea Pig*

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**SUMMARY**

\( \beta,7\alpha \)-Dihydroxy-11-ketotetranor-prostanoic acid was the major metabolite excreted in the urine of guinea pigs injected with tritium-labeled prostaglandin \( \text{E}_1 \), prostaglandin \( \text{E}_2 \), \( 11\alpha,15\beta \)-dihydroxy-9-ketoprost-5-enoic acid, \( 11\alpha \)-hydroxy-9,15-diketoprostanoic acid, and \( 11\alpha \)-hydroxy-9,15-diketoprost-5-en-5-inoic acid.

A method was developed for quantitative determination of \( 5\beta,7\alpha \)-dihydroxy-11-ketotetranor-prostanoic acid in 24-hour samples of guinea pig urine. The basal excretion of the metabolite in 10 male guinea pigs was 1.34 to 2.74 \( \mu \)g per kg of body weight \( \times \) 24 hours. Administration of 50 mg of indomethacin per day inhibited the excretion of the urinary metabolite by about 98%.

The biosynthesis and metabolism of prostaglandin \( \text{E} \) compounds in preparations of organs from guinea pig have earlier been studied in our laboratory. In 1964 the conversion of prostaglandin \( \text{E}_1 \) into \( 11\alpha \)-hydroxy-9,15-diketoprostanoic acid and \( 11\alpha,15\beta \)-dihydroxy-9-ketoprostanoic acid by enzymes present in the soluble fraction of homogenates of guinea pig lung was demonstrated (1). Analogous transformations of prostaglandin \( \text{E}_2 \) and prostaglandin \( \text{E}_3 \) were also described. Later the conversion of PGE\( _2 \) into \( 11\alpha,15\beta \)-dihydroxy-9-ketoprost-5-enoic acid, \( 11\alpha \)-hydroxy-9,15-diketoprost-5-enoic acid, PGE\( _2 \), \( 9\alpha \), \( 11\alpha \)-trihydroxy-9-ketoprost-5-enoic acid, \( 9\alpha,11\alpha \)-dihydroxy-15-ketoprost-5-enoic acid as well as 8-iso-PGE\( _2 \) and 8-iso-PGF\( _2 \) by the high speed supernatant of a homogenate of guinea pig lung was reported in 1965 (2). This conversion also occurred in preparations of guinea pig kidney medulla and liver. Thus, it was clear that the guinea pig possesses the enzymes necessary both for prostaglandin biosynthesis and inactivation. In order to obtain quantitative data on the turnover of PGE compounds in this animal, it was decided to measure the amount of a metabolite of PGE\( _1 \) and PGE\( _2 \) excreted in urine, or feces, or both. In the present work, the structure determination of the major urinary metabolite of PGE\( _1 \) and PGE\( _2 \) is described, as well as a method for quantitative determination of this metabolite in 24-hour portions of guinea pig urine. Data on the basal excretion of the metabolite in normal guinea pigs and in guinea pigs treated with an inhibitor of prostaglandin biosynthesis are also given. Part of the present work has been reported earlier in preliminary form (10).

**MATERIALS AND METHODS**

**Tritium-labeled Prostaglandins**

\( [5,6\text{-H}_2] \text{PGE}_1 \) was prepared by selective reduction of the \( \Delta^5 \) double bond of PGE\( _1 \) with tritium gas as earlier described (11). \( [17,18\text{-H}_2] \text{PGE}_2 \) was obtained in an analogous way by reduction of the \( \Delta^12 \) double bond of PGE\( _2 \) (2). Unlabeled material (generously provided by Dr. J. E. Pike, The Upjohn Company, Kalamazoo, Michigan) was added to make the specific radioactivities indicated. \( [17,18\text{-H}_2] \text{PGE}_2 \) was prepared by incubation of \( [5,6,8\text{-H}_2] \text{PGE}_2 \) with a preparation of sheep vesicular gland microsomes followed by addition of \( [17,18\text{-H}_2] \text{PGE}_2 \) (5).

Mass spectrometric analysis showed the following isotopic composition: 1% undeuterated, 19% hexadeuterated, and 80% heptadeuterated molecules. The specific activity was 2.7 \( \mu \)Ci per \( \mu \)mole.

\( [17,18\text{-H}_2] \text{PGE}_2 \) was obtained by incubation of \( [17,18\text{-H}_2] \text{PGE}_2 \) with the soluble fraction of a homogenate of guinea pig liver (4) and isolated by reversed phase partition chromatography and thin layer chromatography (5). The specific activity of the specimen was 1.8 \( \mu \)Ci per \( \mu \)mole.

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1 The abbreviations used are: PGE\( _1 \), prostaglandin \( \text{E}_1 \) \((11\alpha,15\beta\)-dihydroxy-9-ketoprost-5-enoic acid\); PGE\( _2 \), prostaglandin \( \text{E}_2 \) \((11\alpha,15\beta\)-dihydroxy-9-ketoprost-5-enoic acid\); PGA\( _1 \), prostaglandin \( \text{A}_1 \) \((15\alpha\)-hydroxy-9-ketoprostanoic acid\); MO, O-methylxime.
[5.6-3H]11α-Hydroxy-9,15-diketoprostanoic acid was prepared by manganese dioxide oxidation of [5.6-3H]PGE₉ followed by catalytic hydrogenation (1, 12). The specific activity was 0.7 µCi per pmole.

[17,18-3H]11α-Hydroxy-9,15-diketoprost-5-enoic acid was prepared from [17,18-3H]PGE₉ by incubation with a preparation of guinea pig lung (2) followed by purification as described in detail recently (5). The specific activity was 1.5 µCi per pmole.

The chemical and radiochemical purities of the tritium-labeled prostaglandins were better than 95% as judged by thin layer radiochromatographic analysis of the methyl esters. The RF values found were: methyl esters of PGE₁, PGE₂, and deuterium-labeled PGE₁, RF = 0.79; methyl esters of 11α-hydroxy-9,15-diketoprostanoic acid, RF = 0.66; methyl ester of 11α,15β-dihydroxy-9-ketoprost-5-enoic acid, RF = 0.79; methyl esters of 11α-hydroxy-9,15-diketoprostanoic acid and 11α-hydroxy-9,15-diketoprost-5-enoic acid, RF = 0.89.

[5α-3H]5β,7α-Dihydroxy-11-ketotetranor Prostanoic Acid—Six milligrams (9 PCi) of methyl [5α-3H]5β,11α-dihydroxy-15-ketotetranorprostanoate, obtained from [5α-3H]PGE₃ as earlier described (4), were treated with 10 ml of NaOH in 50% aqueous methanol at room temperature for 18 hours. This treatment gave a virtually complete hydrolysis of the methyl ester as judged by thin layer chromatography. The identity of the acid with [5α-3H]5β,11α-dihydroxy-15-ketoprostanoic acid was confirmed by reesterification of an aliquot of the acid by treatment with diazomethane followed by thin layer chromatographic analysis as well as gas-liquid chromatographic-mass spectrometric analysis of the trimethylsilyl (SiMe₃) ether-O-methyloxime derivative with the authentic derivative as reference.

[9α-3H]9β,11α-Dihydroxy-15-ketoprostanoic acid (800 µg) was incubated with mitochondria from 14 g of rat liver, suspended in 42 ml of the following medium: 0.92 mM KCl-50 mM MgCl₂-50 mM K₂HPO₄-0.072 mM KH₂PO₄-27.6 mM nicotinamide-3.6 mM MgCl₂-50 mM KCl-2.3 mM ATP-0.024 mM cytochrome c 0.10 mM CaO 0.7 mM NAD⁺ 1.2 mM dipotassium fumarate (cf. 12). The mixture was incubated at 37° for 90 min. After addition of 6 volumes of ethanol and removal of precipitated protein, the solution was acidified and extracted three times with ethyl acetate. The residue obtained after evaporation of the solvent (containing about 90% of the incubated radioactivity) was subjected to reversed phase partition chromatography with Solvent System C-38 supplemented with acetic acid. The major peak (about 40% of the incubated radioactivity; 80 to 120 ml of effluent; column, 9 g of hydrophobic Hydro Super-Cel) was due to [5α-3H]5β,7α-dihydroxy-11-ketotetranor-prostanoic acid. This material was isolated by extraction with ethyl acetate and then treated with diazomethane and further purified by silicic acid chromatography (column, 1 g of silicic acid; elution with 60 ml of ethyl acetate-benzene (1:1, v/v)). Thin layer chromatographic analysis of the material obtained showed a single spot with RF = 0.56. In order to prove the identity of the material with methyl 5β,7α-dihydroxy-11-ketotetranor-prostanoate, four derivatives were prepared (SiMe₃, SiMe₃-MO, acetyl, and acety-MO derivatives) and were subjected to gas-liquid chromatographic and mass spectrometric analysis.

The equivalent chain lengths found on gas-liquid chromatography with 1% SE-50 and 1% EGSS-X are given in Table I.

The mass spectrum of the SiMe₃ derivative is shown in Fig. 1. The molecular ion appeared at m/e 456. Prominent ions were seen at m/e 368 (M⁻90; loss of SiMe₃OH), 333 (M⁻90 + 15; loss of SiMe₃OH plus -CH₃), 261 (M⁻90 + 87; loss of SiMe₃OH plus -(CH₂)₂COOCH₃), 278 (M⁻2 x 90; loss of 2 SiMe₃OH), 254 (M⁻114 + 90; elimination of carbons C-10 to C-10 and 1 hydrogen atom plus SiMe₃OH), 241 (M⁻127 + 90); loss of -(CH₂)₂CO(CH₂)₂CH₃ plus SiMe₃OH), 217 (SiMe₃OH=CH-CH=O⁻SiMe₃) (cf. Reference 4), and 179 (M⁻69 + 2 x 90); elimination of -CO(CH₂)₂CH₃ plus 2 SiMe₃OH.

The mass spectrum of the SiMe₃-MO derivative is shown in Fig. 2. The molecular ion appeared at m/e 471. Prominent ions were seen at m/e 369 (M⁻90; loss of SiMe₃OH), 366 (M⁻90 + 31; loss of SiMe₃OH plus -CH₃), 343 (M⁻114; probably by elimination of -CH₂=CH-CH=O⁻SiMe₃) (cf. Reference 4), 307 (M⁻2 x 90; elimination of 2 SiMe₃OH), 276 (M⁻2 x 90 + 31); elimination of 2 SiMe₃OH plus -OCH₃), 253 (M⁻144 + 90); 343-90), 217 (SiMe₃OH=CH-CH=O⁻SiMe₃), and 156 ((CH₂)₂=CH⁻(CH₂)₂SiMe₃⁺).

The mass spectrum of the acetyl-MO derivative of methyl 5β,7α-dihydroxy-11-ketotetranor-prostanoate (Fig. 3) showed a molecular ion at m/e 208 as well as prominent ions at m/e 265 (M⁻113; elimination of -CH₂CO(CH₂)₂CH₃), 278 (M⁻2 x 60; elimination of 2 CH₂COOCH₃), 246 (M⁻2 x 60 + 32); elimination of 2 CH₂COOH plus CH₃OH), 225 (M⁻113 + 60); elimination of -(CH₂)₂CO(CH₂)₂CH₃ plus CH₂COOCH₃), 222 (M⁻2 x 60 + 59); elimination of 2 CH₂COOH plus CH₃=CH⁻-CH=O⁻SiMe₃ by β-cleavage and transfer of 1 hydrogen atom from C-14 to the carbonyl group at C-11), 207 (M⁻2 x 60 + 71); elimination of 2 CH₂COOH plus -(CH₂)₂CH₃), 204, 179 (M⁻2 x 60 + 99); elimination of 2 CH₂COOH plus -CH₃OH), 164 (M⁻2 x 60 + 114); elimination of 2 CH₂COOH plus -(CH₂)₂CH₃), 176 (M⁻144 + 90); 343-90), 217 (SiMe₃OH=CH-CH=O⁻SiMe₃), and 156 ((CH₂)₂=CH⁻(CH₂)₂SiMe₃⁺).

The mass spectrum of the methyl-MO derivative showed prominent ions at m/e 427 (M⁺, 371 (M⁻ - 56; elimination of CH₃=CH⁻-CH=O⁻SiMe₃), 276 (M⁻2 x 60 + 31); loss of 2 CH₂COOCH₃ plus -OCH₃), 251 (M⁻2 x 60 + 56); loss of 2

**TABLE I**

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a. I, 5α,7α-Dihydroxy 11 ketotetranor-prostanoic acid.

b. II, 5β,7α-Dihydroxy-11-ketotetranor-prostanoic acid.

c. III, Major urinary metabolite.
FIG. 1. Mass spectrum of SiMe₃ derivative of methyl 5α,7α-dihydroxy-11-ketotetranor-prostanoate.

FIG. 2. Mass spectrum of SiMe₂-MO derivative of methyl 5α,7α-dihydroxy-11-ketotetranor-prostanoate.

FIG. 3. Mass spectrum of acetyl derivative of methyl 5α,7α-dihydroxy-11-ketotetranor-prostanoate.

CH₃COOH plus CH₂=CH—CH₂—CH₃; 220 (M - (2 × 60 + 56 + 31); loss of 2 CH₃COOH plus CH₂=CH—CH₂—CH₃ plus OCH₃), 156 ([C(=NOCH₃)(CH₃)₄CH₃]⁺), and 128 ([C(=NOCH₃)(CH₂)₃CH₃]⁺). The elimination of CH₂=CH—CH₂—CH₃ in the formations of the ions at m/e 371, 251, and 220 is noteworthy, since it demonstrates that β-cleavage with transfer of one γ-hydrogen, a fragmentation typical for carbonyl compounds (13), also occurs in the mass spectra of compounds containing an O-methyloxime group located in an aliphatic chain.

The identity with 5α,7α-dihydroxy-11-ketotetranor-prostanoic acid of the compound obtained by β-oxidation of 9β,11α-dihydroxy-15-ketoprostanoic acid was mainly based on the mass spectrometric analyses. The differences between the molecular weights of the SiMe₂-MO and SiMe₃ derivatives (487 - 458 = 29) and acetyl-MO and acetyl derivatives (427 - 398 = 29) both indicated the presence of one keto group in the parent compound, since conversion of C=O into C=NOCH₃ increases the molecular weight by 29. Similarly, two hydroxyl groups must be present in the compound, since the differences in the molecular weights between the SiMe₃ and acetyl derivatives (458 - 398 = 60) and SiMe₂-MO and acetyl-MO derivatives (487 - 427 = 60) both were equivalent with twice the difference.
in molecular weights of SiMe₃ and acetyl (73 - 43 = 30). The presence of two hydroxyl groups in the five-membered ring was strongly indicated by the presence of an ion m/e 217 in the mass spectra of the SiMe₃ and SiMes-MO derivatives. This ion corresponds to SiMe₃-O-CH=CH-C=O(SiMe₃) and has earlier been found in the mass spectra of SiMe₃ derivatives of a number of PGF compounds but not in the mass spectra of SiMe₃ derivatives of PGE compounds (4). The presence in the compound of a saturated C₄ chain containing one keto group was shown by the ion m/e 156 ((CH_2)₃C(=NOCH₃)(CH_2)CH(=O)SiMe₃) in the mass spectra of the SiMe₃-MO and acetyl-MO derivatives. The presence of this chain was also indicated by the ions at m/e 241 (SiMe₃ derivative, loss of (CH_2)₂CO(=O)(CH_2)SiMe₃OH) and m/e 152 (acetyl derivative, loss of (CH_2)₂CO-(CH_2)CH₃ plus CH₂COOH plus CH₂COO⁻). Several ions demonstrated that the keto group must be located at C-11, e.g. m/e 170 (SiMe₃ derivative, α-cleavage with loss of CO(CH_2)₃CH₃ plus SiMe₃OH), 170 (acetyl derivative, α-cleavage with loss of CO(CH_2)₃CH₃ plus 2 CH₂COOH), 207 (acetyl derivative, α-cleavage with loss of CH_2=CH-CH_2-CO(OH)CH₂COO⁻). Finally the presence of a (CH_2)₂CH-OOCCH₃ side chain was shown by the ion at m/e 281 (SiMe₃ derivative, loss of (CH_2)₂COOCH₃ plus SiMe₃OH).

\[ ^{53}H \alpha, \beta, 7\alpha, 11\alpha-Dihydroxy-11-ketotetranor-prostanoic \text{ acid} - ^{[95} \text{H}] \alpha, 11\alpha-Dihydroxy-15-ketoprostanic \text{ acid} \] prepared from methyl \( ^{[95} \text{H}] \alpha, 11\alpha-dihydroxy-15-ketoprostanate \) (4) as described above. The compound was isolated by reversed phase partition chromatography (column, 9 g of hydrophobic Hyflo Super-Cel; Solvent System C-41 supplemented with acetic acid; 126 to 168 ml of effluent). The yield was about 20%. An additional 10% was isolated as a compound tentatively identified as the δ-lactone of \( \alpha, \beta, 7\alpha, 11\alpha-dihydroxy-11-ketotetranor-prostanoic \text{ acid} \) and \( \delta \)-lactone of \( \alpha, \beta, 7\alpha, 11\alpha-dihydroxy-11-ketotetranor-prostanoic \text{ acid} \). Column, 4.5 g of hydrophobic Hyflo Super-Cel; Solvent System C-41 supplemented with acetic acid (11); fractions, 3 ml.

**Fig. 4.** Reversed phase partition chromatography of a mixture of \( 5\alpha, 7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid, \( 5\alpha, 7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid, and \( \delta \)-lactone of \( 5\alpha, 7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid. Rf, (CH_2)O(OH)(CH_2)C=CH_2. Column, 4.5 g of hydrophobic Hyflo Super-Cel; Solvent System C-41 supplemented with acetic acid (11); fractions, 3 ml.
with a Barber-Colman series 5000 instrument. The stationary phases used were 1% SE-30 and 1% EGSS-X on Gas-chrom Q. Mass spectra were recorded with an LKB 9000 instrument equipped with a column of 1% SE-30 on Gas-chrom Q.

RESULTS

Isolation of Major Urinary Metabolite of Prostaglandin \( \text{E}_2 \)-\( \text{PGE}_2 \) \( \text{E}_2 \)-Part of the material obtained as described above was refluxed with \( \text{NaOH} \) in 50% aqueous ethanol for 60 min. No absorption band at 278 nm (typical for 9-keto-11,15-dihydroxy-\( \Delta^2 \)) or 237 nm (typical for 9-keto-11,15-dihydroxy prostaglandins) appeared. Mass spectrometric analysis of the methyl ester of prostaglandin \( \text{E}_2 \), RF = 0.56 (reference, methyl ester of prostaglandin \( \text{E}_2 \), RF = 0.66). This compound constituted about 50% of the labeled product excreted in the urine after administration of tritium-labeled prostaglandin \( \text{E}_2 \). Since about 56% of the injected isotope was recovered in the urine, it follows that about 28% of the administered tritium-labeled prostaglandin \( \text{E}_2 \) was excreted as the major urinary metabolite.

In order to obtain larger amounts of the metabolite for structural work, 1 mg of \( [\text{17,18}-\text{H}^3] \)-prostaglandin \( \text{E}_2 \) (2 \( \mu \)Ci, 2 pg) was injected into each of three guinea pigs and the methyl ester of the metabolite was isolated as described above. This afforded 600 \( \mu \)g of essentially pure methyl ester, the structure of which was determined as described below.

Structure of Major Urinary Metabolite of \( \text{PGE}_2 \). Part of the material obtained as described above was refluxed with \( \text{NaOH} \) in 50% aqueous ethanol for 60 min. No absorption band at 278 nm (typical for 9-keto-11,15-dihydroxy-\( \Delta^2 \)) or 237 nm (typical for 9-keto-11,15-dihydroxy prostaglandins) appeared. Mass spectrometric analysis of the methyl ester of \( \text{SiMe}_3 \) and methyl ester-\( \text{SiMe}_3\)-MO derivatives showed that the metabolite possessed one keto group (difference in molecular weights = 29). This keto group was most likely located in a side chain since the ultraviolet data indicated that no keto group was present in the five-membered ring. The mass spectra further indicated the presence of two hydroxyl groups and a carbon skeleton equivalent with tetranor-prostanoic acid. Comparison of the chromatographic behavior of the metabolite and of \( \alpha,\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid and \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid on reversed phase partition chromatography (cf. Fig. 4), thin layer chromatography, and gas-liquid chromatography (Table I) indicated that the metabolite was identical with \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid. The identity was conclusively established by mass spectrometric analysis of the four derivatives discussed above. The mass spectra of the derivatives of the major metabolite were identical with those of the corresponding derivatives of \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid.

Major Urinary Metabolites of \( \text{PGE}_2 \). \( \text{PGE}_2 \)-Part of the material obtained as described above was refluxed with \( \text{NaOH} \) in 50% aqueous ethanol for 60 min. No absorption band at 278 nm (typical for 9-keto-11,15-dihydroxy-\( \Delta^2 \)) or 237 nm (typical for 9-keto-11,15-dihydroxy prostaglandins) appeared. Mass spectrometric analysis of the methyl ester of \( \text{SiMe}_3 \) and methyl ester-\( \text{SiMe}_3\)-MO derivatives showed that the metabolite possessed one keto group (difference in molecular weights = 29). This keto group was most likely located in a side chain since the ultraviolet data indicated that no keto group was present in the five-membered ring. The mass spectra further indicated the presence of two hydroxyl groups and a carbon skeleton equivalent with tetranor-prostanoic acid. Comparison of the chromatographic behavior of the metabolite and of \( \alpha,\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid and \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid on reversed phase partition chromatography (cf. Fig. 4), thin layer chromatography, and gas-liquid chromatography (Table I) indicated that the metabolite was identical with \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid. The identity was conclusively established by mass spectrometric analysis of the four derivatives discussed above. The mass spectra of the derivatives of the major metabolite were identical with those of the corresponding derivatives of \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid.

**Preparation of Deuterium-labeled \( 5\beta,7\alpha\)-Dihydroxy-11-keto-prostaglandins**

To male guinea pigs, and the urine was collected for 24 hours. During this time, 40 to 60% of the administered radioactivity was excreted in the urine. About 90% of the labeled material could be extracted with ethyl acetate after acidification to pH 3. The extracts were subjected to reversed phase partition chromatography (column 9 g; Solvent System C-38 supplemented with acetic acid). In all cases, a major peak of radioactivity appeared (about 80 to 120 ml of effluent, cf. Fig. 5). The labeled material forming this peak was treated with diazomethane and further analyzed by gas-liquid chromatography-mass spectrometry as the \( \text{SiMe}_3 \)-, \( \text{SiMe}_3\)-MO, acetyl, and acetyl-\( \text{SiMe}_3\)-MO derivatives. By this procedure it could be demonstrated that the major urinary metabolites formed from the four prostaglandins were all identical with \( 5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid (Fig. 6).
Metabolism of Prostaglandins \(E_1\) and \(E_2\) in the Guinea Pig

**Fig. 7.** Preparation of deuterium-labeled \(5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid (II, before treatment with sodium hydroxide; III, after treatment with sodium hydroxide) from deuterium-labeled PGE\(_2\) (I).

**Fig. 8.** Lower curve (A), gas-liquid chromatogram of SiMe\(_3\)-MO derivative of methyl \(5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoate in quantitative determination of the amount present in a 24-hour sample of guinea pig urine. Upper curves (B and C), recording of intensities of ions \(m/e\) 456 and \(m/e\) 460 with accelerating voltage alternator (18). LKB 9000 instrument; column, 1\% SE-30 on Gas-chrom Q; column temperature, 210\^\circ; carrier gas, helium.

**Fig. 9.** Calibration curve obtained by multiple ion analysis of SiMe\(_3\)-MO derivatives of mixtures of deuterium-labeled and unlabeled methyl \(5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoate.

**Method for Quantitative Determination of \(5\beta,7\alpha\)-Dihydroxy-11-Ketotetranor-Prostanoic Acid in Guinea Pig Urine—Deuterium- and tritium-labeled \(5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid (3.14 \(\mu\)g) was injected subcutaneously into a male guinea pig, and the urine was collected during 24 hours. Deuterium-labeled \(5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid was isolated in pure form by extraction with ethyl acetate, reversed phase partition chromatography (cf. Fig. 5), and preparative thin layer chromatography (solvent system, organic layer of ethyl acetate-2,2,4-trimethylpentane-acetic acid-water (110:30:20:100, v/v/v/v); \(R_F\) = 0.39). The yield based on injected deuterium-labeled PGE\(_2\) was 15\%.

Mass spectrometric analysis of the SiMe\(_3\)-MO derivative of the methyl ester showed the following isotopic composition: 2\% undeuterated, 9\% trideuterated, 37\% tetradeuterated, and 52\% pentadeuterated molecules. The pentadeuterated molecules apparently contained one readily exchangeable deuterium atom in the side chain attached to C-8. This deuterium was probably located at C-10 (corresponding to C-14 of the deuterium-labeled PGE\(_2\)), in which position it would be readily exchangeable by reversible enolization of the keto group at C-11. The mass spectrum of the methyl ester-SiMe\(_3\)-MO derivative of the deuterium-labeled \(5\beta,7\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid obtained after treatment with sodium hydroxide showed ions of high intensities at \(m/e\) 460 (\(M - 31\)), 370 (\(M - (90 + 31)\)), 369 (\(M - (91 + 31)\)) plus 18 \(\text{CH}_2\)-deuterated (or its equivalent) plus 2 \(\text{CH}_2\)-deuterated (or its equivalent) plus 1 \(\text{CH}_2\)-deuterated (or its equivalent) plus 0 \(\text{CH}_2\)-deuterated (or its equivalent). This preparation was used as internal standard in the quantitative determinations described below. The specific activity of the specimen was 2.8 \(\mu\)Ci per \(\mu\)mole.
The formation of the major urinary metabolite of PGE\textsubscript{2} in the guinea pig, viz. 5\(\beta\),7\(\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid, involves (a) oxidation of the alcohol group at C-15, (b) reduction of the \(\Delta^9\) double bond, (c) two steps of \(\beta\) oxidation, and (d) reduction of the keto group of the five-membered ring. Reactions a and b were originally encountered in the metabolism of PGE compounds in a preparation of guinea pig lung (1-3) and were later found to occur also in guinea pig intestine and kidney (19) as well as guinea pig liver (4). It was recently found that the initial transformation of tritium-labeled PGE\textsubscript{2} injected intravenously to human subjects consists of a rapid conversion into 11\(\alpha\)-hydroxy-9,15-diketoprost-5-enoic acid (20). The corresponding derivative has also been isolated from blood from rats injected with tritium-labeled PGE\textsubscript{2} (11). These data suggest that also in the guinea pig the initial transformation of PGE\textsubscript{2} and PGE\textsubscript{3} consists of formation of the corresponding 15-keto-13,14-dihydro derivatives. The finding that 11\(\alpha\)-hydroxy-9,15-diketoprostanoic acid and 11\(\alpha\)-hydroxy-9,15-diketoprost-5-enoic acid are precursors of the major metabolite is in agreement with this hypothesis.

Degradation of the carboxyl side chain of prostaglandins by \(\beta\) oxidation has been studied with a preparation of liver mitochondria (12). PGE\textsubscript{2} and PGB\textsubscript{2} were mainly converted into the dinor derivatives, whereas PGE\textsubscript{1}, 11\(\alpha\)-hydroxy-9,15-diketoprostanoic acid, 11\(\alpha\),15-dihydroxy-9-ketoprostanoic acid and PGE\textsubscript{2} (21, 22) yielded mixtures of the dinor and tetrnor derivatives. Reduction of the keto group of the five-membered ring of PGE\textsubscript{2}, 11\(\alpha\)-hydroxy-9,15-diketoprostanoic acid and 11\(\alpha\)-hydroxy-9,15-diketoprost-5-enoic acid to 9\(\alpha\)-hydroxy derivatives by an enzyme present in the soluble fraction of a homogenate of guinea pig liver was recently demonstrated (4, 5). This enzyme, however, is apparently not operating in the in vivo metabolism of administered PGE\textsubscript{2} in the guinea pig since the new hydroxyl group in the five-membered ring of the major urinary metabolite had the \(\beta\) configuration. Furthermore, no trace of 5\(\beta\),7\(\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid, the major urinary metabolite of PGE\textsubscript{2} in the guinea pig (14), could be found among the urinary metabolites excreted after injection of tritium-labeled PGE\textsubscript{2}.

The method chosen for quantitative determination of 5\(\beta\),7\(\alpha\)-dihydroxy-11-ketotetranor-prostanoic acid consisted of addition of a known amount of deuterium-labeled metabolite to a 24-hour portion of guinea pig urine, purification by reversed phase partition chromatography and thin layer chromatography, and subsequent multiple ion analysis of the SiMe\textsubscript{3}-MO derivative of the methyl ester. The lower limit for analysis of the metabolite was about 30 ng. This limit was mainly set by the presence of 2\% of unlabeled material in the deuterium standard. The basal excretion of 10 normal male guinea pigs was in the range 1.34 to 2.74 \(\mu\)g per kg of body weight. The individual guinea pigs, however, excreted rather constant amounts of the metabolite (Table II). Recently a gas-liquid chromatographic method was developed for quantitative determination of two urinary metabolites of PGE\textsubscript{2} in the rat, viz. tetranor-PGE\textsubscript{2} and tetrnor-PGB\textsubscript{1} (23). By treatment of the urine with sodium hydroxide, the former metabolite was converted into the latter. The methyl ester-SiMe\textsubscript{3} derivative of the tetranor-PGB\textsubscript{1} was analyzed by gas-liquid chromatography with an electron-capture technique. The values found for the sum of

**Table II**

<table>
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<tr>
<th>Guinea pig</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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<td>1.34</td>
<td>1.57</td>
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</table>
the basal excretion of these metabolites in male rats were 2.04 to 2.63 μg per kg of body weight × 24 hours (23).

The sum of the secretion rates of PGE₁, PGE₂, and their metabolites which serve as precursors of the major urinary metabolite in the guinea pig may be estimated from the observed basal excretion of the urinary metabolite and the fact that 28% of subcutaneously administered tritium-labeled PGE₂ was recovered in form of the metabolite in urine. The figures obtained were 5 to 10 μg per kg of body weight × 24 hours.

Inhibition of the biosynthesis of PGE₂ and PGE₃ from arachidonic acid by indomethacin and aspirin were recently reported (24–26). In cell-free homogenates of guinea pig lung, the concentrations required for 50% inhibition were 0.75 μM and 35 μM, respectively (24). It appeared to be of interest to establish whether these drugs also acted as inhibitors of the prostaglandin biosynthesis in the intact animal. To this end a number of male guinea pigs were fed once a day with a suspension of 50 mg of indomethacin (Indomee, Merck, Sharp and Dohme) in 0.9% NaCl solution. This treatment inhibited the basal excretion of 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid by 98% (Fig. 10). The inhibition was rapidly relieved when administration of the drug was discontinued. Indomethacin in doses of 15 mg per day inhibited the excretion by about 65%. That the biosynthesis and not the metabolism of prostaglandins was inhibited was indicated by the finding that tritium-labeled PGE₂ administered to a guinea pig receiving indomethacin (50 mg per day) was converted into 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid to the same extent as in normal guinea pigs. Aspirin administered in a dose of 50 mg per day did not result in any significant reduction of the excretion of the major metabolite.

Use of indomethacin to inhibit the biosynthesis of prostaglandins in the intact animal may be valuable in studies on the physiological roles of prostaglandins. In addition, the demonstrated 5α-reduction inhibition is of interest in connection with speculation on the participation of prostaglandins in provoking various symptoms alleviated by indomethacin and other analogs (24).

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