Prostaglandins and Related Factors. XLII. Metabolism of Prostaglandin E_3 in Guinea Pig Lung*

Erik Änggård and Bengt Samuelsson

ABSTRACT: [^{14}C]Prostaglandin E_3 was prepared biosynthetically from 5,8,11,14,17-eicosa[^{14}C]pentaenoic acid using homogenates of sheep vesicular glands. Enzymes in the supernatant fraction of a homogenate of guinea pig lung centrifuged at 100,000 × *g* transformed the labeled prostaglandin E_3 (11α,15-dihydroxy-9-ketoprosta-5,13,17-trienoic acid) into two less polar metabolites. These metabolites have been assigned the structures 11α,15-dihydroxy-9-ketoprosta-5,17-dienoic and 11α-hydroxy-9,15-diketoprosta-5,17-dienoic acids.

The prostaglandins[1] are a group of structurally related compounds with smooth muscle–stimulating activity and effects on lipids metabolism (see Bergström and Samuelsson, 1965, for references). Although first discovered in semen and male genital glands, the prostaglandins have lately been identified in a variety of other animal tissues, e.g., calf thymus (Bergström and Samuelsson, 1963), sheep iris (Änggård and Samuelsson, 1964a), beef brain (Samuelsson, 1964a), human menstrual fluid (Eglington *et al.*, 1963), and lungs of cattle, sheep, guinea pig, monkey, and man (Bergström *et al.*, 1962a; Änggård and Samuelsson, 1963; Samuelsson, 1964b; Änggård, 1964).

Recent work has shown that certain essential fatty acids can act as precursors in the biosynthesis of prostaglandins. In sheep vesicular glands 8,11,14-eicosatrienoic acid was converted to prostaglandin E_1 (PGE_1) (Van Dorp *et al.*, 1964a; Bergström *et al.*, 1964a), and PGE_2 and PGE_3 were formed from 5,8,11,14-eicosatetraenoic acid (arachidonic acid) and 5,8,11,14,17-eicosapentaenoic acid, respectively (Bergström *et al.*, 1964a,b; Van Dorp *et al.*, 1964b). In homogenates of guinea pig lung arachidonic acid was transformed mainly to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) but also to PGE_2 (Änggård and Samuelsson, 1965).

It has also been shown that lung tissue participates in the metabolism of PGE_1 and PGE_2. Enzymes in the particle-free fraction of a homogenate of guinea pig lung catalyze the reduction of the Δ^{13} double bond and the oxidation of the secondary alcohol at C-15 in both PGE_1 and PGE_2 (Änggård and Samuelsson, 1964b; Änggård *et al.*, 1965).

In this paper we report the biosynthetic preparation of ^{14}C-labeled PGE_3 and studies on its metabolism in guinea pig lung. The experiments showed that PGE_3 is transformed into two less-polar metabolites. The structures of these metabolites have been determined.

Experimental Procedure

Methods

Chromatographic Methods. Reversed-phase partition chromatography was performed on columns of hydrophobic Super-Cel as described earlier (Norman, 1953). The solvent systems are given in Table I.

TABLE I: Solvent Systems in Reversed-Phase Partition Chromatography.[a]

System	Moving Phase	Stationary Phase
C-45	Methanol-water (135:165)	Chloroform-isooctyl alcohol (15:15)
F-55	Methanol-water (165:135)	Chloroform-heptane (45:15)

[a] All volume ratios are given in milliliters.

Thin-layer chromatography was performed on silica gel with and without $AgNO_3$ (Gréen and Samuelsson, 1964). The solvent systems are given in Table II. The substances, with the exception of 2,4-dinitrophenylhydrazones, were detected by spraying with concentrated sulfuric acid or phosphomolybdic acid (10% in ethanol) followed by heating to 150–200°.

* From the Department of Chemistry, Karolinska Institutet, Stockholm, Sweden. *Received April 24, 1965.* This work was supported by grants from the Swedish Medical Research Council (Project No. F-0217-B-A).

[1] The structures of the prostaglandins have been determined (Bergström *et al.*, 1962b, 1963; Abrahamsson *et al.*, 1962; Samuelsson, 1963) and a systematic nomenclature has been introduced with the use of prostanoic acid for the parent C-20 acid numbered from the carboxyl end (Figure 1). Prostaglandin E_1 (PGE_1), 11α,15-dihydroxy-9-ketoprost-13-enoic acid; prostaglandin E_2 (PGE_2), 11α,15-dihydroxy-9-ketoprosta-5,13-dienoic acid; prostaglandin E_3 (PGE_3), 11α,15-dihydroxy-9-ketoprosta-5,13,17-trienoic acid; prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), 11α,9,15-trihydroxyprost-13-enoic acid; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 11α,9,15-trihydroxyprosta-5,13-dienoic acid; prostaglandin $F_{3\alpha}$ ($PGF_{3\alpha}$), 11α,9,15-trihydroxyprosta-5,13,17-trienoic acid; PGE_1-H_2, 11α,15-dihydroxy-9-ketoprostanoic acid; PGE_3-278, 15-hydroxy-9-ketoprosta-5,8(12),13,17-tetraenoic acid.

TABLE II: Solvent Systems in Thin-Layer Chromatography.[a]

System	Composition	Adsorbent
A VIII	Ethyl acetate–acetic acid–2,2,4 trimethylpentane–water (110:15:30:100)[b]	Silica gel G[c]–$AgNO_3$ (30:1)
M IV	Ethyl acetate–methanol–water (100:5:100)[b]	Silica gel G–$AgNO_3$ (30:1)
D I	Diisopropyl ether–*n*-pentane (40:40)	Silica gel G
F I	*n*-Pentane–ethyl ether–acetic acid (40:40:10)	Silica gel G–$AgNO_3$ (25:1)

[a] All volume ratios are given in milliliters. [b] The solvent mixtures were equilibrated for 1 hour and the upper phase was used. [c] Merck, AG, West Germany.

TABLE III: Conditions for Gas-Liquid Chromatography.

Condition	Column	Carrier Gas Pressure	Temperatures		Instrument
			Column	Flash Heater	
A	8% EGSS-K[a]	3.0 kg/cm^2	170°	210°	F & M
B	1% SE-30[a]	0.6 kg/cm^2	210°		See Ryhage (1964)[b]
C	8% EGSS-K[a]	3.0 kg/cm^2	98°	142°	F & M
D	1% SE-30[a]	0.6 kg/cm^2	118°		See Ryhage (1964)[b]

[a] On 100–120 mesh Gas-Chrom P. [b] Gas-liquid chromatography in conjunction with mass spectrometry.

Gas-liquid chromatography was performed with an F & M Biomedical gas chromatograph, Model 400, with a hydrogen-flame ionization detector and with glass U-tube columns, 3 mm × 1.6 m. The column support was washed with acid, silanized, and coated with the liquid phase as described by Horning *et al.* (1963). The column conditions are given in Table III.

Silicic acid chromatography was performed on columns of silicic acid (Mallinckrodt, 100 mesh) activated for 1 hour at 115°. For purification of 5,8,11,14,17-eicosapentaenoic acid, 30-g columns were prepared in *n*-pentane and eluted with increasing concentrations (5–10%) of ether in *n*-pentane. Metabolites of PGE_3 were chromatographed on 1-g columns prepared with ethyl acetate–benzene (1:19) and eluted with increasing concentrations of ethyl acetate in benzene.

Infrared Spectrometry. The spectra were recorded with a Perkin-Elmer Model 221 infrared spectrophotometer on chloroform solutions (15–25 μg/μl) in a microcell (sodium chloride window, path length 0.1 mm, Type M-5 ON, Research and Industrial Instruments, England).

Measurement of Radioactivity. Radioactivity was measured in a gas-flow counter (Frieseke-Hoepfner, FH 51) operated in the proportional range, or in a liquid-scintillation spectrometer (Packard Tri-Carb, Model 314).

Catalytic Hydrogenation. Unless otherwise stated the compounds (0.05–1 mg) were dissolved in 3 ml of 96% ethanol and were hydrogenated for 30 minutes in a microhydrogenation vessel (Clauson-Kaas and Limborg, 1947) with 1 mg of 5% rhodium on carbon (Baker Platinum Division, Englehard Industries, Ltd.) as the catalyst.

Reduction with $NaBH_4$. The compounds (0.1–0.5 mg) were dissolved in 1 ml of 96% ethanol, and 1 mg of $NaBH_4$ in 1 ml of 96% ethanol was added. After 15 minutes at room temperature, the reaction mixture was diluted with water, acidified to *p*H 3, extracted with ether, and evaporated. Reductions with $NaBD_4$ (Metal Hydrides Inc., 92.1% purity) were carried out in the same way.

Mass Spectrometry. Mass spectrometry in conjunction with gas-liquid chromatography was performed using the instrument described by Ryhage (1964). The column conditions are given in Table III.

Oxidative Ozonolysis. The compounds (0.1–1 mg) were esterified by treatment with diazomethane and acetylated. The product was dissolved in 3–5 ml of ethyl acetate, and an excess of ozone was passed through the solution for 15 minutes at −70°. The solvent was at once evaporated under reduced pressure and the residue was dissolved in 0.2 ml of acetic acid and 0.2 ml of hydrogen peroxide (Perhydrol, 30% H_2O_2 Merck AG; Germany) and kept at 50° overnight. The solution was evaporated almost to dryness under reduced pressure. The residue was treated with diazomethane before gas-liquid chromatography.

Reductive Ozonolysis. The procedure was adapted after that described by Privett *et al.* (1963). The compounds were esterified with diazomethane and acetylated. This material was transferred to a small hydro-

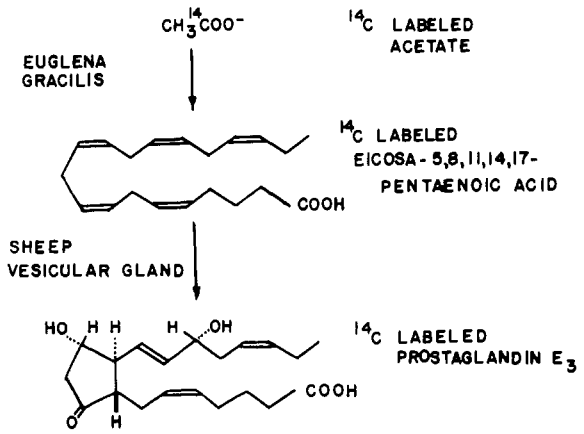


FIGURE 1: Scheme showing the biosynthetic preparation of labeled prostaglandin E_3.

genation vessel, dissolved in 0.2 ml *n*-pentane, and cooled to −70°, and 0.5–1 ml of cold (−70°) ozone-saturated *n*-pentane was added. The *n*-pentane had been pretreated with ozone and washed with concentrated sulfuric acid to remove olefinic impurities.

The *n*-pentane was immediately evaporated and the ozonides were dissolved in 0.2 ml of methyl caprylate or dichloromethane and reduced with hydrogen at 0° using 3 mg of palladium (5%) on carbon as a catalyst. The reaction mixture was agitated by magnetic stirring. After 20 minutes 0.1 ml was removed for conversion of the product to 2,4-dinitrophenylhydrazones.

Preparation of 2,4-Dinitrophenylhydrazones. The aldehydes obtained on reductive ozonolysis of the metabolites were converted to the 2,4-dinitrophenylhydrazones by the addition of 0.1–0.4 ml of 0.1% (w/v) 2,4-dinitrophenylhydrazine in 0.12 N hydrochloric acid. These derivatives were then compared by thin-layer chromatography (solvent system D 1) with authentic 2,4-dinitrophenylhydrazones of aliphatic aldehydes (C_1–C_6).

Lung Preparation. Lungs of thirty to forty male guinea pigs weighing 300–700 g were rapidly excised and transferred to an ice-cooled Bucher medium (0.02 M KH_2PO_4, 0.072 M K_2HPO_4, 0.0276 M nicotinamide, 0.0036 M $MgCl_2$, *p*H7.4). The lungs were minced and then homogenized in an Ultra-Turrax homogenizer (Model TP 18/2, Janke and Kunkel KG, Staufen, West Germany). The volume was adjusted to yield a tissue-to-buffer ratio of 1:4. The homogenates were centrifuged for 15 minutes at 900 × *g* and the supernatant fluid was centrifuged for 60 minutes at 105,000 × *g*. All procedures were carried out at 0–4°. The final clear supernatant was divided into 10-ml portions and incubated with shaking Erlenmeyer flasks at 37°. Labeled prostaglandin E_3 dissolved in Bucher medium was added to each flask at the beginning of the incubation period. After 30 minutes the incubation was terminated by the addition of nine volumes of 96% ethanol. The protein precipitate was filtered off. After evaporation the aqueous residue was acidified to *p*H 3 and extracted three times with ether. The combined extracts containing 90–100% of the added radioactivity were evaporated to dryness and the residue was analyzed by chromatography.

Materials

[^{14}C]Prostaglandin E_3 was prepared biosynthetically from 5,8,11,14,17-eicosa[^{14}C]pentaenoic acid with homogenates of the vesicular gland of sheep (Figure 1). For the preparation of the labeled pentaenoic acid we made use of the observation by Erwin and Bloch (1963) that *Euglena gracilis* grown in the dark incorporates substantial amounts of ^{14}C from labeled acetate into 5,8,11,14,17-eicosapentaenoic acid.

Preparation of 5,8,11,14,17-Eicosa[^{14}C]*pentaenoic Acid. Euglena gracilis* Z. was grown in the dark in 250 ml medium containing 0.5 mc of [1-^{14}C]acetate (New England Nuclear Corp.), as described by Hulanicka

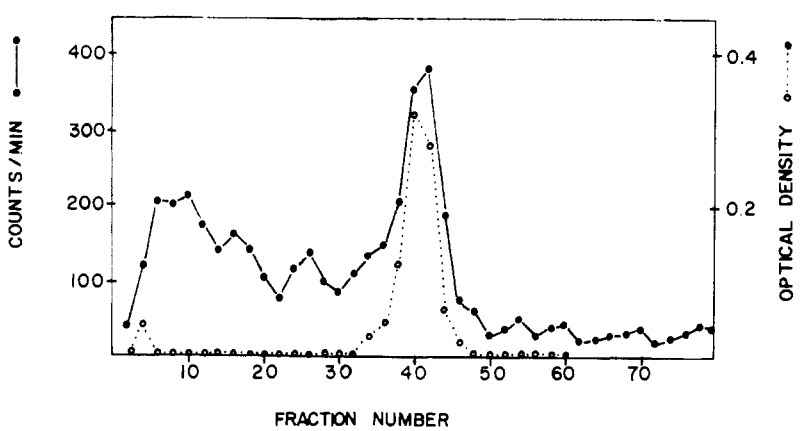


FIGURE 2: Reversed-phase partition chromatography of 5% of the ether extract from an incubation of 5,8,11,14,17-eicosa[^{14}C]pentaenoic acid with a homogenate of sheep vesicular glands. The unlabeled PGE_3 added as a reference was detected by treating aliquots of the fractions with dilute alkali and measuring the ultraviolet absorption at 278 mμ. Column, 4.5 g of hydrophobic Hyflo SuperCel; solvent, C-45; fraction volume, 2.3 ml.

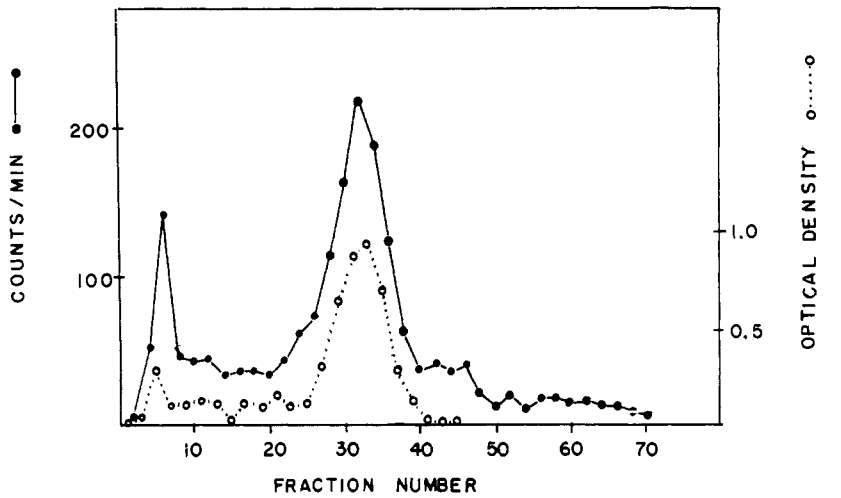


FIGURE 3: Chromatographic separation of products formed by treating labeled PGE_3 (Figure 2, fractions 36–46) with 0.5 N alcoholic NaOH (see under Materials). Column, 4.5 g of hydrophobic Hyflo SuperCel; solvent, F-55; fraction volume, 2.5 ml.

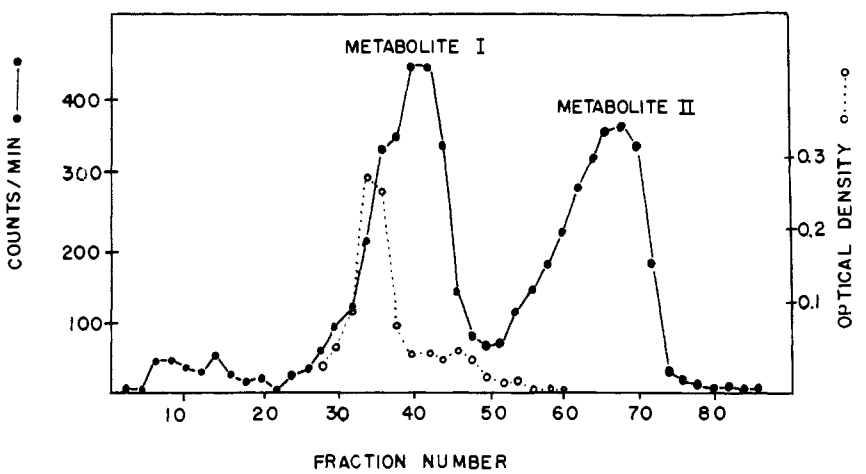


FIGURE 4: Reversed-phase partition chromatography of radioactive material from incubation of labeled PGE_3 with the supernatant fraction of a homogenate of guinea pig lung. Unlabeled PGE_3 (1 mg) was added as a reference. This compound was detected by measuring the absorption at 278 mμ after treating aliquots of the fractions with 0.5 N NaOH. Column, 4.5 g of hydrophobic Hyflo SuperCel; solvent, C-45; fraction volume, 2.5 ml.

et al. (1964). The cells were harvested 48 hours later and the fatty acid fraction was isolated after alkaline hydrolysis.

One-third of the fatty acids was separated by silicic acid chromatography, and the fractions containing 5,8,-11,14,17-eicosapentaenoic acid (as determined by gas-liquid chromatography [condition A] with a reference obtained from the Hormel Institute) were combined. The purified acids were subjected to preparative thin-layer chromatography on $AgNO_3$-impregnated silica gel using solvent F I. The area of the plate corresponding to the reference 5,8,11,14,17-eicosapentaenoic acid was scraped off and eluted with ether. The material obtained on evaporation of the solvent by a stream of nitrogen weighed 3.2 mg and contained 8% of the radioactivity of the processed material. Analysis of the methyl esters by gas-liquid chromatography (condition A) gave a main peak with the retention time of 5,8,11,14,17-eicosapentaenoic acid. A mass spectrum (condition D) of this peak was identical to the mass spectrum of authentic 5,8,11,14,17-eicosapentaenoic acid. The specific activity was about 2 μc/mg.

Preparation of Labeled PGE_3. The isolated 5,8,11,14,-17-eicosa[^{14}C]pentaenoic acid was converted into PGE_3 using homogenates of vesicular gland from sheep (5 g/mg acid). The preparation of the homogenate and the isolation of the product by extraction was performed as described previously (Bergström *et al.*, 1964a).

Five per cent of this material together with 1 mg of unlabeled PGE_3 was subjected to a pilot chromatog-

TABLE IV: Mass Spectrometric Data.[a]

Fragment	$PGF_{1\alpha}$	$PGF_{2\alpha}$	$PGF_{3\alpha}$	Metabolite I Reduced with $NaBH_4$	Metabolite I Reduced with $NaBD_4$	Metabolite II Reduced with $NaBH_4$	Metabolite II Reduced with $NaBD_4$
M − (2 × 60)	376	374	372	374	375	374	376
M − (3 × 60) = B	316	314	312	314	315	314	316
B − 69			243	245	246	245	247
B − 71	245	243					
B − 101	215	213	211	213	214	213	215
B − 127		187	185	187	188	187	
B − 129	187	185	183	185	186	185	187
B − 141		173	171	173	174	173	175
B − 143	173	171	169	171	172	171	

[a] The compounds were converted to the methyl esters by treatment with diazomethane and acetylated (Änggård and Samuelsson, 1964b) before the analysis (condition B).

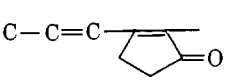
raphy using solvent C-45. Aliquots of the fractions were analyzed for radioactivity, and also for ultraviolet absorption at 278 mμ after treatment with 0.5 N sodium hydroxide at room temperature (Samuelsson, 1963). The chromatogram is seen in Figure 2. A peak of radioactivity coincided with that of the ultraviolet absorption due to the added PGE_3. Part of this material was subjected to thin-layer chromatography in solvent A VIII. After development, zones of the adsorbent were scraped off the plate and eluted with methanol for determination of radioactivity. It was found that 90% of the radioactivity was present in the zones corresponding to PGE_3. Another part of the radioactive PGE_3 fraction (Figure 2, fractions 36–46) was treated with alcoholic 0.5 N sodium hydroxide for 30 minutes at room temperature for conversion into PGE_3-278. The solution was acidified with 2 N hydrochloric acid and extracted twice with ether. The combined ether phases were washed to neutrality with water and evaporated to dryness under reduced pressure, and the residue was analyzed by reversed-phase partition chromatography in solvent F-55. The fractions were analyzed for radioactivity and ultraviolet absorption at 278 mμ. The chromatogram is shown in Figure 3. Most of the radioactivity appeared in a peak which coincided with the ultraviolet absorption due to PGE_3-278. The results showed that the radioactive compound was identical with PGE_3. The overall conversion of 5,8,11,14,17-eicosapentaenoic acid to PGE_3 was found to be 17%. The radioactive PGE_3 fraction of the remaining part of the extract was isolated as described above and was dissolved in benzene and kept at −20° under nitrogen until used.

Results

Enzymes present in the supernatant fraction of guinea pig lung homogenates centrifuged for 60 minutes at 105,000 × *g* converted labeled PGE_3 into two less polar compounds (metabolite I and metabolite II). To obtain sufficient amounts of these metabolites for structural work, large-scale incubations were carried out as described under Experimental Procedure. Almost complete conversion occurred with concentrations of PGE_3 of 25–30 μg/ml of supernatant. After preliminary extraction a pilot chromatography was run on 5% of the extract of the incubation together with 1 mg of unlabeled PGE_3. One of these chromatographies is shown in Figure 4. Most of the radioactivity was present in the two peaks appearing after PGE_3. From 10 to 20% of the chromatographed radioactivity remained in the stationary phase. The nature of this material was not investigated. The two metabolites were isolated in larger amounts by reversed-phase partition chromatography (solvent C-45) of the remaining 95% of the extract without added PGE_3, followed by silicic acid chromatography. Metabolites I and II were eluted from the silicic acid columns with ethyl acetate–benzene in the volume ratios of 2:3 and 1:3, respectively.

The purified metabolites gave single spots on thin-layer chromatography in solvent A VIII and were used in the structural work to be described later. The best yields (based on incubated amount of PGE_3) of pure compounds were 32% for metabolite I and 35% for metabolite II.

Structure of Metabolite I. The infrared spectrum of metabolite I showed absorption at 5.77 μ (C=O in five-membered ring) and at 5.87 μ. However, the absorption band at 10.3 μ (*trans* double bond) observed for PGE_3 had disappeared, indicating that the Δ^{13} double bond (*trans*) had been saturated. This supposition was further supported by the finding that metabolite I gave no ultraviolet absorption after treatment with dilute alkali at room temperature. PGE_3 similarly treated is transformed into the chromophore



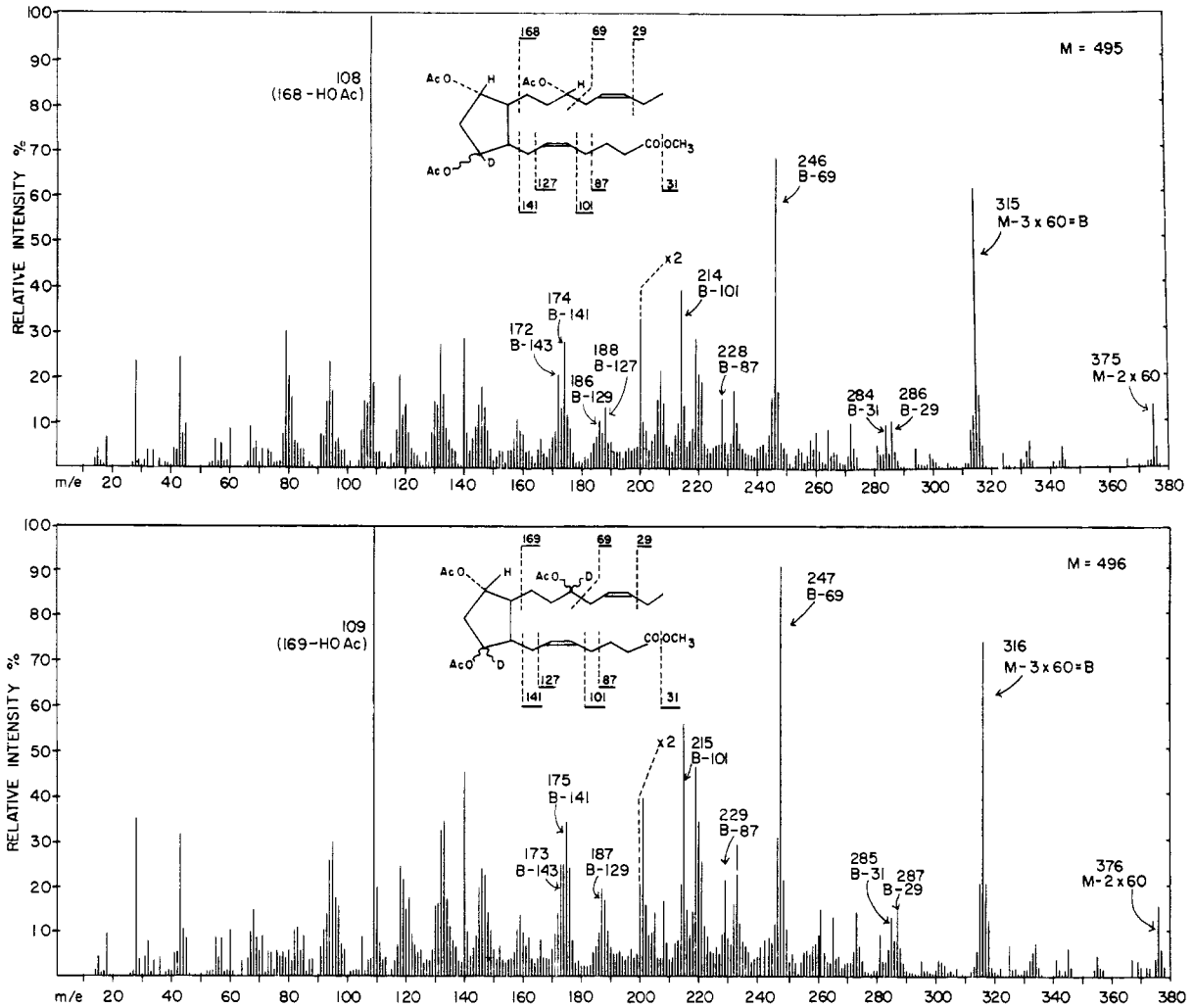


FIGURE 5: Mass spectra of metabolite I reduced with $NaBD_4$ (top) and of metabolite II reduced with $NaBD_4$ (bottom). The compounds were converted to the methyl esters by treatment with diazomethane and acetylated (Änggard *et al.*, 1965). The spectra were recorded on the substances as they emerged from a gas-chromatography column (Ryhage, 1964). The relative intensities at all *m/e* values above 199 have been doubled. The main features of the spectra are summarized in Table IV.

(λ_{max}^{EtOH} 278 mμ, ε 26,800) by dehydration and isomerization of the introduced double bond (Samuelsson, 1963).

To convert metabolite I into a known derivative, suitable for identification, it was esterified by treatment with diazomethane and subjected to catalytic hydrogenation. The product was analyzed by thin-layer chromatography on $AgNO_3$-impregnated plates using solvent M IV. The reference compounds were the methyl esters of PGE_3 (11α,15-dihydroxy-9-ketoprosta-5,13,-17-trienoic acid) and its saturated analog PGE_1-H_2 (11α,15-dihydroxy-9-ketoprostanoic acid). Before hydrogenation the methyl esters of metabolite I and PGE_3 had R_F values of 0.19 and 0.10, respectively. After hydrogenation, however, both these compounds and PGE_1-H_2 had identical R_F values (0.78). These results indicated that metabolite I contained two double bonds and showed that it had the parent structure of 11α,15-dihydroxy-9-ketoprostanoic acid.

Strong evidence for this structure was obtained by mass spectrometry of derivatives prepared by reduction with either sodium borohydride or sodium borodeuteride. The compounds were converted to the methyl esters with diazomethane and acetylated (Änggård *et al.*, 1965) before they were analyzed (condition B). The mass spectrum of metabolite I reduced with $NaBD_4$ is seen in Figure 5. In addition the mass spectrometric data are summarized in Table IV. For comparison the corresponding values for $PGF_{1\alpha}$, $PGF_{2\alpha}$, and $PGF_{3\alpha}$ are included.

The *m/e* values in the spectrum of metabolite I reduced with $NaBH_4$ showed that its molecular weight was two units higher than that of corresponding reduction product of PGE_3, i.e., $PGF_{3\alpha}$ (Table IV). This

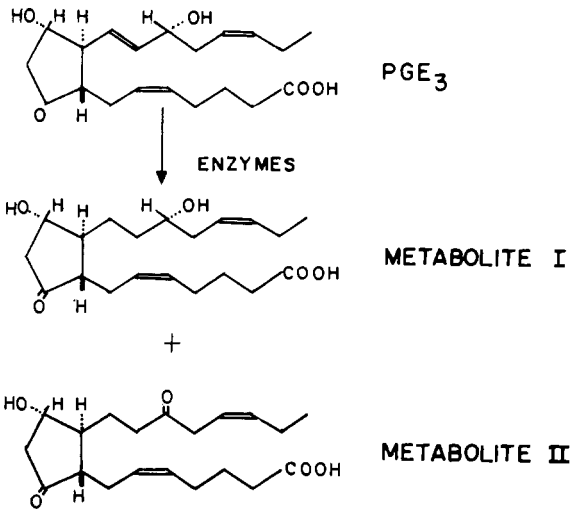


FIGURE 6: Structures of metabolites of PGE_3.

demonstrated that one of the double bonds of PGE_3 was reduced in the formation of metabolite I.

Reduction of metabolite I with $NaBD_4$ instead of $NaBH_4$ resulted in an increase of the *m/e* values of most fragments with *one* unit (Table IV). This demonstrated that *one* deuterium had been incorporated during the reduction, and that metabolite I therefore contained only *one* keto group (Figure 5).

The mass spectra also indicated the location of the two double bonds in metabolite I. The large fragment with a *m/e* value of 246 (B − 69) (Figure 5) arises from the loss of the terminal pentenyl group (C-16 to C-20) from B. Elimination of corresponding carbon atoms from $PGF_{1\alpha}$ and $PGF_{2\alpha}$, which have no Δ^{17} double bond, involves loss of 71 *m/e* units (Table IV). These results therefore indicate that the Δ^{17} double bond is retained in metabolite I.

The position of the other double bond in metabolite I was also implied by its mass spectrum (Figure 5). The strong peaks with *m/e* values of 214, 200, 188, and 174, owing to loss of 101, 115, 127, and 141, respectively, from B, are probably caused by the elimination of these fragments from the carboxyl side chain as indicated in Figure 5. Significant peaks are also seen at 186 and 172 owing to elimination of 129 and 143 from B. Both types of fragments are eliminated from $PGF_{2\alpha}$ and $PGF_{3\alpha}$, which have double bonds in the Δ^5 position. However, in $PGF_{1\alpha}$, which lacks this double bond, *only* fragments corresponding to loss of 129 and 143 are seen (Table IV). Elimination of 129 and 143 in derivatives having a Δ^5 double bond might be due to isomerization of the double bond toward the ring during electronic impact prior to the fragmentation. These data therefore indicate that the Δ^5 double bond is retained in metabolite I. Direct evidence for the position of the two double bonds in metabolite I was obtained by reductive and oxidative ozonolysis followed by identification of two of the fragments formed.

Metabolite I was reduced with $NaBH_4$, converted to the methyl ester by treatment with diazomethane, and acetylated. Part of this material was subjected to reductive ozonolysis using methyl caprylate as solvent during the reduction. One of the products was identified as propionic aldehyde by thin-layer chromatography of the 2,4-dinitrophenylhydrazone (solvent D I).

Another part of the reduced and acetylated methyl ester of metabolite I was subjected to oxidative ozonolysis. The products were treated with diazomethane and analyzed by gas-liquid chromatography. One fragment was identified as the dimethyl ester of glutaric acid (condition C). A larger fragment, which was not identified, was also noticed.

The identification of propionic aldehyde and glutaric acid after reductive and oxidative ozonolysis showed, together with the mass spectrometric data, that the double bonds of metabolite I were located in positions Δ^5 and Δ^{17}. The complete structure of metabolite I is therefore 11α,15-dihydroxy-9-ketoprosta-5,17-dienoic acid (Figure 6).

Structure of Metabolite II. The infrared spectrum of metabolite II showed absorption bands in the carbonyl region at 5.77 μ (C=O in five-membered ring) and 5.87 μ. However, no absorption at 10.3 μ (*trans* double bond) was observed, indicating that the Δ^{13} double bond (*trans*) of PGE_3 was saturated in the formation of metabolite II. This was also consistent with the finding that metabolite II did not exhibit any ultraviolet absorption at 278 mμ after treatment with alkali at room temperature (cf. discussion under Structure of Metabolite I).

To obtain further information on the number of double bonds in metabolite II, and at the same time to convert it into a known derivative, the metabolite was subjected to catalytic hydrogenation. The product was analyzed by thin-layer chromatography (solvent A VIII). The reference compounds were 11α-hydroxy-9,15-diketoprost-5-enoic acid (Änggård *et al.*, 1965) and its saturated analog, 11α-hydroxy-9,15-diketoprostanoic acid. Before hydrogenation metabolite II and 11α-hydroxy-9,15-diketoprost-5-enoic acid had R_F values of 0.48 and 0.71, respectively. After hydrogenation, however, metabolite II had the same R_F value (0.82) as 11α-hydroxy-9,15-diketoprostanoic acid. These data indicated that metabolite II had two double bonds and showed that it had the parent structure of 11α-hydroxy-9,15-diketoprostanoic acid. These structural features were confirmed by mass spectrometric analyses (condition B) of derivatives prepared by reduction of metabolite II with either sodium borohydride or sodium borodeuteride.

The analyses of these derivatives and of reference compounds are summarized in Table IV, and the mass spectrum of the derivative prepared by $NaBD_4$ reduction of metabolite II is illustrated in Figure 5. The molecular weight (as judged from the fragments shown in Table IV) of metabolite II reduced with $NaBH_4$ was two units higher than that of $PGF_{3\alpha}$. This showed that metabolite II contained one double bond less than its precursor, PGE_3.

Reduction of metabolite II with $NaBD_4$ resulted in

incorporation of *two* atoms of deuterium (Figure 5 and Table IV), showing that metabolite II contained *two* keto groups. The spectrum of metabolite II reduced with $NaBH_4$ was identical to the spectrum of metabolite I treated in the same way. Together these data indicated that the only difference between the two metabolites was the presence of an additional keto group at C-15 in the former compound. This also implies, according to the foregoing discussion of the mass spectrum of metabolite I, that the two double bonds of metabolite II are located in positions Δ^5 and Δ^{17}. These locations of the double bonds in metabolite II were confirmed by ozonolysis followed by identification of the C_3 and C_5 fragments as described above for metabolite I. The complete structure of metabolite II is therefore 11α-hydroxy-9,15-diketoprosta-5,17-dienoic acid (Figure 6).

Discussion

Labeled PGE_3 was prepared biosynthetically from 5,8,11,14,17-eicosapentaenoic acid using homogenates of vesicular glands from sheep. These experiments confirm previous studies (Bergström *et al.*, 1964a) demonstrating the conversion with the unlabeled precursor.

The experiments reported here show that PGE_3 is metabolized by enzymes in the particle-free fraction of a homogenate of guinea pig lung to 11α,15-dihydroxy-9-ketoprosta-5,17-dienoic acid and 11α-hydroxy-9,15-diketoprosta-5,17-dienoic acid. These transformations, which are analogous to those previously observed for PGE_1 and PGE_2 (Änggård and Samuelsson, 1964b; Änggård *et al.*, 1965), involve reduction of the Δ^{13} double bond and also oxidation of the alcohol group at C-15.

Several observations indicate that the enzymatic transformations reported in this and previous papers (Änggård and Samuelsson, 1964b; Änggård *et al.*, 1965) are of importance for the metabolism of PGE compounds. Both enzyme activities have thus been found in kidney and intestine of sheep and guinea pig.[2] Furthermore, *in vivo* experiments using rats showed the presence of labeled 11α,15-dihydroxy-9-ketoprostanoic acid and 11α-hydroxy-9,15-diketoprostanoic acid in the blood after intravenous infusion of labeled PGE_1 (Samuelsson, 1964c). The biological significance of these transformations will be reported later.

Our data also provide experimental evidence on the stereochemistry of the Δ^5 and Δ^{17} double bonds of PGE_3. The selective reduction of the Δ^{13} double bond (*trans*) of PGE_3 in the formation of metabolites I and II was accompanied by disappearance of the infrared absorption band at 10.3 μ (*trans* double bond). This finding shows that the remaining double bonds at Δ^5 and Δ^{17} have the *cis* configuration. Since *cis-trans* isomerizations are unlikely to occur in the formation of the metabolites, the *cis* configuration for the Δ^5 and Δ^{17} double bonds can also be inferred for PGE_3.

[2] E. Änggård, and B. Samuelsson, unpublished results.

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