

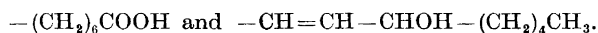
Degradation Studies on Prostaglandins

Prostaglandins and Related Factors 10

SUNE BERGSTRÖM, R. RYHAGE, B. SAMUELSSON and J. SJÖVALL

Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

Oxidative degradation of PGE, PGF_{1α} and PGF_{1β} has shown that the molecules contain the side chains



In a previous investigation¹⁻³ it was demonstrated that PGE is a C₂₀ monocarboxylic acid containing two hydroxyl groups, one keto group and one *trans* double bond. The presence of a cyclopentane ring structure was also suggested on the basis of the IR-spectrographic data and the elemental analysis. Reduction of the keto group with sodium borohydride yielded two epimeric monounsaturated trihydroxy acids, PGF_{1α} and PGF_{1β}, the former of which is identical with the previously isolated PGF.

In the present communication further studies on the structures of PGE, PGF_{1α} and PGF_{1β} are described.

EXPERIMENTAL

Oxidation with chromium trioxide in acetic acid

Preliminary oxidation experiments with chromium trioxide indicated that PGE rapidly consumed 20-30 oxidation equivalents under mild conditions (20-50°) in 90-99 % acetic acid (*cf.* Fig. 1). A preparative run that was followed analytically will be described.

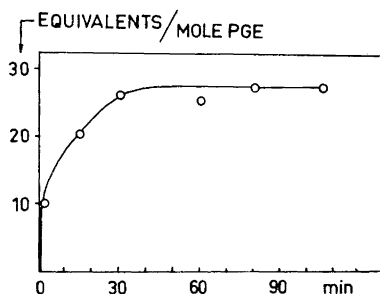


Fig. 1. Oxidation of PGE with chromium trioxide in 97.5 % acetic acid at 50°C (see text).

Preparative oxidation of PGE and gas chromatography of the products. PGE (20 mg, m.p. 115°) was dissolved in 10 ml of glacial acetic acid and 10 ml of 95 % acetic acid containing 200 mg of chromium trioxide was added. A corresponding control was prepared and both were kept in a water bath at 50°. Samples of 10 μ l were taken at times indicated on the curve in Fig. 1 and titrated with 0.005 N thiosulfate after addition of potassium iodide.

After 2 h methanol was added and the mixture was taken to dryness *in vacuo*. Nine tenths of the residues was dissolved in about 4 ml of water that was acidified with hydrochloric acid and continuously extracted with ether for 20 h. The ether was taken to dryness and the residue was dissolved in a drop of methanol and treated with diazomethane. Gas chromatography showed the presence of one main component that behaved like dimethyl suberate. The mass spectrum of a collected specimen also proved to support this contention when compared with that of an authentic specimen, *cf.* Figs. 2 and 3.

A minor peak corresponded to the dimethylester of pimelic acid. Larger fragments were not found and smaller molecules might have been lost during the evaporation.

We are most grateful to Dr. Stina Ställberg-Stenhagen for the gas chromatographic analysis of the first sample of suberic acid ester and to prof. Einar Stenhagen for the

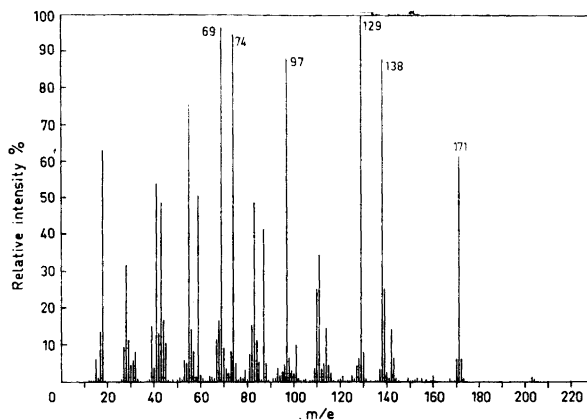


Fig. 2. Mass spectrum of dimethyl suberate isolated from chromic acid oxidation of PGE.

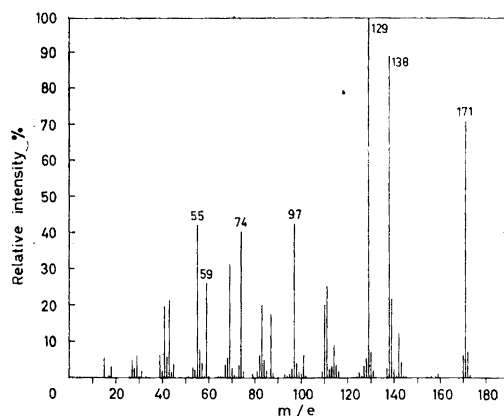


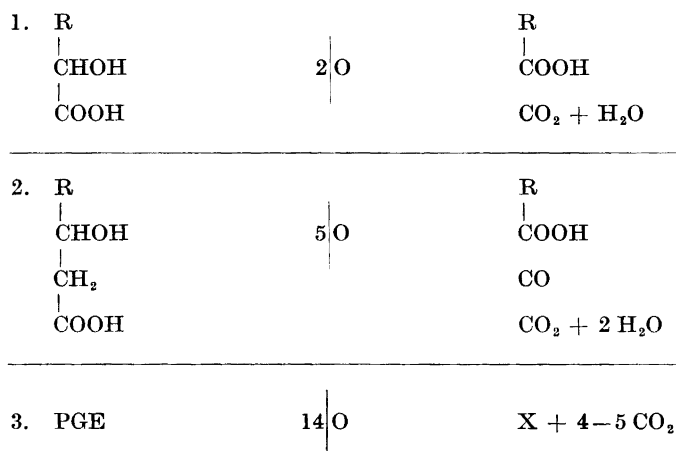
Fig. 3. Mass spectrum of authentic dimethyl suberate.

comparison of the mass spectrographic analysis with at that time unpublished mass spectrographic data on dicarboxylic acid esters.⁴

1. Formation of carbon dioxide in the oxidation of PGE with chromic acid.

As other observations indicated that several of the substituents and the double bond might be grouped together and as the consumption of oxidation equivalents was relatively large, a quantitative investigation was done to ascertain if some carbon dioxide was formed in the oxidation with chromium trioxide.

The substance (0.4–0.8 mg) in 0.5 ml of glacial acetic acid was pipetted into a Warburg flask the side arm of which contained 6 mg of chromium trioxide dissolved in 0.3 ml of 95 % acetic acid. After equilibration at 45°C the chromium trioxide solution was added to the sample by tilting the flask and the evolution of gas was measured. Under these conditions α -hydroxystearic acid yielded 1.0 mole of CO₂ per mole of acid within one hour, β -hydroxytridecanoic acid yielded 2.1 moles of CO₂ in about 5 h, whereas PGE was more slowly oxidized giving 4–5 moles of CO₂ in about 20 h (cf. the formulas, Ref.⁵).



Oxidation of PGF_{1 α} -trimethyl ether with permanganate-periodate

The trimethyl ether of PGF_{1 α} methyl ester was prepared with diazomethane and boron trifluoride catalyst as described earlier.³ The product was purified by GLC and the compound used in the experiments described below was collected from the column. This material (5.3 mg) was dissolved in 0.5 ml of methanol and hydrolysed with 0.2 ml of 0.1 N NaOH for 15 h at room temperature. The solvent was removed *in vacuo* and the resulting sodium salt of the trimethoxy acid was dissolved in 2 ml of water and oxidized essentially as described by Lemieux and Rudloff.¹¹ A solution of 5.8 mg of potassium carbonate, 4.5 mg of sodium hydroxide, 22 mg of periodic acid and 0.31 mg of potassium permanganate in 4 ml of water was added. The solution was stirred for 30 h at room temperature and treated with excess of sodium bisulfite and 4 N sulfuric acid. The solution was exhaustively extracted with ether. The ether solution was dried over sodium sulfate and evaporated. The acids formed were esterified with diazomethane and separated by GLC. The fragment of higher molecular weight was collected * and subjected to mass-spectrometry (Fig. 9). The fragment of lower molecular weight was unfortunately lost.

* Samples were collected from the gas chromatographic effluent by a technique developed by Dr. Stina Ställberg-Stenhagen.¹²

Oxidative ozonolysis of PGE

The methyl ester of PGE (50 mg, m.p. 113–114°) was prepared with diazomethane and acetylated with 1.5 ml of acetic anhydride and 1.0 ml of pyridine at room temperature for 18 h. The reaction mixture was taken up in ether and ice and the ether phase washed with 2 N hydrochloric acid, 5 % sodium hydrogen carbonate and water. After drying over sodium sulfate the solvent was evaporated *in vacuo*. The residue was dissolved in 30 ml of chloroform and cooled to –15°. Ozone, 2.4 mole/mole of PGE, was carried through the solution during 4 min. 10 min later the solvent was removed under reduced pressure.

The oxidative splitting of the ozonide was carried out under conditions similar to those used by Klenck *et al.*⁶ The following procedure was found most satisfactory as judged by gas-liquid chromatography (GLC) of the oxidation products formed in test runs from the ozonide of methyl oleate.

The ozonide from the acetylated methyl ester of PGE was dissolved in 5 ml of glacial acetic acid and treated with 0.5 ml of perhydrol (Merck A. G., Darmstadt, Germany, 30 % H₂O₂). The mixture was kept in a thermostat at 50° for 24 h after which time the solvent was distilled off *in vacuo* at 50°. The residue was dissolved in water and extracted continuously with ether for 6 h. Evaporation of the dried ether solution yielded 53.5 mg of a yellow oil. Part of this material was esterified with diazomethane and analyzed by GLC.

Gas-liquid chromatography

A Pye gas chromatograph with an argon ionization detector was used. Silicone grease (15 %) was supported on 100–120 mesh Celite. An argon pressure of 1.0 kg/cm² was applied giving a gas flow of approximately 40 ml/min.

The columns were standardized by running a mixture of the methyl esters of normal fatty acids (C₆ to C₁₈). A semilogarithmic diagram was constructed with the retention times found on the logarithmic scale and the number of carbon atoms in the acids on the normal scale. The straight line obtained by connecting these points (*cf.* James and Martin⁷) could be conveniently used to relate the observed retention times of the isolated products to the size of the molecule. The retention times were expressed as "M-values", M 10 and M 9 being equivalent to the methyl esters of capric acid and pelargonic acid, respectively. When straight chain mono- or dicarboxylic acids containing different functional groups, *e.g.* hydroxy, keto, acetoxy, and methoxy groups, were run, it was found that the influence of these groups on the M value was constant for each substituent. It was thus found that a hydroxyl group or a keto group was equivalent to an increase of 0.8 of the M value of the parent acid ester. The introduction of an acetoxy group increased the M value with 2.5 and the conversion of the terminal methyl group to a carboxymethyl group raised the M value with 3 units when compared with the corresponding unsubstituted monocarboxylic acid methyl ester.

A GLC analysis of the esterified ozonolysis products at 100°C with silicone grease as the stationary phase yielded one main peak corresponding to M 9.5. A separation at 200° (Fig. 4) revealed the presence of two fragments with M 16.0 and 16.7, respectively. No additional compounds were found by chromatography at 250° or 100°. The compounds must be acids since no peaks were found when a sample was chromatographed before treatment with diazomethane.

A sample of the original products of ozonolysis was hydrolysed at room temperature for 18 h with 3 ml of 0.5 N NaOH in 3 ml of methanol. After acidification and exhaustive ether extraction the residue was treated with diazomethane. The GLC analysis at 200° showed that the smaller peak (M 16.7) had disappeared and only one peak at M 16.0 was found.

Analysis of the shorter fragment at 100° before and after hydrolysis demonstrated that this treatment caused a decrease of the M value from 9.5 to 7.7.

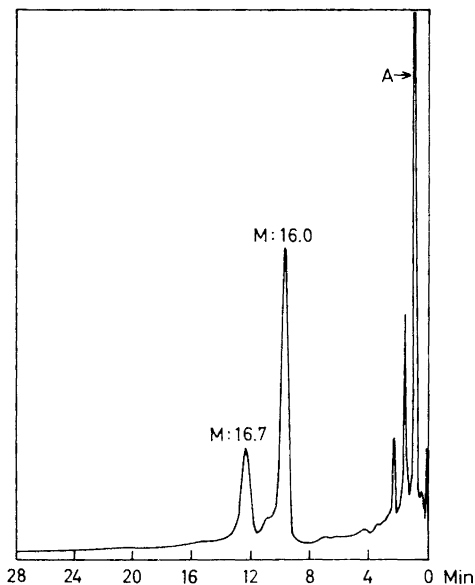


Fig. 4. GLC at 200° of products from the ozonolysis of acetylated PGE methyl ester after treatment with diazomethane. Peak A corresponds to the compound with M 9.5.

Reversed phase partition chromatography

In order to get larger amounts of the pure degradation products for chemical identifications column partition chromatography was used. The following solvent systems were employed.

Phase system	Moving phase	Stationary phase
C	135 ml methanol:165 ml water	15 ml chloroform:15 ml isoctanol
D	300 ml water	100 ml butanol-1

Siliconized Hyflo Supercel (Johns Manville and Co., USA) was used as supporting material. A part (42 mg) of the original reaction product from the ozonolysis was chromatographed on 13.5 g of hydrophobic Hyflo Supercel with solvent system C (Fig. 5). The material in the different peaks and the material remaining in the stationary phase were analyzed by GLC after treatment with diazomethane.

The front peak I was due to acetic acid that had not been completely removed. Analysis of material from peak II and III at 200° revealed the presence of compounds with M 16.7 and 16.0, respectively.

The material retained in the stationary phase being almost exclusively of the M 16 type was hydrolysed with 0.5 N methanolic NaOH at room temperature for 18 h. Chromatography of the resulting material with solvent system D is shown in Fig. 6. The material in the main peak was homogeneous (M 16.0) as judged by GLC of the diazomethane treated compound. Peak IV in the chromatogram shown in Fig. 4 was demonstrated by GLC to be made up of one single component with M equal to 9.5 after diazomethane treatment.

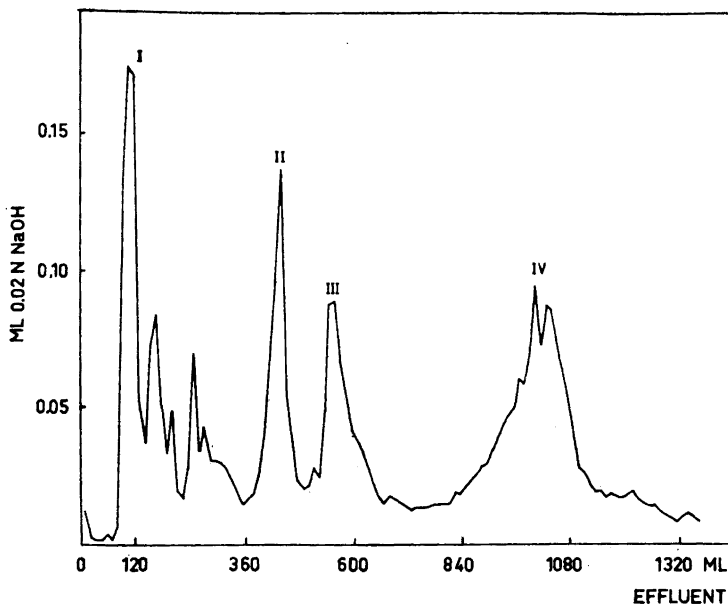


Fig. 5. Reversed phase partition chromatogram (phase system C) of products from oxidative ozonolysis of PGE methyl ester acetate.

Identification of α -acetoxy-heptylic acid

The material contained in peak IV of the chromatogram shown in Fig. 5 was found to have an equivalent weight of about 190 by titration with 0.02 N NaOH.

Hydrolysis. An excess of 0.1 N sodium hydroxide was added and back titration was performed after 18 h at room temperature. This revealed the liberation of 1.08 equivalents of an acidic group per equivalent of acid. In combination with the above-mentioned GLC analysis of the acid before and after hydrolysis this result strongly indicated the presence of an acetoxy group.

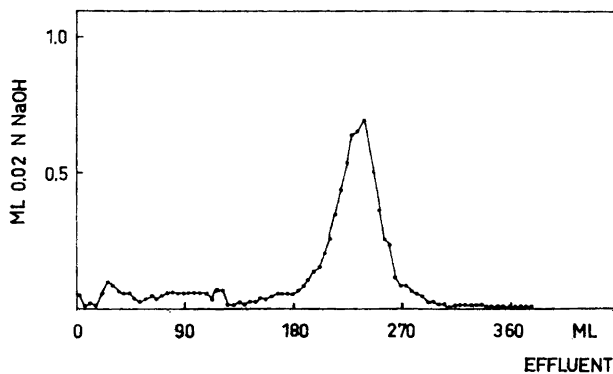


Fig. 6. Partition chromatogram of the hydrolyzed material retained in the stationary phase of the run shown in Fig. 5.

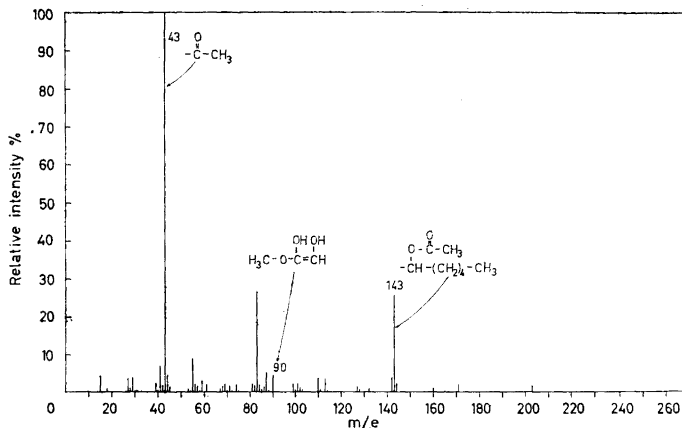


Fig. 7. Mass spectrum of the methyl α -acetoxyheptylate isolated from the ozonolysis of PGE methyl ester acetate (see text).

Acid treatment of the hydrolyzed acid failed to give a lactone as judged by GLC, thus excluding the presence of a γ or δ hydroxyl group.

Quantitative chromic acid oxidation in 95% aqueous acetic acid at 50° showed a consumption of 4.24 oxidation equivalents after 30 min, thereby excluding a hydroxyl substituent in β -position.⁵

Quantitative lead tetraacetate oxidation of the free acid gave an uptake of 1.72 oxidation equivalents after 40 min at 50° whereas the methyl ester only consumed 0.09 equivalents. This result indicated the presence of an α -hydroxy-acid structure in the isolated degradation product.

As an acetoxy group is equivalent to M 2.5 in the GLC-system used the parent acid should have 9.5–2.5 = M 7.0, i.e. heptylic acid.

Preparation of α -acetoxy-heptylic acid. Heptylic acid was brominated and esterified according to the method described by Schwenck and Papa⁸ and the ethyl ester of α -bromoheptylic acid was isolated by distillation (b.p. 110–111° at 15 mm, cf. Staudinger and Ruzicka⁹). Hydrolysis followed by treatment with diazomethane and acetylation with

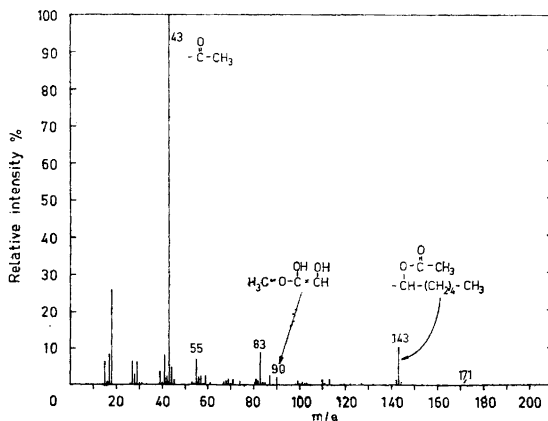


Fig. 8. Mass spectrum of authentic methyl α -acetoxyheptylate.

acetic anhydride in pyridine yielded methyl α -acetoxy heptanoate. This compound behaved identically with the isolated compound (M 9.5) in GLC and was collected for masspectrographic analysis.

The identity of this compound with that obtained by oxidative ozonolysis of the acetylated methyl ester of PGE could be established by comparing the mass spectrograms which are shown in Figs. 7 and 8.

Optical rotation. 33.7 mg of the α -hydroxyheptylic acid peak obtained from the chromatogram of the ozonolysis products of acetylated PGE methyl ester were dissolved in ethyl acetate and decolorized with Norite. 10.1 mg of the partly crystalline material were dissolved in 1 ml of 1 M sodium hydroxide and the rotation determined in an 1 dm tube. $[\alpha]_D^{23}$ was found to be $+ 9.0^\circ$ indicating the acid to be the D - α -hydroxyheptylic acid (*cf.* Baker and Meister¹⁰).

Oxidative ozonolysis of PGF_{1 α} and PGF_{1 β}

The methyl esters of PGF_{1 α} and PGF_{1 β} prepared with diazomethane,³ were acetylated with pyridine and acetic anhydride for 15 h at room temperature. A sample of each were treated with ozone and oxidized with acetic acid and hydrogen peroxide under conditions identical with those used for degradation of PGE.

GLC analysis of the oxidation products after treatment with diazomethane revealed the presence of two main fragments from each of PGF_{1 α} and PGF_{1 β} . The M-values of these compounds and of those derived from PGE are given in Table 1.

Table 1.

Compound	Products	
PGE	M = 9.5	M = 16.0
PGF _{1α}	M = 9.5	M = 19.1
PGF _{1β}	M = 9.5	M = 20.3

The compounds with M equal to 9.5 were identified as α -acetoxyheptylic acid methyl ester by masspectrometric analysis of material collected by GLC.

The masspectrometric data of the larger fragments obtained from PGF_{1 α} and PGF_{1 β} were too complex to allow a direct unequivocal interpretation.

DISCUSSION

The isolation of a high yield of suberic acid when PGE was oxidized with chromic acid showed that six unsubstituted methylene groups are present in one chain, and the isolation of α -hydroxy-heptylic acid after oxidative ozonolysis proves that seven other carbon atoms are located in a side chain with the configuration $=\text{CH}\cdot\text{CHOH}\cdot(\text{CH}_2)_4\text{CH}_3$.

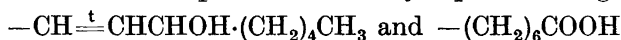
IR-data indicate that a cyclopentanone ring is present in PGE. One possibility would then be that the five carbons unaccounted for constitute this ring. In such a case the hydroxyheptyl residue is connected directly to the cyclopentane ring with a double bond and the eight carbon atoms might then all be present in another side chain provided that the cyclopentane ring did not contain a third side chain. Another possibility would be that it is connected through a methine group to the cyclopentane ring but then one of the carboxyl

As is evident from the mass spectrum in Fig. 9 the highest peaks are at 315, 298, 283, 266, 251, 239 which all fit a molecular weight of 330 but are incompatible with 300.

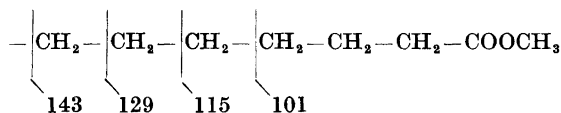
$$\begin{aligned} 330 &= M \\ 315 &= M-15 \\ 298 &= M-32 \\ 283 &= M-(32+15) \\ 266 &= M-(2 \times 32) \\ 251 &= M-(2 \times 32+15) \\ 239 &= M-(32+59) \\ 207 &= M-(2 \times 32+59) \text{ etc.} \end{aligned}$$

The compound as expected easily loses methanol (32) from the remaining methyl ether groups. The facile loss of 59 is in accordance with the presence of a carbomethoxy grouping directly on a ring. The fragmentation of a carbomethoxy grouping from a straight chain unsubstituted acid is not a marked reaction under these conditions.

The data obtained at this stage thus support the following structural details of PGF_{1α}. It contains a central cyclopentane ring on which two hydroxyls are present. Two side chains are present on the cyclopentane ring:



The presence of the latter side chain is further supported by the marked peaks at 298-143 = 155, 298-129 = 169 and 298-101 = 197 etc.



The difference between PGF_{1α} and PGF_{1β} is the stereoisomerism of the hydroxyl group that is formed on reduction of the keto group that is present on the cyclopentane ring in PGE.

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