Prostaglandins and related factors: XIX. Thin-layer chromatography of prostaglandins

KRISTER GRÉEN and BENGT SAMUELSSON

Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

SUMMARY Methods are described for separation of all known prostaglandins and some derivatives as free acids and methyl esters by thin-layer chromatography both on analytical and preparative scales. The use of silica gel containing silver nitrate was required for separations of compounds differing in the degree of unsaturation whereas ordinary silica gel was suitable for group separations of prostaglandin E, prostaglandin F_α, and prostaglandin F_β compounds.

THE LIPID-SOLUBLE acidic material with smooth muscle and vasodepressor activity present in seminal plasma and in accessory genital glands (1–3) (prostaglandin) has recently been shown to include several closely related compounds. Thus, prostaglandin E_1 (PGE_1) and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) were isolated from sheep vesicular glands (4) and later two new biologically active compounds, prostaglandin E_2 (PGE_2) and prostaglandin E_3 (PGE_3), were obtained from the same source (7). The structures of these compounds[1] have been elucidated (5, 7, 8). Recently, a reduction product of prostaglandin E_2, called prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), was isolated in small amounts from lungs of sheep and pig (9).

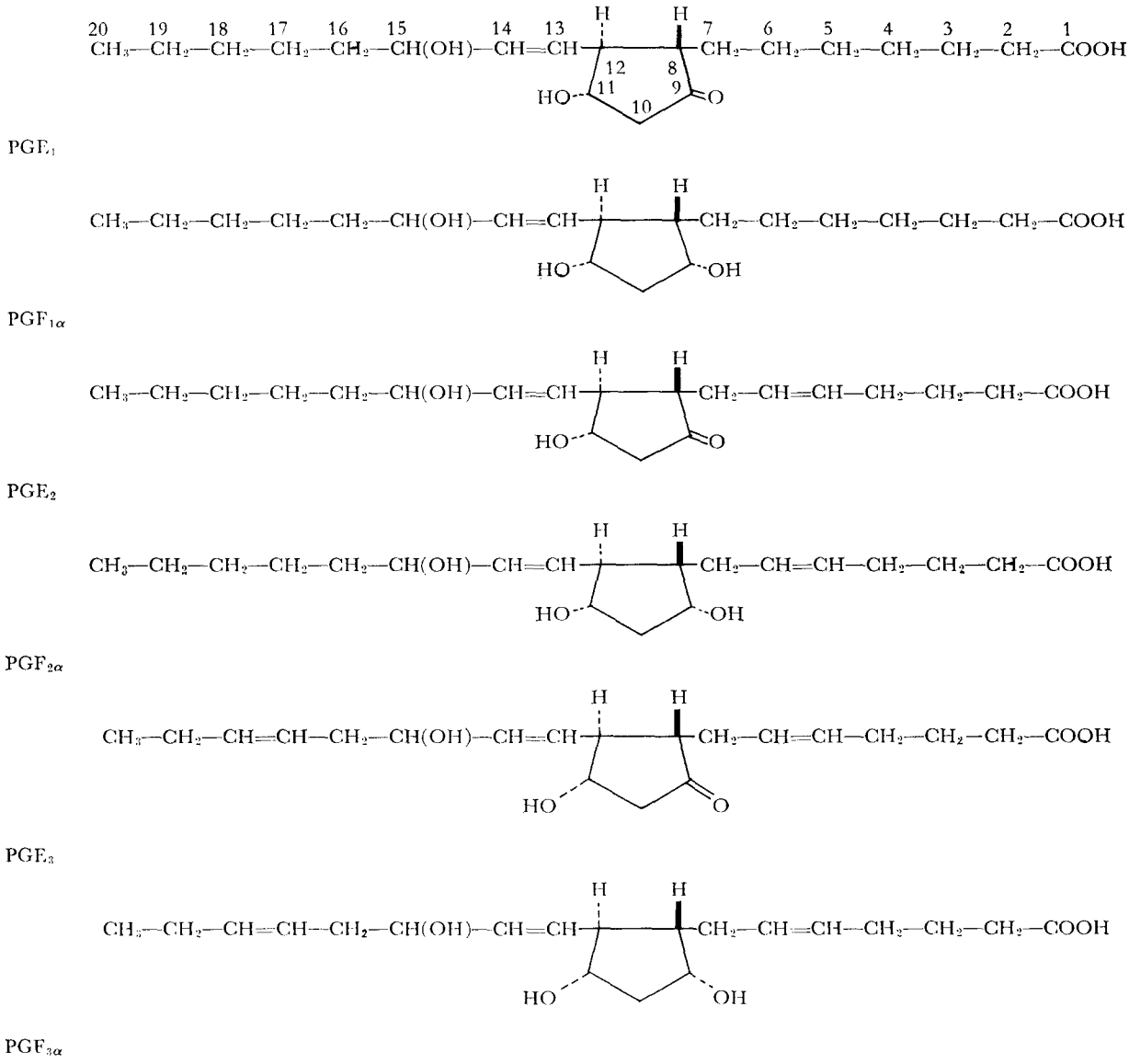
Earlier work on the separation of prostaglandins has mainly been concerned with fractionations on a preparative scale. Thus, reversed phase partition chromatography (4, 7) allows an efficient separation of the different PGE compounds and silicic acid chromatography effects the group separation of PGE compounds from the PGF compounds (10). The latter fractionation is of importance since PGE and PGF compounds occur together in material obtained from natural sources and since there is overlapping of the PGF compounds with compounds of the PGE type in separations by reversed phase partition chromatography.

On an analytical scale, descending paper chromatography separates the different PGE compounds whereas there is no appreciable separation of the compounds of the PGF type except for separation of the epimeric alcohols produced by chemical means (7).

Gas-liquid chromatography of methyl esters or methyl ester acetates of compounds of the PGF type separates $PGF_{1\alpha}$ from those containing either two or three double bonds; however, no separation of the two latter derivatives has been achieved (7). Gas chromatography of PGE compounds has so far not been possible without at least partial degradation.

The work described in the present paper is concerned with the separation of prostaglandins by thin-layer chromatography (TLC). The use of silica gel containing silver nitrate, essentially as described by Barret et al. (11), has greatly facilitated the separation of derivatives with different degrees of unsaturation. These methods allow separation of all known prostaglandins and have proved useful for analytical purposes and, on a preparative scale, for the final isolation of prostaglandins in pure form from material obtained after preliminary purifications.

[1] Prostaglandin E_1 (PGE_1) gives, on reduction of the keto group $PGF_{1\alpha}$ and $PGF_{1\beta}$, which are epimeric at CA. Corresponding derivatives are obtained from PGE_2 and PGE_3; i.e., $PGF_{2\alpha}$ and $PGF_{2\beta}$ and $PGF_{3\alpha}$ and $PGF_{3\beta}$. The hydroxyl group at C9 is *cis* to the carboxyl side chain in α-derivatives, and *trans* in β-derivatives (6). The systematic nomenclature recently introduced is based on the trivial name prostanoic acid for the parent C_{20} acid. Prostaglandin E_1 (PGE_1) 11α,15-dihydroxy-9-keto-prost-13-enoic acid. Prostaglandin E_2 (PGE_2) 11α, 15-dihydroxy-9-keto-prosta-5,13-dienoic acid. Prostaglandin E_3 (PGE_3) 11α,15-dihydroxy-9-keto-prosta-5,13,17-trienoic acid. Prostaglandin $F_{i\alpha}$ (earlier PGF_{1-1}) 9α,11α,15-trihydroxy-prost-13-enoic acid. Prostaglandin $F_{1\beta}$ (earlier PGF_{2-1}) 9β,11α,15-trihydroxy-prost-13-enoic acid. Prostaglandin $F_{2\alpha}$ (earlier PGF_{1-2}) 9α,11α,15-trihydroxy-prosta-5,13-dienoic acid. Prostaglandin $F_{2\beta}$ (earlier PGF_{2-2}) 9β,11α,15-trihydroxy-prosta-5,13-dienoic acid. Prostaglandin $F_{3\alpha}$ (earlier PGF_{1-3}) 9α,11α,15-trihydroxy-prosta-5,13,17-trienoic acid. Prostaglandin $F_{3\beta}$ (earlier PGF_{2-3}) 9β,11α,15-trihydroxy-prosta-5,13,17-trienoic acid.



EXPERIMENTAL METHODS

The glass plates (0.40 x 20 x 20 cm or 0.40 x 5 x 20 cm) were coated by spreading a mixture of 30 g Silica Gel G[2] and 60 ml water using the applicator[3] designed by Stahl (thickness of coating approximately 0.25 mm). In some cases, silver nitrate (1–7 g as indicated) was dissolved in the water before addition to the silica gel. This procedure was found to give more reproducible results than spraying the plates with an alcoholic solution of silver nitrate. The plates were activated by heating for 30 min at 110–115° and the plates were subsequently kept in a closed box. The materials to be analyzed were applied on the plates as single spots (10–100 μg) or as bands (up to 10 mg) using a Hamilton syringe.

The chromatoplates were placed in glass jars covered with ground glass plates. The plates were developed using the ascending technique, which was interrupted when the solvent front reached a point 2–4 cm from the top (50–100 min).

After development, the plates were dried at 100° and sprayed with 10% phosphomolybdic acid in ethanol and heated for 15 min at 120°, which resulted in the appearance of blue spots on a yellow-green background. This was the preferred method on chromatoplates containing silver nitrate since spraying of these plates with sulfuric acid followed by heating at 250° gave very weak spots with compounds containing several double bonds.

Preparative TLC. In preparative runs, the plates were dried at room temperature and the substances visualized by spraying with water. For recovery, the zones were scraped off with a spatula and transferred to test tubes.

[2] E. Merck, A. G., Darmstad, Germany.
[3] C. Desaga, G.m.b.H., Heidelberg, Germany.

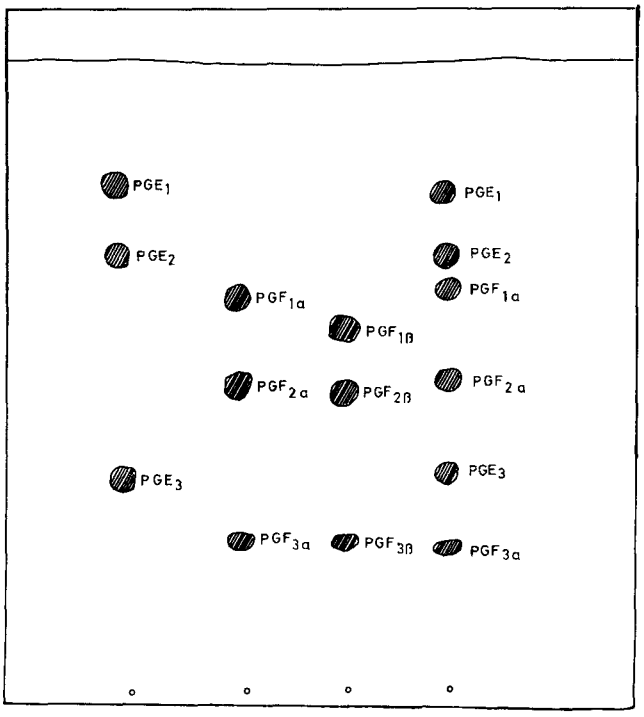


FIG. 1. Separation of prostaglandins as free acids with solvent system A II. The abbreviations used are given in footnote 1.

Methanol (5 ml) was added to each tube, the tubes were swirled, and the contents were allowed to sediment. The almost clear supernatant solution was pipetted off and filtered. This procedure was repeated twice using 2 ml methanol, and the combined extracts were evaporated to dryness in vacuo. The residue was extracted with chloroform (3 x 2 ml) and the solution evaporated to dryness. The methyl esters were recovered in 90–95% yield by this procedure as tested with tritium-labeled PGE_1.

An alternative method for the free acids was worked out and evaluated by use of tritium-labeled PGE_1. The silica gel was extracted with methanol as described above and the extract evaporated to about 1 ml. This solution was diluted with 5 ml water, acidified with 1 N hydrochloric acid, and extracted three times with equal volumes of ether. The ether phases were washed repeatedly with water (until neutral), and evaporated to dryness in vacuo. Recoveries of 80–90% were obtained.

Solvent Systems. All solvents except acetic acid (analytical grade, Merck, A.G., Germany) were redistilled before use. The systems used were:

M I	Benzene–dioxane	5:4
M II	Ethyl acetate–methanol–water	8:2:5
M III	Ethyl acetate–methanol–water	16:2.5:10

TABLE 1 R_F VALUES OF PROSTAGLANDINS IN FIVE SOLVENT SYSTEMS

	R_F of Methyl Esters			R_F of Free Acids	
	Solvent System			Solvent System	
Compound	M I*	M II	M III	A I*	A II
PGE_1	0.58	0.65	0.62	0.62	0.80
PGE_2	0.57	0.57	0.49	0.62	0.70
PGE_3	0.58	0.29	0.20	0.63	0.35
$PGF_{1\alpha}$	0.38	0.47		0.46	0.64
$PGF_{1\beta}$	0.25	0.43		0.35	0.58
$PGF_{2\alpha}$	0.37	0.35		0.47	0.49
$PGE_{2\beta}$	0.26	0.33		0.36	0.48
$PGF_{3\alpha}$	0.38	0.18		0.47	0.23
$PGF_{3\beta}$	0.26	0.18		0.36	0.23
PGE_1-278			0.90		
PGE_2-278			0.84		
PGE_3-278			0.50		

* Without silver nitrate.

A I	Benzene–dioxane–acetic acid	20:20:1
A II	Ethyl acetate–acetic acid–methanol–2,2,4-trimethyl pentane–water	110:30:35:10:100

In separations with solvent systems M II, M III, and A II, the phases were equilibrated for 2 hr and the less polar phase was used.

Prostaglandins. Prostaglandins E_1, E_2, and E_3 were obtained from sheep vesicular glands as described earlier (7). Prostaglandin $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$, $F_{2\beta}$, $F_{3\alpha}$, and $F_{3\beta}$ were prepared by sodium borohydride reduction of the corresponding E compounds and purified by reversed phase partition chromatography.

Prostaglandin E_1-278, E_2-278, and E_3-278 were prepared by treatment of the PGE compounds with alkali (7). Methyl esters were prepared by treatment of the acids with diazomethane. Tritium-labeled prostaglandin E_1 was synthesized according to Samuelsson.[4]

RESULTS AND DISCUSSION

Preliminary experiments demonstrated that PGE and PGF compounds can be separated by thin-layer chromatography on silica gel with solvent systems A I or M I. No separation of the different PGE and PGF compounds occurred. When silver nitrate was incorporated into the plates, however, the individual derivatives of both the PGE and PGF type were separated. These derivatives differ in the number of double bonds in the molecule (7, 8) and the separation is due to formation of charge transfer complexes between the olefinic double bonds and silver ions (cf ref. 11, 12).

[4] B. Samuelsson. Data to be published.

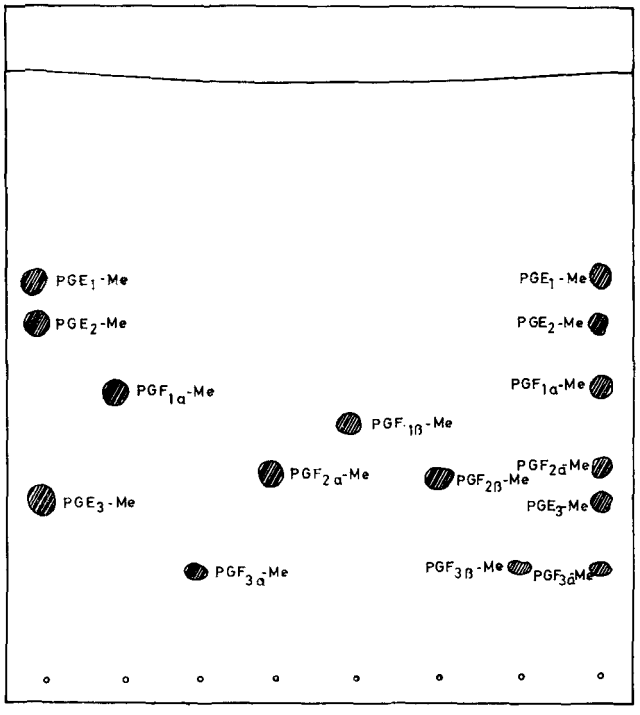


FIG. 2. Separation of methyl esters of prostaglandins with solvent system M II. The abbreviations used are given in footnote 1.

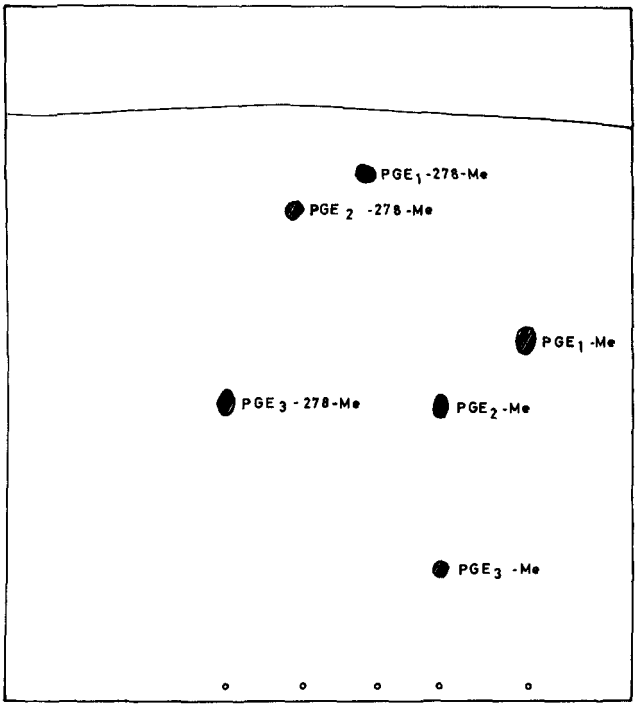


FIG. 3. Separation of methyl esters of prostaglandins and derivatives with solvent system M III. PGE_1-278-Me, PGE_2-278-Me, and PGE_3-278-Me are methyl esters of compounds obtained by treatment of PGE_1,[1] PGE_2, and PGE_3, respectively, with alkali (7).

A chromatogram of all the known prostaglandins with solvent system A II on a plate containing silver nitrate is shown in Fig. 1. All the derivatives except for $PGF_{2\alpha}$–$PGF_{2\beta}$, and $PGF_{3\alpha}$–$PGF_{3\beta}$ are separated from one another. The complexing with silver ions dominates here and abolishes the effect of the positions of the hydroxyl groups on the adsorption to the silica gel. However, a complete separation of the stereoisomeric compounds is achieved when chromatoplates without silver nitrate are used and developed with solvent system A I (Table 1).

A corresponding separation of methyl esters of the different prostaglandins has been achieved with solvent systems M II (Fig. 2) and M I (Table 1).

When prostaglandins E_1, E_2, or E_3 are treated with alkali, less polar compounds are formed by dehydration and isomerization of the double bond (7). These derivatives (PGE_1-278, PGE_2-278, and PGE_3-278), which sometimes are of interest for identification purposes, have been separated as the methyl esters with solvent system M III (Fig. 3).

The separations with respect to the degree of unsaturation can be further accentuated by increasing the amount of silver nitrate. This has occasionally been used for preparative purposes when difficulties were encountered in removing contaminating material.

Isolation of prostaglandins from the silica gel was done by extraction with methanol. Methyl esters were reextracted with chloroform after evaporation of the solvent. This procedure gave a 90–95% recovery of the methyl esters. The free acids were extracted with ether after dilution with water and acidfication. In this way 80–90% of the free acids were recovered.

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