ACTA CHEMICA SCANDINAVICA 14 (1960) 1693–1700

# The Isolation of Prostaglandin F from Sheep Prostate Glands

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A smooth muscle stimulating factor present in vacuum-dried sheep prostate glands has been isolated in crystalline form. The formula $C_{20}H_{36}O_5$ has been suggested.

Goldblatt [1] and U. S. von Euler [2,3] described the presence of a smooth muscle stimulating and blood pressure reducing activity in sperm and in extracts of the prostate glands of certain species. The activity was ascribed to a factor named "Prostaglandin" by von Euler, who made studies on the distribution of this type of activity in various organs and species. He also made extensive physiological studies on a concentrate prepared from sheep prostate glands [4–6].

This raw material was used by Bergström [7] for further concentration of the factor. We have continued this work and have succeeded in isolating a crystalline compound, PGF, from vacuum-dried sheep prostate glands. This compound has a stimulating effect on smooth muscles of rabbit intestinal strips but does not show any effect on the blood pressure of rabbits. This paper describes the isolation procedure and some of the properties of the compound. The isolation of a second factor from frozen sheep prostate glands is described in a following paper.

## EXPERIMENTAL AND RESULTS

*Starting material.* The glands (*glandula vesicalis*) located in the area where the urethra leaves the bladder, were removed at slaughter, immediately frozen in dry ice and stored at —20°C. They were then ground through a quarter-inch plate in a semifrozen condition and promptly dried in a vacuum mixer. The dehydrated material was then shipped to our laboratory and stored at —20°C for several years before being processed. Only this type of starting material was used for the work described in this paper.

We are greatly indebted to Dr. Walter Lundberg of the Hormel Institute, to Mr. Murphy, and Hormel & Co., Austin, Minn. U.S.A., for the collection of the sheep prostate glands used in this investigation.

*Extraction procedure.* During the various stages of the isolation procedure, the physiological activity was determined on duodenal intestinal strips of rabbits in a bath of 30 ml (*cf.* Ref.[5]).

The dried glands were suspended with a mechanical stirrer in 4 liters of tap water per kg of dried glands and 15 min later 12 liters of 95 % ethanol were added. The suspension was stirred mechanically for about one hour and then left to sediment overnight. The supernatant, clear, ethanol solution was siphoned off and the insoluble residue strained through cheese cloth and filtered. The combined extracts were evaporated *in vacuo* to about one twentieth of their original volume.

This crude extract was first extracted once with its own volume of ether. The aqueous phase was then acidified to about pH 3.5 with 6 N hydrochloric acid and extracted once with its own volume and then twice with half the volume of ether. The aqueous phase was discarded and the combined ether extracts were extracted 6 times with half the volume of 0.2 M sodium phosphate buffer of pH 8. During the first extraction, the pH of the buffer had to be adjusted back to pH 8 with 2 M sodium carbonate. The ether phase was discarded and the combined buffer phases were acidified to pH 3 with hydrochloric acid. They were extracted once with one volume of ether and then twice with half the volume of ether. The aqueous phase was discarded. The combined ether extracts were washed until free of chloride ions with small portions of water, each of which passed a second ether phase. The combined ether phases were then evaporated to dryness *in vacuo* leaving a dark, brown paste.

*Preliminary purification.* The residue obtained as described in the previous section was subjected to a five or ten-stage counter-current distribution between equal volumes (200 ml per 5 g of extract) of ether and 0.5 M sodium phosphate buffer pH 6.4. The buffer phases were acidified and extracted three times with ether. All the phases were evaporated and weighed and the physiological activity of each phase was determined. An example of a five-stage, counter-current distribution is given in Table 1, though a ten-stage procedure is more efficient and has more often been used.

*Table 1.*

| Phase number | Weight (g) | Physiological activity Relative units per phase | per mg |
|---|---|---|---|
| Ether 1 | 6.40 | 7 200 | 1 |
| » 2 | 0.80 | 2 200 | 3 |
| » 3 | 0.25 | 1 300 | 5 |
| » 4 | 0.15 | 1 000 | 7 |
| » 5 | 0.10 | 1 200 | 12 |
| Buffer 5 | 0.06 | 1 700 | 28 |
| » 4 | 0.05 | 1 900 | 38 |
| » 3 | 0.05 | 2 400 | 48 |
| » 2 | 0.06 | 2 200 | 37 |
| » 1 | 0.80 | 2 100 | 3 |

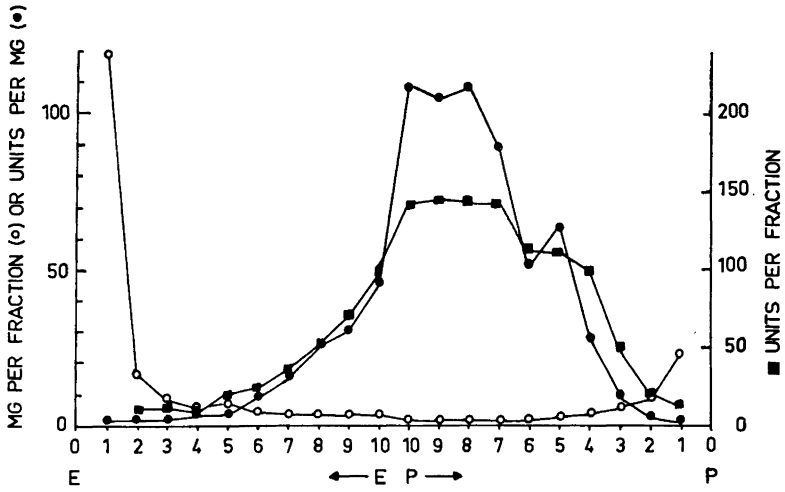


*Fig. 1.* Purification of PGF with a ten-stage, counter-current distribution of the crude extract of prostate glands. E = ether phases, P = buffer phases.

The material corresponding to that contained in ether 4 to buffer 2 was combined and used for the further purification of PGF. Different lots of prostate glands gave somewhat different distributions of physiological activity in the counter-current distribution procedure. The activity in the earlier ether phases only occurred in certain batches of dried glands apparently due to the handling during collection and drying. In Fig. 1 a ten-stage distribution of material that did not contain any activity in the first ether phases is

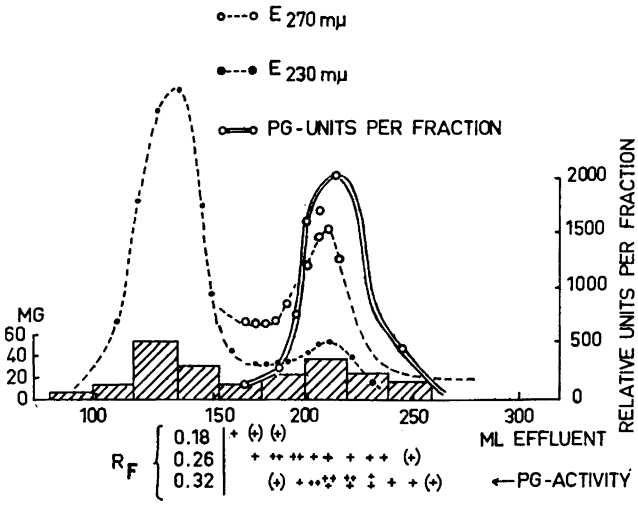


*Fig. 2.* Reversed phase chromatography of material (prostaglandin concentrate, sheep) from the countercurrent distribution. For explanations see text.

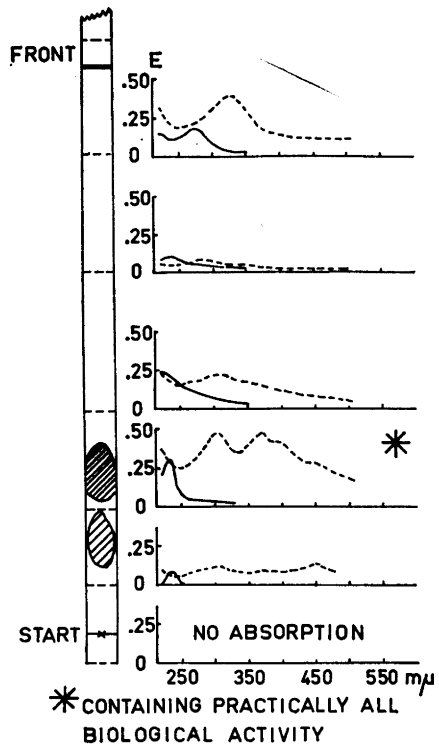


*Fig. 3.* Paper chromatographic analysis of the active material obtained after the purification with reversed-phase chromatography. For explanations see text.

shown. A study of the more lipophilic factor will be described in a following paper.

*First partition chromatography.* The material from the phases ether 4 to buffer 2 in a five-stage, counter-current distribution, or the corresponding phases in a ten-stage procedure, was subjected to reversed phase partition chromatography. 300 ml of 50 % (v/v) methanol/water was equilibrated with 30 ml of 50 % (v/v) *iso*octanol/chloroform in a separatory funnel. 4 ml of the less polar phase were supported on 4.5 g of hydrophobic Supercel (silane treated kieselguhr) which was then slurried in the aqueous phase, poured into a chromatography tube and allowed to settle. A one cm layer of sand was put on the top of the column. About 100 mg of the material was then dissolved in the smallest possible amount of moving phase and added onto the top of the column. When larger amounts of material were run, the size of the column was correspondingly increased and it was found that columns with 90 g of Supercel gave good results with 2—3 g of material.

A typical result of a chromatographic separation with an 18 g column is shown in Fig. 2. Besides the distribution of physiological activity (determined in every third fraction) and weight, the light absorption at 270 and 230 mμ of diluted aliquots of the fractions are shown.

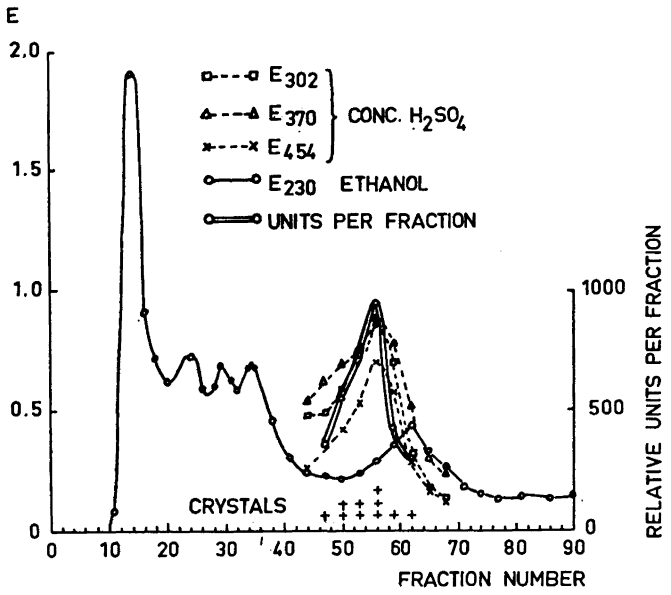


*Fig. 4.* Chromatography with the acid solvent system of the active material (purified PG) obtained after reversed-phase chromatography. For explanations see text.

*Paper chromatography.* The phase system used for the paper chromatography of prostaglandin concentrates has been utilized before for the separation of bile acids [8,9]. Ethylene chloride/heptane 50/50 was used as moving phase after equilibration with an equal volume of 70 % (v/v) acetic acid/water. A suitable aliquot of the fractions (usually about 50 $\mu$g of material) was put on the starting line of the filter paper (Whatman 3 MM). After equilibration overnight in the chromatographic tank in the vapors of both phases, the papers were brought into contact with the moving phase and developed for 6 h using the ascending technique. After drying, the papers were sprayed with a 15 % ethanolic solution of phosphomolybdic acid. Heating for a few minutes at 80°C revealed blue spots on the chromatograms. Ethanol elution of zones on unsprayed papers, corresponding to these spots, revealed that practically all of the physiological activity was confined to the zone of one of the spots. The results of paper chromatographic analyses of fractions from the chromatography shown in Fig. 2 are shown in the same figure below the chromatogram. The $R_F$ values of the spots varied in different runs, but the relative position of the spots and the physiological activity was unchanged.

The fractions from the reversed-phase partition chromatography that contained the physiological activity were combined. Aliquots of this material were used for a spectrophotometric analysis after paper chromatography, according to the method described by Sjövall [9]. Paper chromatography, with the phase system described above, was performed on washed filter paper strips with purified solvents. After the development of the chromatograms, unsprayed

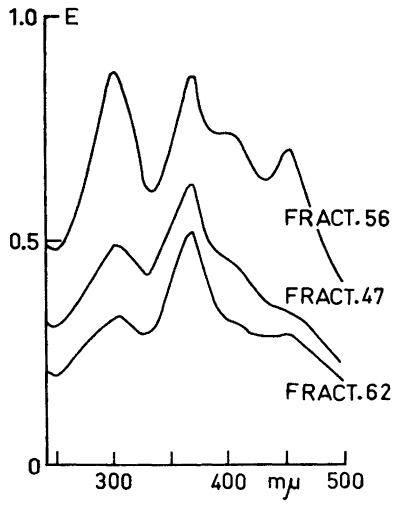


*Fig. 5.* Sulphuric acid spectra of active material (fractions from K 25) from the chromatography shown in Fig. 4. The fraction numbers refer to this figure.

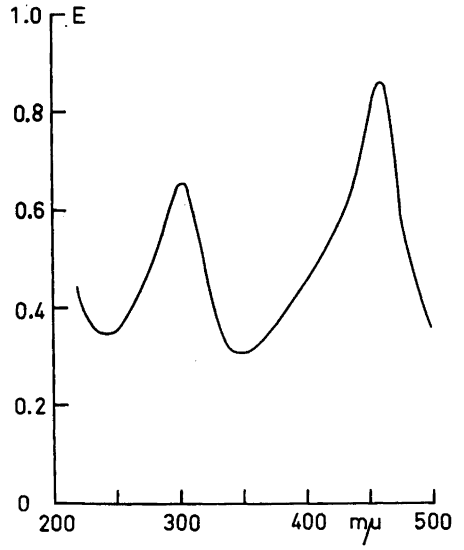


*Fig. 6.* Sulphuric acid spectrum of crystalline PGF, (31.2 μg after 2 h at room temperature in 1 ml conc. $H_2SO_4$).

strips were cut into pieces with the guidance of the spots on the sprayed strip. These pieces were eluted with ethanol and the eluates evaporated to dryness *in vacuo*. Light absorption spectra were then recorded in ethanol solution and in 96 % sulphuric acid after 2—3 h at room temperature. An automatic spectrophotometer (Perkin-Elmer, Spectracord 4 000) was used. The physiological activity of the eluates was also determined; the results of a typical analysis are shown in Fig. 3. The area containing the physiological activity had a typical sulphuric acid spectrum, and corresponded to a blue spot obtained with phosphomolybdic acid.

*Second partition chromatography.* It was evident (Fig. 3) that the material from the reversed-phase partition chromatography was inhomogeneous. A column chromatography with an acid solvent system was therefore developed. Ethylene chloride/heptane 50/50, after equilibration with 1/3 of its volume of acetic acid/water 60/40 was found to be a suitable moving phase. 4 ml of the acetic acid/water phase were supported on 4.5 g of Hyflo Supercel and served as stationary phase. The column was prepared as described for the reversed phase column. 50 mg of the material from the reversed-phase partition chromatography were run on a column of this size. With larger amounts, the column was correspondingly increased. The material was transferred onto the top of the column dissolved in the smallest possible amount of moving phase.

Fig. 4 illustrates a run of about 200 mg of material on a column with 18 g of Supercel. The peak of physiological activity appeared at approximately 280 ml effluent. Suitable aliquots of every third fraction were evaporated to

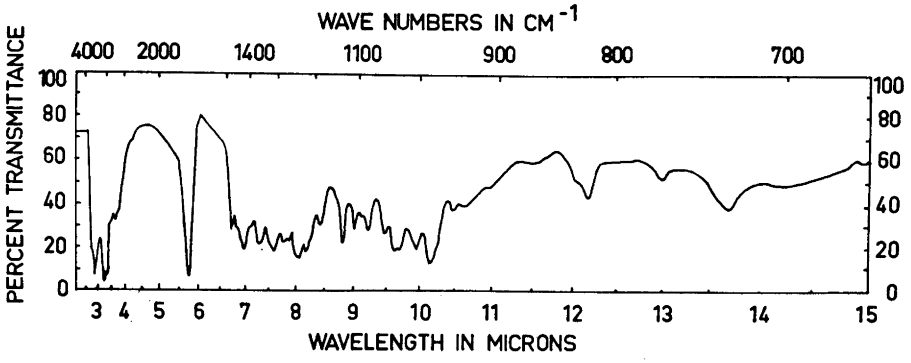


*Fig. 7.* Infrared spectrum of the methyl ester of PGF (KBr disc).

dryness *in vacuo* and were dissolved in one ml of ethanol. The U.V. spectra were recorded and it was found that most fractions had an absorption maximum at 230 mμ. The extinction of the different fractions at this wavelength is shown in Fig. 4. It is evident that there is no correlation to the physiological activity. One-thirtieth of each of these fractions was then evaporated to dryness and dissolved in 1 ml of 96 % sulphuric acid. After 2—3 h at room temperature, the U.V. spectra were recorded. The spectra of fractions at the beginning, middle and end of the band of physiological activity are shown in Fig. 5. Three absorption maxima are found at 302, 370 and 454 mμ, respectively, and the extinctions at these wavelengths are recorded in Fig. 4. As seen from Fig. 5, the band of physiological activity does not consist of a pure compound since the ratios between the extinctions at the three maxima are different in the beginning, middle and end of the band. The best correlation between activity and light absorption was found for the maxima at 320 and 454 mμ.

Paper chromatography of aliquots of the fractions showed just one spot with the phosphomolybdic acid reagent and physiological activity was confined to the same zone as this spot. By making spot tests with approximately 20 μl of the effluent from the acetic acid columns, the material containing the physiological activity could be detected. This method was sometimes used instead of testing on intestinal strips.

*Crystallization.* After evaporation in a vacuum oven at 50°C, the fractions containing the main part of the physiological activity crystallized partly in needle-shaped crystals. The relative distribution of crystals is shown in Fig. 3. The amount of crystals in each tube was less than 1 mg in this run.

The crystals were found to be relatively insoluble in ether. Trituration of each fraction with a few drops of ether at 0° dissolved the oily substance, leaving most of the crystals. After pipetting off the ether, the crystalline residues were combined and recrystallized several times from ethyl acetate/light petroleum until a constant m.p. of 102—103°C was reached. The crystals were small, colourless needles. In all, we have had about 20 mg of this material. The sulphuric acid spectrum of pure PGF is shown in Fig. 6.

Elementaly analysis yielded the following results:

| | | C | H | O | N |
|---|---|---|---|---|---|
| Found: | | 67.2 | 10.0 | 22.6 | 0.0 |
| Calc. for | $C_{20}H_{32}O_5$ (352.45) | 68.2 | 9.2 | 22.7 | |
| | $C_{20}H_{34}O_5$ (354.47) | 67.8 | 9.7 | 22.6 | |
| | $C_{20}H_{36}O_5$ (356.49) | 67.4 | 10.2 | 22.4 | |

CHN-analysis was done with an ultramicro gasometric technic by Dr. W. Kirsten, Uppsala, and oxygen determinations by Dr. K. J. Karrman, Lund.

A titration of 2.50 mg in 50 % ethanol yielded a curve indicating a p$K$ of 6.3 and an equivalent weight of 357 ($\pm$ 20).

A titration of cholic acid under the same conditions yielded the half equivalent point at 6.3 and an equivalent weight of 412 (calc. 409).

A microhydrogenation in ethanol-acetic acid and Adams-Shriner platinum catalyst at room temperature resulted in the uptake of slightly more than one mole of hydrogen per an assumed molecular weight of 356.

The infrared spectrum of the methylester is shown in Fig. 7.

Dr. Sixten Abrahamsson, Uppsala, has kindly determined the interplanar spacings by X-ray diffraction of crystalline PGF. The values obtained were as follows (Ångström units; intensities put in brackets with the scale *v*ery *s*trong, *s*trong, *m*edium, *w*eak, *v*ery *w*eak): 16.44 (s), 9.46 (w), 8.16 (w), 6.19 (m), 5.46 (m), 5.05 (m), 4.71 (vw), 4.63 (vs), 4.54 (m), 4.46 (m), 4.08 (m), 4.02 (s), 3.74 (w), 3.56 (w), 3.45 (vw), 3.07 (vw), 2.93 (w), 2.80 (w), 2.67 (vw), 2.56 (vw), 2.48 (vw), 2.39 (vw), 2.34 (vw) 2.02 (w), 1.48 (w).

One mg of the compound was treated with diazomethane and the resulting methyl ester subjected to cracking in a mass spectrometer (Dr. R. Ryhage, Stockholm). The masspectrum indicated a molecular weight of 356 for PGF. The details will be discussed in a forthcoming publication.

The results obtained would thus suggest the compound to be an unsaturated acid with the formula $C_{20}H_{36}O_5$.

*Acknowledgements.* The technical assistance of Mr. L. Krabisch, Miss Anita Ivarsson and Miss Berit Persson is gratefully acknowledged.

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Received June 16, 1960.