

## The Isolation of Prostaglandin E from Sheep Prostate Glands

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A smooth muscle stimulating and blood pressure depressing factor present in sheep prostate glands — Prostaglandin E — has been isolated in crystalline form. The most likely formula is  $C_{20}H_{34}O_2$ .

We have described the isolation of prostaglandin F from vacuum dried sheep prostate glands in the preceding paper<sup>1</sup>.

In that work it was noticed that a more lipid soluble factor was sometimes present which showed activity both on intestinal strips and on rabbit blood pressure. It has been found that this fraction is responsible for most of the activity of the fresh or frozen glands.

The isolation of this factor, prostaglandin E (PGE) in crystalline form, will be described in this paper.

### EXPERIMENTAL AND RESULTS

*Starting material.* Frozen sheep prostate glands were obtained from various slaughter houses.

*Extraction procedure.* The frozen glands were minced in a meat grinder. After addition of 4 liters of 95 % ethanol per kg, the suspension was stirred mechanically for one hour and then left to sediment over night. The extract was separated and concentrated as described in the preceding paper<sup>1</sup>. The crude concentrate was then extracted into ether and transferred into phosphate buffer as described in the same paper.

*Partition chromatography.* In this work, we left out the counter-current distribution procedure for the preliminary purification of the crude extract. Instead, this extract was directly subjected to reversed-phase chromatography with 50 % methanol/water as moving phase and 50 % *isooctanol*/chloroform as stationary phase, supported on Hostalen (Hoechst). In most cases the extract from 12.5 kg of prostate glands weighed 5—7 g and this material was put on a column with 67 ml of stationary phase on 100 g of Hostalen. The result of such a chromatography is shown in Fig. 1. The peak of physiological activity appeared at about 1.5 l effluent. This position varied somewhat depending

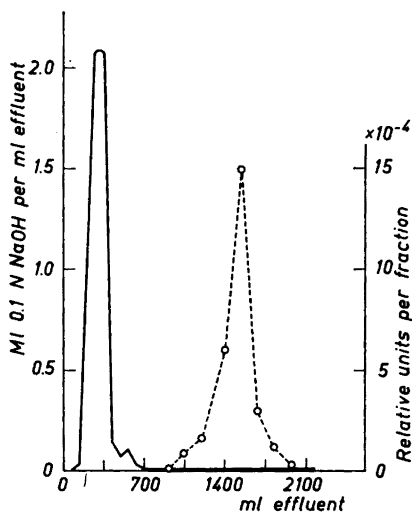


Fig. 1. Reversed phase partition chromatography of the crude extract of prostate glands.

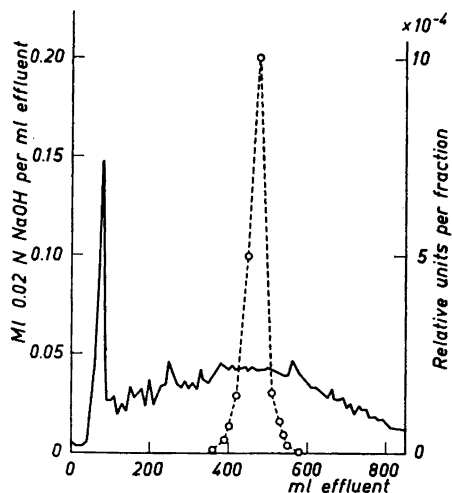


Fig. 2. Reversed phase chromatography of the active material obtained from the chromatography shown in Fig. 1.

upon the amount of material put on the column. Good separations have been obtained with 24 g of extract put on a column with 200 g of Hostalen. A 10 to 30-fold purification of the active material was obtained with the reversed phase chromatography.

The material obtained was found to lower rabbit blood pressure. This effect is not obtained with previously isolated PGF. It was found, however, that PGE, although being eluted somewhat later than PGF, was not separated from this compound. Therefore, a second reversed-phase chromatography was run using 35 % (v/v) methanol/water saturated with 1/10 of its volume of 40 % (v/v) *iso*amyl acetate/chloroform as moving phase. The hydrophobic phase was used as stationary phase, 4 ml being supported on each 4.5 g of silane treated kieselguhr. With this system, PGF is eluted just after the front, separated from PGE which is eluted at about 50 ml effluent from a 4.5 g column. For preparative purposes, 250—500 mg of material from the first chromatography were usually run on a column with 45 g of hydrophobic kieselguhr. Columns with up to 200 g of kieselguhr have been used for separation of larger amounts of material (3 g). Fig. 2 shows the chromatogram of the active material obtained from the chromatography shown in Fig. 1. No activity was found at the place of PGF in this case and an approximate 4-fold purification was obtained.

*Paper chromatography.* During the chromatographic purifications, paper chromatography was used to test the purity of the fractions eluted from the columns. This method was described in the preceding paper<sup>1</sup>. PGE was found to move somewhat faster than PGF on the chromatograms and clearly separated from this compound. However, attempts to use the column chroma-

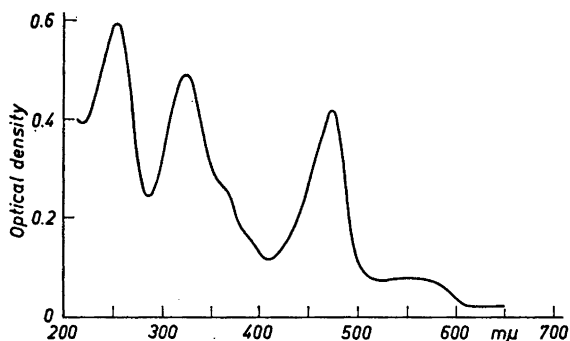


Fig. 3. Absorption spectrum of 40  $\mu$ g of PGE after 85 min in 96 % sulphuric acid.

tography with acid solvent systems<sup>1</sup> for the preparative separation of PGF and PGE failed; the compounds being eluted together in one peak with a displacement of only a few fractions. Paper chromatograms of the fractions from the second reversed-phase chromatography showed the highest purity of PGE in the last part of the peak of activity. Besides PGE, the first part of the active peak contained a compound giving a spot on the chromatograms between the positions of PGE and PGF.

*Crystallization of prostaglandin E.* The fractions from the second reversed phase chromatography, containing the last half of the active band, were combined as also were those containing the first half. After evaporation to dryness *in vacuo*, the residues were dissolved in a few ml of ethyl acetate and allowed to stand overnight at room temperature. Needle-shaped crystals were usually obtained from the material contained in the last half of the active band in contrast to the material from the first half of this band. The latter material, from several chromatograms, was therefore again subjected to reversed phase chromatography with 35 % methanol as moving phase and 40 % *isoamyl* acetate/chloroform as stationary phase and crystallization procedure repeated. In some cases, the noncrystalline material was subjected to chromatography with the acid solvent system<sup>1</sup> prior to the second reversed phase chromatography.

The crystalline material was recrystallized several times from ethyl acetate and ethyl acetate/heptane until a constant m.p. of 115–117°C was reached. The sulphuric acid spectrum<sup>1</sup> of the crystalline compound is shown in Fig. 3.

Ultramicro C, H analysis and oxygen determinations yielded the following results:

Found	C 68.0	H 9.7	O 22.9
Calc. for C <sub>20</sub> H <sub>34</sub> O <sub>2</sub> :	67.8	9.7	22.6

C, H analysis was done with an ultramicro gasometric technique by Dr. W. Kirsten, Uppsala, and oxygen determinations by Dr. K. J. Karrman, Lund.

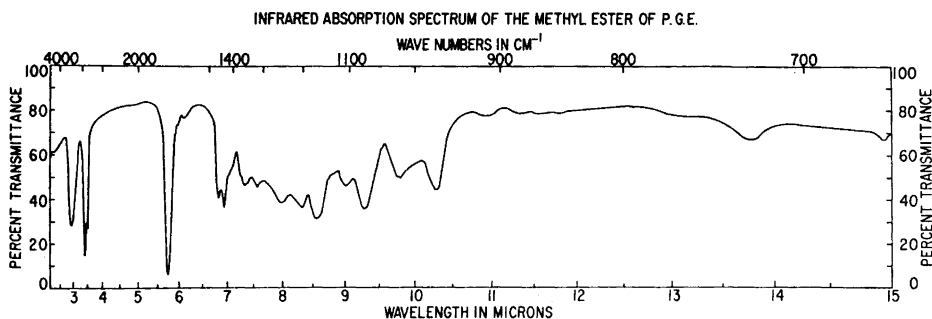


Fig. 4. Infrared spectrum of the methyl ester of PGE (KBr disc).

A microtitration curve obtained in 50 % ethanol indicated a  $pK$  of 6.5 and a molecular weight of  $350 \pm 20$ .

The methyl ester was prepared from one mg of the compound and subjected to cracking in a mass spectrometer (Dr. R. Ryhage, Stockholm). The results indicated a molecular weight of 354.

In a single crystal X-ray study done for us by Dr. S. Abrahamsson, Uppsala, it was found that there were eight molecules per unit cell. The unit cell dimensions were:  $a = 8.23 \pm 0.04$ ;  $b = 19.4 \pm 0.1$ ; and  $c = 25.9 \pm 0.1$  Å. The specific gravity, as determined by flotation in potassium bromide solution, was  $1.135 \text{ g/cm}^3$ . These results give a molecular weight of  $353 \pm 5$ .

The interplanar spacings in Ångström units obtained by X-ray diffraction of crystalline PGE (Dr. S. Abrahamsson, Uppsala), were as follows (the intensities are denoted with *very strong*, *strong*, *medium*, *weak*, *very weak*): 13.00 (s), 6.45 (vw), 6.13 (vw), 5.80 (w), 5.75 (m), 5.01 (m), 4.26 (vw), 4.20 (vw), 4.14 (vs), 3.97 (s), 3.91 (m), 3.75 (vw), 3.48 (w), 3.25 (w), 3.18 (w), 3.06 (w), 2.87 (vw), 2.75 (w), 2.65 (vw), 2.59 (vw), 2.50 (w), 2.44 (vw), 2.39 (vw), 2.38 (vw), 2.35 (vw).

The infrared spectrum of the methyl ester of PGE is shown in Fig. 4.

In a microhydrogenation of PGE in ethanol-acetic acid and Adams-Shriner platinum catalyst at room temperature, hydrogen was taken up corresponding to approximately one mole of hydrogen per molecule of 354.

The results, taken together, strongly indicate a  $C_{20}$  formula. The elementary analysis, the mass spectrometer data, and the X-ray data together support the formula  $C_{20}H_{34}O_5$  (354.5). The C values obtained are a little low, but ultramicro analysis of cholic acid also yielded carbon values that were about 0.5 % low.

The mass spectrometer data indicate that the peaks at 332 and 350, differing by 18 units, correspond to fragments of the methyl ester that have lost two and one molecule of water, respectively. The whole ester molecule would then have the weight 368 and the corresponding free acid a molecular weight of 354. This is also made very likely by the mass spectra of the ethyl ester.

Results of investigations of the physiological activity of PGE have been published separately <sup>2,3</sup>.

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