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SHORT COMMUNICATION

Smooth muscle stimulating lipids in sheep iris. The identification of prostaglandin $F_{2\alpha}$.* Prostaglandins and related factors 21.

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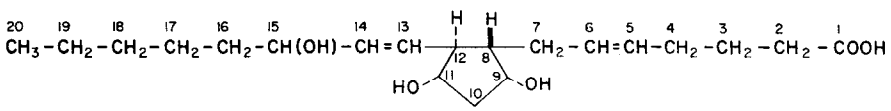
THE SMOOTH muscle stimulating action of extracts of rabbit and ox iris was first discovered by Ambache,[1] who named the active principle irin. On the basis of further investigations by the same author[2,3] it was suggested that irin was an unsaturated hydroxy acid.

Recent work from this laboratory has established the structures of a new class of smooth muscle stimulating compounds called prostaglandins.[5,7] These were first isolated from sheep vesicular glands and seminal plasma.[4,6] However, the isolation of prostaglandin $F_{2\alpha}$† (I) from lung tissue[8] seemed to indicate a more widespread occurrence of these compounds in the body. Since the properties of irin, as reported by Ambache, to some extent resembled those of the prostaglandins it was felt of interest to further investigate the nature of the smooth muscle stimulating lipids in sheep iris. The present report demonstrates the presence of several smooth-muscle stimulating factors in this tissue. One of them has been identified as prostaglandin $F_{2\alpha}$ (Fig. 1).



Prostaglandin $F_{2\alpha}$ (I).

The sheep irides were collected at slaughter and immediately frozen to −20°. This material (6,878 irides; 1·4 kg wet weight) was homogenized by pressing at −20 to −40° according to Edebo[9] and then extracted at room temperature with 4 l. of 95% ethanol for 12 hr (extract I). After filtration the insoluble residue was extracted once more with 3 l. of chloroform–methanol (1:1) at room temperature for 12 hr (extract II). The smooth muscle stimulating activity present in the two extracts was equivalent to respectively 45,000 μg and 3,800 μg of prostaglandin E_1 when tested on the isolated rabbit duodenum. Assay on the atropinized hamster colon gave the same proportions of spasmogenic activity in the two extracts. Each extract was then concentrated to a small volume at 40° under N^2 at reduced pressure followed by acidification to pH 3 with 2 N hydrochloric acid and three extractions with equal volumes of ethyl acetate. The ethyl acetate phases were washed with distilled water until neutral and evaporated to dryness. The residue of extract I weighed 7·5 g and that of extract II 0·5 g. The combined residues containing a smooth muscle stimulating activity equivalent to 39,000 μg prostaglandin E_1 were distributed four times between equal volumes of 67% aqueous ethanol and petroleum ether. The alcoholic phase contained 50 per cent of the recovered smooth muscle stimulating activity (tested on rabbit duodenum). This solution was concentrated to a small volume, acidified to pH 3 and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate phases were washed with distilled water until neutral and evaporated to dryness.

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† Prostaglandin $F_{2\alpha}$ (earlier PGF_{1-2}) is called 9α, 11α, 15-trihydroxyprosta-5, 13-dienoic acid according to the recently introduced systematic nomenclature. This is based on the trivial name prostanoic acid for the parent C_{20} acid numbered as shown in Fig. 1.

An efficient fractionation of this material (4·3 g) was achieved by silicic acid chromatography (250 g silicic acid, Mallinckrodt; activated at 120°) with the elution sequence shown in Fig. 2. At least four different peaks appeared, one of which was eluted with ethyl acetate-hexane (70:30), two with pure ethyl acetate and one upon elution with methanol.

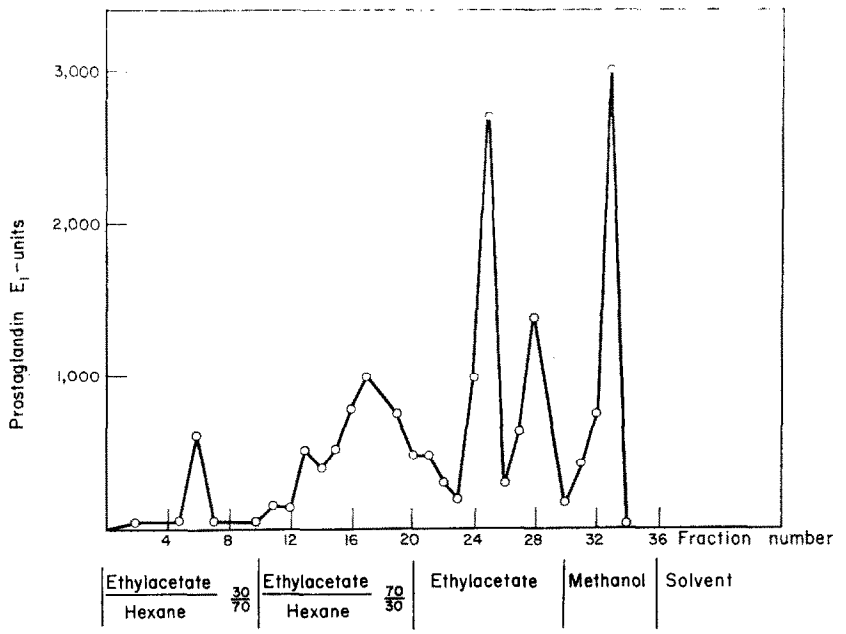

FIG. 2. Silicic acid chromatography of a lipid extract from sheep iris. Column: 250 g of silicic acid Fraction: 600 ml. The smooth muscle stimulating activity was assayed on rabbit duodenum. One prostaglandin E_1 unit is equivalent to the effect produced by 1 μg of prostaglandin E_1.

Fractions 24–29 (27 mg) were then subjected to reversed phase partition chromatography. The moving phase (300 ml of 47·5% aqueous methanol) and the stationary phase (15 ml of isooctanol and 15 ml of chloroform) were equilibrated for 12 hr at 23°. The less polar phase (4 ml) was supported on 4·5 g of hydrophobic Supercel.[10] Every fourth fraction (3 ml) was anlysed for rabbit duodenum stimulating activity. A peak with a retention volume characteristic of prostaglandin $F_{2\alpha}$ appeared.

Further proof as to the identity of this material was obtained by the comparison with pure prostaglandins with various thin layer chromatographic procedures.[11]

Chromatoplates coated with silica gel (Silicagel G, Merck AG, Germany) and developed with benzene–dioxane–acetic acid (20:20:1) separate prostaglandin E compounds from prostaglandin F compounds. The factor isolated from sheep iris behaved as a compound of the prostaglandin F type in this system.

Thin layer chromatography on silica gel containing $AgNO_3$ (1 g $AgNO_3$/30 g adsorbent) separates all the known prostaglandins both as free acids and methyl esters. The material from the peak of the reversed phase partition chromatography (1·8 mg) was run on a chromatoplate containing $AgNO_3$ in ethyl acetate–acetic acid–methanol–2,2,4-trimethylpentane–water (110:30:35:10:100, the less polar phase was used). After spraying with 10% alcoholic phosphomolybdic acid and heating to 90° for 15 min a spot appeared with the same R_f value as the standard prostaglandin $F_{2\alpha}$. In another run the material was applied along a line with pure prostaglandin $F_{2\alpha}$ on either side. After development the lipids were visualized by spraying with water. The opaque areas were scraped off the plate, eluted with methanol and the smooth muscle stimulating activity assayed on the isolated rabbit duodenum. Of the total spasmogenic activity recovered, 83 per cent was found in the zone corresponding to prostaglandin $F_{2\alpha}$.

Irin has previously been defined as the smooth muscle stimulating lipid soluble principle present in extracts of iris. The present investigation shows that several such factors occur in sheep iris and that

one of them is identical with prostaglandin $F_{2\alpha}$. The identification of prostaglandin $F_{2\alpha}$ in sheep iris further indicates that the prostaglandins are widely distributed also in mammalian tissues outside the genital sphere. It also stresses the importance of further work in order to establish the physiological role of these biologically highly active compounds.

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