THE ISOLATION OF AN ADDITIONAL PROSTAGLANDIN DERIVATIVE FROM THE ENZYMATIC CYCLIZATION OF HOMO-γ-LINOLENIC ACID

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The biosynthesis of prostaglandins from C-20 essential fatty acids using homogenates of sheep seminal vesicles (vanDorp, et al., 1964; Bergstrom, et al., 1964) and acetone powders of beef seminal vesicles (Wallach, 1965) has been well established. Recent studies have concerned the mechanism of the cyclization and the origin of the oxygen incorporated during the biosynthesis of prostaglandin E_1** (Ryhage, et al., 1965; Nugteren, et al., 1965; Samuelsson, 1965). In addition to prostaglandin E_1, the conversion of homo-γ-linolenic acid-1-^{14}C has afforded prostaglandin $F_{1\alpha}$ in low yield (Kupiecki, 1965) as well as a major product less polar than prostaglandin E_1. The isolation, properties and elucidation of the structure of this compound are described in the present communication.

Experimental details of bioconversions using homo-γ-linolenic acid-1-^{14}C and sheep seminal vesicular glands have been previously described (Kupiecki, 1965). In larger scale conversions, 5 kg. of frozen sheep seminal vesicles was homogenized with 5 l. of 0.1 M ammonium chloride buffer, pH 8.5, to which was added 5 l. of the same buffer containing 2.5 g. of homo-γ-linolenic acid. After adjusting the pH to 8.0 with 6 N ammonium hydroxide the homogenate was incubated for 1 hour at 37°. At the end of this period the pH

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** PGE_1 or 11α,15-dihydroxy-9-oxoprost-13-enoic acid (for nomenclature, see Hamberg and Samuelsson, 1965).

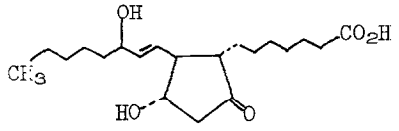
was adjusted to 7.0 with 6 N hydrochloric acid. After addition of 45 l. of cold acetone, the mixture was filtered and the filtrate extracted with Skellysolve B. The aqueous acetone was then concentrated to one fourth of the original volume, adjusted to pH 3.0 with hydrochloric acid and extracted with methylene chloride. The prostaglandin was extracted from the methylene chloride with 0.2 M sodium phosphate buffer, pH 8.0. The aqueous extract was acidified to pH 3.0 and re-extracted with methylene chloride. Concentration of the methylene chloride fraction followed by cooling yielded crystalline prostaglandin E_1. Typically, between 250 and 500 mg. of once recrystallized product was obtained, with a melting point 113-115° C.

From the pooled mother liquors of several large scale biosynthetic runs, a residue of 56 grams was obtained, redissolved in 0.4 M sodium phosphate buffer, pH 8.0, and washed with Skellysolve B. The aqueous solution was acidified to pH 3.0 and re-extracted with methylene chloride. Concentration of this extract yielded a residue of 44 g. which was chromatographed on silica gel (Silica Gel 0.05-0.2 mm. for chromatography, E. Merck Darmstadt, treated with hydrochloric acid, washed with water, dried and reactivated at 100° C.) using increasing concentrations of ethyl acetate in Skellysolve B as the eluant. With ethyl acetate:Skellysolve B 3:2, a 10.04 g. fraction was obtained.

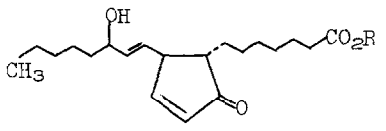
Rechromatography in the same system yielded 5.57 g. of a material which gave a single spot on thin layer chromatography and exhibited an ultraviolet absorption maximum at λ_{max}^{EtOH} 218 mμ suggesting structure 2 (R = H). This structure was supported by the infrared spectrum (Nujol mull) which showed principle absorption peaks at 3400, 2640, 1700, 1580, and 1180 $cm.^{-1}$ and by the nmr spectrum (run in $CDCl_3$ on a Varian A-60 spectrophotometer operating at 60 MC) which exhibited principle absorption peaks at 6.17δ and 7.52δ (10,11-vinyl-H's), at 5.6δ (13,14-vinyl-H's), at 4.1δ (15-H) and at 3.25δ (12-H).

Esterification of 2 (R = H) with diazomethane gave the methyl ester

(2, R = CH_3). The mass spectrum showed a molecular ion peak at m/e 350 with fragmentation patterns in complete accord with the assigned structure.*



(1) PGE_1



(2) PGE_1-217 (R = H)

The preparation of the acetate of the methyl ester of PGE_1-217 by treatment of the methyl ester of PGE_1 with acetic anhydride has previously been described (Bergstrom, et al., 1963). We have found that treatment of prostaglandin E_1 (1) with 90% aqueous acetic acid (acetic acid:water 9:1) for 18 hours at 60° C. gave 2 (R = H) which was identical by thin layer chromatography and infrared spectroscopy with PGE_1-217 obtained from the bioconversion of homo-γ-linolenic acid.**

In studies employing homo-γ-linolenic acid-1-^{14}C, an autoradiography of the thin layer chromatogram was run as described earlier (Kupiecki, 1965). A radioactive spot, less polar than prostaglandin E_1, had the same R_f as authentic PGE_1-217 and failed to separate from it in two solvent systems (Table I). Under the conditions of these experiments, the average yield of radioactive PGE_1-217 was 9.8% and the yield of PGE_1 was 7.1%.

The biological properties of the PGE_1-217 (2, R = H) are of special interest. On the guinea pig ileum and on the rabbit duodenum PGE_1-217 had 1/100th the smooth muscle stimulating activity of PGE_1. In antagonizing the epinephrine-induced mobilization of free fatty acids from rat adipose tissue (Steinberg, et al., 1963) PGE_1-217 was 1/200th as effective as PGE_1. However, in the anethestized, normotensive, vagotomized, pentolinium-treated dog (Muirhead, et al., 1965), PGE_1-217 has marked vasodepressor activity, a

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* We are indebted to Dr. M. Grostic and R. Wnuk of the Physical and Analytical Chemistry Unit at Upjohn for running and interpreting the mass spectrum. Details of this aspect of the structure elucidation will be reported by them in a later publication. The mass spectra were recorded on an Atlas CH-4 instrument equipped with a TO-4 source (ionization voltage 70 e.v.).

** Following chromatography this sample crystallized to give PGE_1-217, m.p. 42-44° C, and λ_{max}^{EtOH} 217 mμ (ε, 11650).

Table I - R_f* of PGE_1-217

Solvent System	from PGE_1	from homo-γ-linolenic acid-^{14}C
Methanol; acetic acid; chloroform 5:5:90	0.69	0.69
Methanol; ethyl acetate; water 2:8:5 (upper phase)	0.41	0.41

dose of 1/20 μg/kg, producing a blood pressure depression of 36 mm Hg for 5 minutes. This vasodepressant effect appeared to be at least as active as PGE_1 in the same system.** PGE_1-217 thus appears to have the blood pressure lowering activity associated with the prostaglandins of the E series but with markedly less smooth muscle stimulating properties and much reduced effects on free fatty acid metabolism. It is of considerable interest that recent work on the isolation of materials from human seminal fluid has led to the identification of some substituted 9-oxoprost-10-enoic acids as naturally occurring products (Hamberg and Samuelsson).*** The present evidence does not preclude the possibility that at least part of the PGE_1-217 is formed by a non-enzymatic process.

REFERENCES

Bergstrom, S., Danielsson, H., Klenberg, D. and Samuelsson, B., J. Biol. Chem. 239, PC 4006 (1964).
Bergstrom, S., Ryhage, R., Samuelsson, B. and Sjovall, J., J. Biol. Chem. 238, 3555 (1963).
Hamberg, M. and Samuelsson, B., Biochim. Biophys. Acta 106, 215 (1965).
Kupiecki, F. P., Life Sciences 4, 1811 (1965).

* PGE_1-217 was made visible on thin layer plates by spraying with a solution of vanillin in phosphoric acid. The radioactive material was located by autoradiography.

** Vasodepressor tests were conducted by Dr. W. A. Freyburger and R. D. Hamilton (Pharmacology Unit), and smooth muscle tests by Dr. G. F. Cartland and also by W. E. Brown (Pharmacology Unit).

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Muirhead, E. E., Daniels, E. G., Booth, E., Freyburger, W. A. and Hinman, J. W., Archives of Pathology 80, 43 (1965).
Nugteren, D. H. and vanDorp, D. A., Biochim. Biophys. Acta 98, 654 (1965).
Ryhage, R. and Samuelsson, B., Biochem. Biophys. Res. Comm. 19, 279 (1965).
Samuelsson, B., J. Biol. Chem. 238, 3229 (1963).
Samuelsson, B., J. Amer. Chem. Soc. 87, 3011 (1965).
Steinberg, D., Vaughan, M., Nestel, P. and Bergstrom, S., Biochem. Pharmacol. 12, 764 (1963).
vanDorp, D. A., Beerthuis, R. K., Nugteren, D. H., VonKeman, H., Nature 203, 839 (1964).
Wallach, D. P., Life Sciences 4, 361 (1965).