# THE OCCURRENCE OF 19-HYDROXY F PROSTAGLANDINS IN HUMAN SEMEN

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## 1. Introduction

Since the original demonstration by Von Euler [1] and Goldblatt [2] of the smooth muscle stimulating activity of human semen, the structure and function of prostaglandins in this fluid have been the subject of much research. Samuelsson et al. originally determined the structure of 5 prostaglandins ($E_1$, $E_2$, $E_3$, $F_{1\alpha}$ and $F_{2\alpha}$) in human semen [3] and later claimed that eight more compounds were present ($A_1$, $B_1$, $A_2$, $B_2$ and the corresponding 19-hydroxylated compounds) [4]. We have recently shown [5] that these dehydrated prostaglandins are possibly artifacts of the naturally occurring 19-hydroxy $E_1$ and 19-hydroxy $E_2$ prostaglandins which together with $E_1$ and $E_2$ comprise the four main prostaglandins in human semen. These findings have recently been confirmed [6]. As a continuation of this work we examined semen extracts for compounds which were yet more polar than the 19-OH Es. Such compounds would almost certainly have been missed using previous techniques.

## 2. Materials and methods

During the purification of natural 19-OH E prostaglandins from semen we observed that some of the reactions contained compounds whose mass spectra implied a 19-OH F prostaglandin structure (fig.1). It proved difficult to separate these compounds from the much larger amounts of 19-OH E prostaglandins. Therefore we used old semen samples in which the 19-OH Es had dehydrated to the less polar 19-OH As and Bs

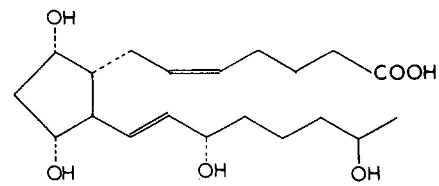


Fig.1. 9,11,15,19-tetrahydroxy prosta-5,13-dienoic acid (19-hydroxy prostaglandin $F_{2\alpha}$.)

[7], which were rendered still less polar by oximation prior to chromatography.

Human semen (200 ml) which had been stored for 12 months at $-20^\circ C$ was added to acetone (800 ml). The precipitate was removed and the supernatant evaporated to dryness. The residue was redissolved in sodium phosphate buffer (100 ml) (0.5 M, pH 5.). This was extracted first with ether (100 ml) and then three times with ethyl acetate (300 ml). The ethyl acetate extracts were combined and evaporated to dryness. The residue was dissolved in pyridinium acetate buffer (50 ml) (1.5 M, pH 5.0) containing 10 mg/ml ethoxyamine hydrochloride. The solution was left in an ultrasonic bath for 45 min, then extracted with ethyl acetate (3 $\times$ 150 ml). The extract was evaporated and redissolved in 0.5 ml chloroform/ethyl acetate/acetic acid (50:50:1) prior to running on a 400 $\times$ 5 mm Sephadex LH20 column using the same solvent. 10 ml fractions were collected and a 0.1 ml aliquot from each derivatised for analysis by gas chromatography-mass spectrometry. Fresh semen samples were stored in a large excess of acetone at $-20^\circ C$ until required. The existence of the 19-OH Fs and accompanying isomers was demonstrated by thin layer chromatography.



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*Analysis of fractions*

Samples were methylated using diazomethane and silylated using Bis-trimethylsilyl trifluoroacetamide or *t*-butyl dimethyl chlorosilane and imidazole in dimethylformamide [8]. *N*-Butyl boronates were formed using the method published elsewhere [9]. Derivatised samples were analysed for prostaglandins using a Hewlett Packard 402 gas chromatograph coupled via a Watson–Bieman separator to an AEI MS 12 mass spectrometer. The column used was a 1700 X 6 mm U tube packed with 1% Dexsil on 100/120 mesh gas chrom. Q. The column oven temperature was 260°C and the helium carrier gas flow rate was 40 ml/min. The energy of the ionising electron beam was 20 eV and the source temperature was 280°C.

Thin layer chromatography was carried out on Merck precoated 10 X 20 cm plates using ethyl acetate:acetic acid (9:1) as the developing solvent. The plates were run twice to the same mark and the prostaglandins were visualised by sulphuric acid charring.

Synthetic 19-hydroxy $F_{2\alpha}$ (9α, 11α, 15α, 19-tetrahydroxy prosta-5, 13-dienoic acid) (both 15R and 15S isomers) were the gift of Dr. N. Crossley of I.C.I. (Pharmaceuticals Ltd.).

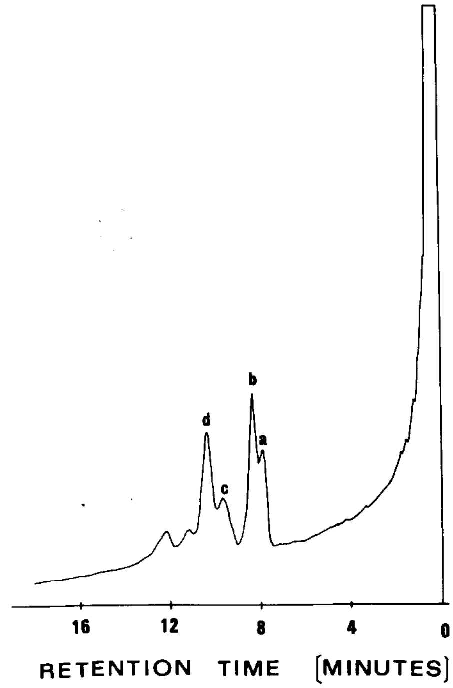


Fig.2. GLC trace of 19-hydroxy F prostaglandins as the methyl ester, Trimethyl silyl derivative. (a) iso 19-hydroxy $F_{2\alpha}$. (b) iso 19-hydroxy $F_{1\alpha}$. (c) 19-hydroxy $F_{2\alpha}$. (d) 19-hydroxy $F_{1\alpha}$.

## 3. Results

Fractions 10–18 from the LH20 column were shown to contain four compounds whose mass spectra were consistent with a 19-hydroxy F prostaglandin structure (fig.1). The g.l.c. trace (fig. 2) shows that these are separable and the mass spectra indicate the presence of two pairs of isomeric compounds corresponding to two isomers of 19-OH $PGF_{1\alpha}$ and two of 19-OH $PGF_{2\alpha}$. Some separation between these isomers was obtained on the LH20 column; fractions 10–12 contained mainly the pair of isomers which gave the longer retention time (r.t.) on g.l.c. and fractions 13–18 the shorter r.t. pair. Comparison of g.l.c. retention times and t.l.c. $R_f$ values (fig.3) with those of authentic 19-OH $F_{2\alpha}$ indicated that the compounds in fractions 10–12 were 19-OH $F_{2\alpha}$ and 19-OH $F_{1\alpha}$ (9α, 11α, 15α, 19-tetrahydroxy prost-13-enoic acid). The yield was approximately 1 mg distributed equally among the four compounds.

The mass spectra of the normal and iso 19-OH Fs were very similar in all the derivatives studied. Gas liquid

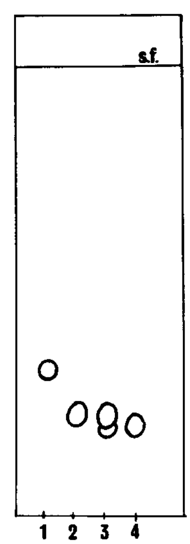


Fig.3. TLC separation of 19-hydroxy F prostaglandins. (1) 15R 19-hydroxy $PGF_{2\alpha}$ (synthetic). (2) 15S 19-hydroxy $PGF_{2\alpha}$ (synthetic). (3) 19-hydroxy F PGs from semen (fractions 10–12). (4) iso 19-hydroxy F PGs from semen (fractions 13–18).

Table 1
Assignment of ions in the mass spectra of 19-hydroxy prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ as the methyl ester trimethyl silyl ether derivative

| 19OH $F_{1\alpha}$ | 19OH $F_{2\alpha}$ | Probable origin of ion |
|---|---|---|
| 584 | 582 | M – TMSi OH |
| 515 | 513 | M – ($C_{16-20}$) |
| 494 | 492 | M – $(\text{TMSi OH})_2$ |
| 425 | 423 | M – ($C_{16-20}$ + TMSi OH) |
| 404 | 402 | M – $(\text{TMSi OH})_3$ |
| 235 | 235 | M – ($C_{1-7} + C_{10-11} + (\text{TMSi OH})_3$) |
| 191 | 191 | M – ($C_{1-7} + C_{16-20} + (\text{TMSi OH})_3$) |
| 117 | 117 | ($CH_3$: CH: :O: Si: $(Me)_3$) |

chromatography of the methyloxime methylester trimethyl ether derivative of fresh semen shows the isomers of 19-OH Fs well separated although 19-OH $F_{2\alpha}$ and 19-OH $F_{1\alpha}$ are masked by the first isomers of the 19-OH Es. Estimation of the concentration of total 19-OH Fs in typical fresh semen indicates 20 μg/ml of this material. This level should be compared with a combined total of 5 μg/ml for $F_{1\alpha}$ and $F_{2\alpha}$, a total of 200 μg/ml for 19-OH $E_1$ and 19-OH $E_2$ and a total of 40 μg for $E_1$ and $E_2$.

Table 1 gives the assignment of the main present in the spectra of both isomers as the methyl ester trimethyl silyl ether derivative. Figure 4 gives the spectra of synthetic and natural 19-OH $F_{2\alpha}$ and Iso 19-OH $F_{2\alpha}$ as this derivative. All four compounds form *n*-butyl boronates and their spectra as the methyl ester, *n*-butyl boronate, trimethyl silyl ether resemble those of the PGFs [9] having a strong ion at m/e 435 ($F_{2\alpha}$ compounds) or 437 ($F_{1\alpha}$ compounds) corresponding to the loss of $C_{16-10}$. The spectra of all four compounds as the methyl ester, *t*-butyl dimethylsilyl ether are spectacular, having their base peak at m/e 783 ($F_{2\alpha}$ compounds) and 785 ($F_{1\alpha}$ compounds) this ion representing the loss of a tertiary butyl radical.

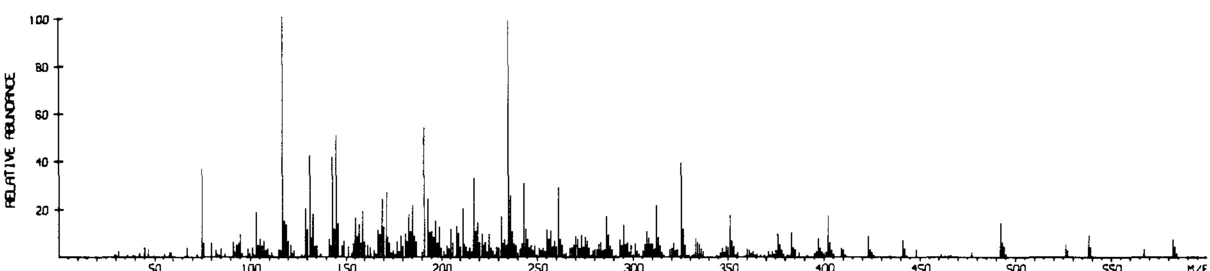


Fig.4a
19 OH PG F'S EX SEMEN ME TMS


Fig.4b
19 OH ISO PG F'S EX SEMEN ME TMS

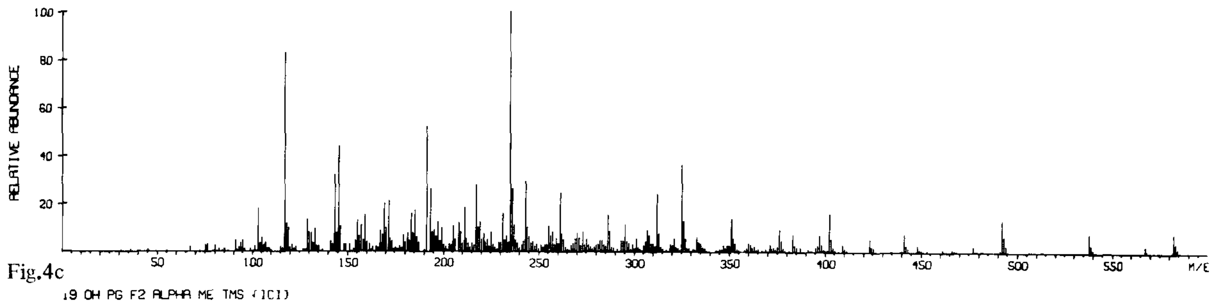


Fig.4c

Fig.4. Mass spectra of 19-hydroxy $F_{2\alpha}$ methyl ester trimethyl silyl ether. (a) 19-hydroxy $F_{2\alpha}$ from semen. (b) iso 19-hydroxy $F_{2\alpha}$ from semen. (c) synthetic 15S 19-hydroxy $F_{2\alpha}$.

## 4. Discussion

The identity of 19-hydroxy $F_{2\alpha}$ has been established by comparison of mass spectra, t.l.c. and g.l.c. properties with those of the authentic compound and the existence of a compound corresponding to 19-hydroxy $F_{1\alpha}$ has also been demonstrated. However, the identity of accompanying isomeric compounds has not been established with certainty. The close similarity of the mass spectra indicates that two pairs of epimers are present and the possible sites of epimerisation are 8, 12 or 9 and 11 together. Epimerisation at 15 is not a possibility since the authentic 15S and 15R compounds have indistinguishable g.l.c. retention times. Similarly epimerisation at 19 is not possible as the authentic compounds were a mixture of epimers at 19. Epimerisation at 9 only or 11 only is ruled out, since the formation of a butyl boronate of all species found implies *cis* configurations of the 9 and 11 hydroxyls.

Of the remaining possibilities, epimerisation at both 9 and 11 together seems improbable if the two forms arise from a common precursor, as such a precursor would have to have two enolizable functions. Furthermore, one would not expect the g.l.c. retention time of a $9\beta$ $11\beta$ F prostaglandin to be significantly different from that of a $9\alpha$ $11\alpha$ compound. The shorter g.l.c. retention time of the iso compounds is compatible with either the 8-iso or 12-iso structure as both these compounds would have the side chains on the same side of the ring, giving a more compact and hence more volatile compound. Epimerisation of the 8 position is readily achieved in the E prostaglandins and it is possible that these 19-hydroxy Fs are derived from 19 hydroxy E precursors. Furthermore 8-iso prostaglandin E has been reported as a product of the incubation of arachidonic acid with sheep vesicular glands [10] and this compound was shown to have a distinctive spectrum of biological activity. The appearance of the 19-hydroxy F prostaglandins in fresh semen precludes the possibility that they are artefacts of storage and because they are more abundant than the F prostaglandins themselves it is important that their role in reproduction should be investigated. Work is at present in progress to determine the biological activity of these compounds.

## Acknowledgements

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