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MASS SPECTROMETRY OF PROSTAGLANDINS

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ABSTRACT

A detailed mass spectrometric study was undertaken to elucidate the genesis of ions produced in the electron ionization mass spectra of prostaglandin derivatives of the A. B, E and F families. Stable isotope derivatives, metastable defocusing, accurate mass determinations and low resolution mass spectra provided data to facilitate elucidation of the majority of ions occurring in mass spectra of these four prostaglandin families. During this study two new prostaglandins, 19-hydroxy PGE and 19-hydroxy PGE_2 were found and quantified in human seminal fluid. A preliminary study was undertaken to determine if any correlation exists between endogenous levels of these two new prostaglandins and human male infertility.

INTRODUCTION

Prostaglandins participate in a wide variety of physiological processes (Samuelsson, 1976). Other metabolites of arachidonic acid recently discovered, namely prostacyclins (Dusting *et al*, 1977) and thromboxanes (Hamberg *et al*, 1975), have extended to an even greater degree the range of physiological processes in which these arachidonic acid metabolites participate. This detailed mass spectrometric fragmentation study was undertaken to investigate in greater detail the mass spectral genesis of various ions formed in the mass spectra of trimethylsilyl and alkyloxime-trimethylsilyl derivatives of prostaglandins A, B, E and F (Middleditch *et al*, 1973).

Other workers have performed low resolution mass spectral studies on alkyloxime-TMS ether-methyl esters of prostaglandins (Green, 1969). In addition, structures of many metabolites of arachidonic acid have been elucidated by means of gas chromatography-mass spectrometric methods. (Wong *et al*, in press; Wong *et al*, submitted) Also, even though radioimmunoassay techniques have been employed to quantify primary prostaglandins, metabolites and other arachidonic acid metabolites (Granstrom *et al*, 1978), quantification by gas chromatography-mass spectrometric methods offers the assurance of combining a gas chromatography retention time with monitoring a unique structurally significant ion arising from the compound of interest. At the minimum, quantification by gas chromatography-mass spectrometry methods should be performed once to establish that the compound of interest is the one being quantified.

The current study was based on this extensive background of mass spectral studies. For the first time stable isotope derivatives, metastable defocusing, accurate mass determinations and low resolution mass spectra were combined in a concerted effort to rationalize the genesis and structure of every ion occurring in the mass spectra of alkyloxime-TMS ether, TMS ester derivatives of prostaglandins. As a general rule this combination of methods should be employed whenever feasible on a class of compounds to provide a level of assurance before investigating a presumed unknown member of that family. As this manuscript will illustrate, collecting background information of this type facilitates recognition of, and subsequent structural elucidation of unknown biologic compounds.

METHODS

TMS ester, TMS ether derivatives were synthesized by dissolving prostaglandins (100 µg) in *N, O* bis-trimethylsilyl trifluoroacetamide (BSTFA, 100 µl) and allowing the solution to stand at 50° for three hours. d_0-TMS ester d_9-TMS ether derivatives were prepared by adding *N, O,* bis d_9-TMS acetamide (10 µl) to prostaglandins (100 µg) in dry pyridine (100 µl). Selectively labeled TMS ester, d_9-TMS ether derivatives were prepared by preferential on-column exchange of the more labile ester TMS group during GC-MS: one µl of the d_9-TMS ester, d_9-ether (1 µg) reaction mixture was injected onto the GC column followed by injection of *N*-TMS imidazole (10 µl). Ester TMS exchange took place to the extent of 40%-70%. No exchange of ether TMS was observed. Alkyloxime TMS derivatives were prepared by a two-stage method. Either methoxyl or ethoxyl amine hydrochloride (1 ml) was added to the prostaglandin (100 µg) in dry pyridine (250 µl) and the solution was allowed to stand at 50° overnight before being blown to dryness under nitrogen. Trimethylsilylation with BSTFA was performed as above. GC-MS was carried out using an LKB 9000 instrument fitted with 6' X 0.25" silinized glass GC columns containing 1% SE30 on Gas Chrom Q (100-120 mesh). The column temperature was 200°; separator 250°; ion source 250°; electron energy 22.5 or 40 eV; accelerating voltage 3.5 kV. High resolution spectra were obtained using a CEC 21-110B instrument with a resolution of 15-20,000 and an electron energy of 70 eV. Samples were introduced using a direct insertion probe. Spectra were recorded on Ilford Q2 photoplates and measured using a Gaertner model M1205C microdensitometer-linear comparator which, in turn, was coupled to a PDP 8/I-BM 360-50 Data Reduction System. (Desiderio *et al*, 1972).

RESULTS

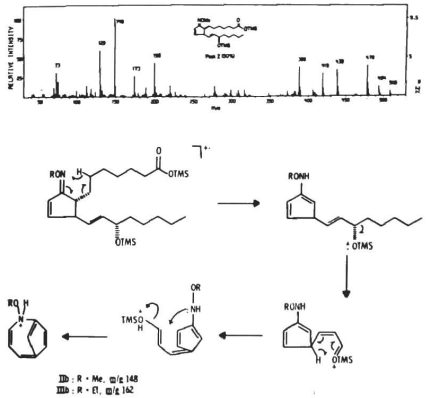
PGA Mass Spectra

The alkyloxime derivatives of the PGA family produced two GC peaks, presumably due to formation of syn-anti isomers. The mass spectrum of the methoxime-TMS derivatives of PGA_1 (second GC peak) is given in Figure 1. The molecular ion occurs at m/z 509. The methyl group involved in loss of 15 mass units originates from both ester and ether groups but not the methoxyl group of the methoxime. The $(M-31)^+$ at m/z 478 is due to loss of the *O-methyl* group from the methoxime. $(M-71)^+$ occurs at m/z 438 and loss of 90 (TMSOH) from $M^{+\cdot}$ produces m/z 419, the latter due to ether and not ester TMS groups. The peak at m/z 388 is due to loss of CH_3O plus TMSOH. Formation of the base peak at m/z 148 is rationalized in Scheme 1. The high abundance of this ion may be accounted for by the structure shown in which charge is delocalized over nine atoms. Formation of the abundant ion 199 involves fragmentation of the C 12/13 bond with concomittant cyclization followed by loss of acetylene to form ion 173. (Scheme 2).

FIG. 1. *The mass spectrum of methoxime-TMS derivatives of* PGA_1.

SCHEME 1: *Formation of ion at m/z 148 in the spectrum of methoxime-TMS ether-TMS ester* PGA_1.

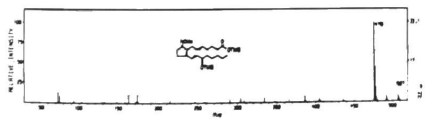
SCHEME 2: *Formation of ions at m/z 173 and 199 in spectrum of methoxime-TMS ether-TMS ester* PGA_1.



PGB Mass Spectra

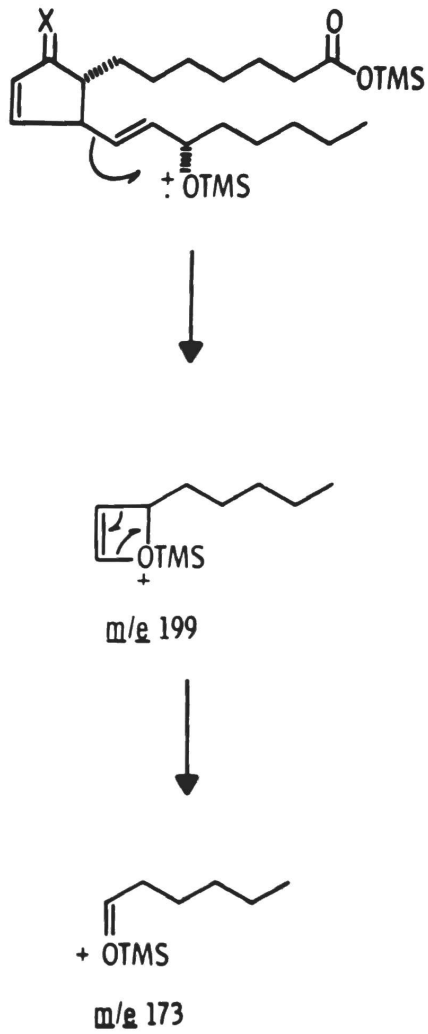
Alkyloxime TMS PGBs display one GC peak in contrast to PGA and PGE derivatives. In addition, minimal fragmentation occurs. In Figure 2, $M^{+\cdot}$ occurs at 507, while $(M-15)^+$ at m/z 478 comprises 37.5% of the total ion current. This is an outstanding candidate for selected ion monitoring for PGB, for example, after methanolic KOH treatment of PGE.

FIG. 2. *The mass spectrum of alkyloxime-TMS derivatives of PGB.*



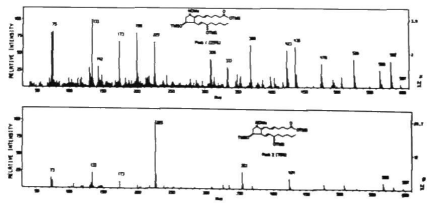
PGE Mass Spectra

Prostaglandins of the E series are widely distributed in body fluids of man and of many other animals. The 1, 3 keto moiety of the ring of these compounds is particularly unstable. Therefore, oxime-TMS derivatives were formed and the mass spectra of the resulting pair of syn-anti isomers analyzed. The mass spectra of the two PGE syn-anti isomers (Figures 3A, 3B) illustrate the different qualitative effects that isomerization provides upon EI fragmentation. The smaller (25%) first GC peak contains many more fragment ions than the second (75%) peak. The molecular ion



occurs at m/z 597. Loss of a methyl group occurs only from ether TMS groups; $(M-31)^+$ occurs at 566; no metastable ion was found for $(M-71)^+$ indicating a fragmentation process occurring more rapidly than could be observed in μsec; $[M\text{-}TMSOH\text{-}CH_3O]^+$ occurs at 476. Long range interactions between TMS groups on ether and ester functions provide ions at m/z 204, 217, and the base peak 133. (Middleditch *et al,* 1973)

FIG. 3A & 3B. *The mass spectrum of oxime-TMS derivatives of two PGE syn-anti isomers.*



It is interesting to note that, in addition to the ion structure for m/z 199 and 173 rationalized in Scheme 2 containing only ether TMS groups, minor components of different accurate mass, and thus elemental composition occurred containing ester TMS groups. Data in Table 1 collect accurate mass, elemental composition and stable isotope data for these ions occurring at nominal mass 199 and 173.

TABLE 1: *Accurate mass, elemental composition and isotope data for ions 199 and 173.*

$C_8H_{14}OTMS^a$	199.1518	$C_6H_{12}OTMS^a$	173.1361
$C_7H_{10}O_2TMS^b$	199.1154	$C_5H_8O_2TMS^b$	173.0998

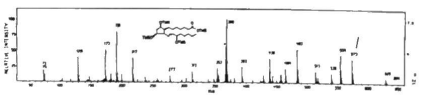
[a]*ether stable* [b]*ester*

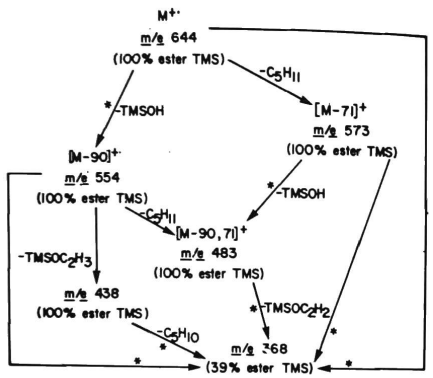
PGF mass spectra

The mass spectrum of TMS ether, TMS ester PGF_1 is given in Figure 4. The molecular ion occurs at m/z 644; $(M—71)^+$ at 629 is due to loss of an ether TMS methyl group. $(M—71)^+$ occurs at 573, $(M—90)^{+\cdot}$ at 544; further loss of 90 yields 464. Loss of 90 from $(M—31)^+$ provides 539. Metastable defocusing data for the base peak at 368 indicates five different processes contributing to this nominal mass. For example, $M^{+\cdot}$ can lose the C_5H_{11} side chain to produce 573 which either yields 368 directly or loses TMSOH to produce 483 which then loses $TMSOC_2H_2$ to produce 368. Other steps occur in various sequences. (Scheme 3)

FIG. 4. *The mass spectrum of TMS ether, TMS ester PGF_1.*

SCHEME 3: *Metastable defocussing data rationalizing fragmentations producing* m/z *368 in TMS ether, TMS ester PGF_1 .*

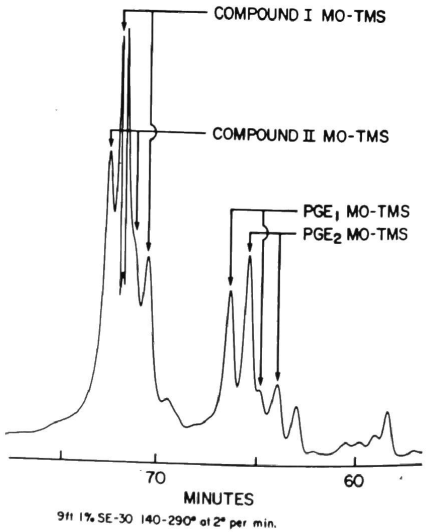




Chemical Ionization Data

Chemical ionization processes transfer less energy to molecules of the compound under investigation and less fragmentation occurs. Maximum structural information is retained in the molecular ion region resulting in a concomittant increase in sensitivity during quantification. Data from CI studies employing reagent gases NH_3 and $(CH_3)_4$ Si have been published. (Desiderio *et al,* 1973)

FIG. 5: *The GC trace from silicic acid chromatography of two new compounds.*

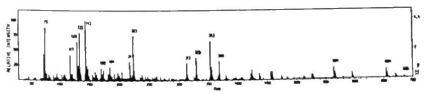


Extraction From Natural Sources

The preceeding work was performed to elucidate the genesis of significant ions in the mass spectra of prostaglandins A, B, E and F, and was a necessary prelude to various studies such as quantifying endogenous levels of PGs by selected ion monitoring (SIM) methods and elucidation of structures of unknown prostaglandins. The model system these GC-MS techniques were applied to was human seminal fluid, which contains the highest concentration of prostaglandins known. To avoid interconversion of prostaglandins, caution was taken by freezing the sample and avoiding oxygen and enzymatic interconversions (Jonsson *et al,* 1975: Jonsson *et al,* 1976). Silicic acid chromatography preceded oxime formation and trimethylsilylation. The GC trace (Figure 5) indicates four new peaks (corresponding to two new compounds, each compound forming a pair of syn-anti isomers) eluting later than the PGEs. The spectrum (Figure 6) of compound 1A shows $M^{+\cdot}$ at m/z 685, 88 mass units more than PGE_2. Structurally significant peaks occur at 117, 129 and 143 (Scheme 4). These data led to the structures 19-hydroxy PGE_1 and 19-hydroxy PGE_2 for the structures of the two new compounds found in human seminal fluid. (Jonsson *et al,* 1975; Jonsson *et al,* 1976) GC retention index data corroborated location of the hydroxyl group on C-19 vs. C-20.

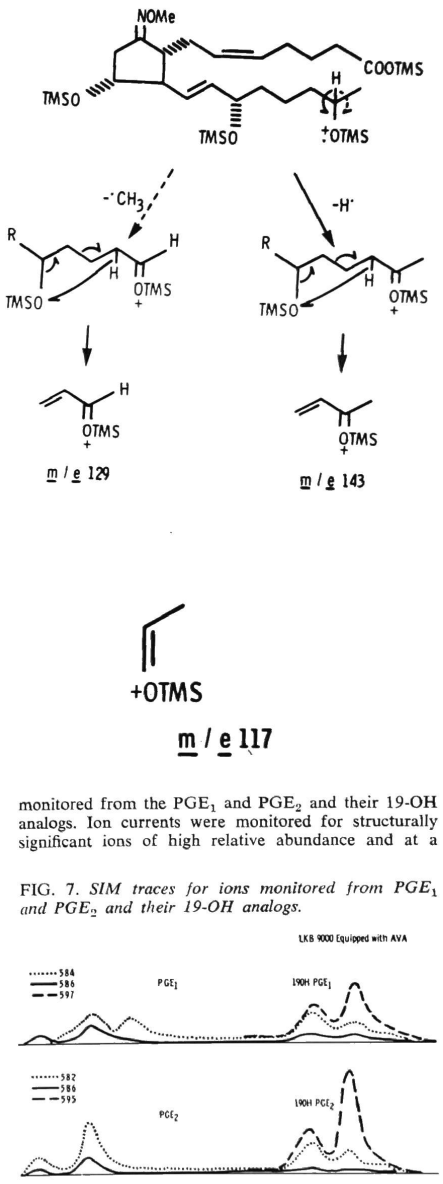
The question arose why previous workers did not find 19-hydroxy prostaglandins in human seminal fluid. (Bygdeman, 1970) To provide an answer, we divided a pooled seminal fluid sample into two equal portions. One-half was allowed to metabolize at ambient laboratory conditions while the other half was frozen and then extracted with the method described above. The sample left at ambient temperature contained decreased levels of PGEs and 19-hydroxylated PGEs and increased levels of PGA, PGB and 19-hydroxy PGA. On the other hand, the sample frozen immediately contained no PGA, PGB or 19-hydroxy PGA or PGB. This study indicated for the first time the need to specially handle biologic samples more carefully to avoid chemical and/or enzymatic interconversions of PGEs and 19-OH PGEs to corresponding PGAs, PGBs, 19-OH PGAs and 19-OH PGBs.

FIG. 6. *The mass spectrum of compound 1A.*
SCHEME 4: *Structure of ions at* m/z *117, 129 and 143 from compound 1A.*



Quantification of Prostaglandins in Human Seminal Fluid

A pilot study was undertaken to quantify concentrations of individual prostaglandins in three clinical groups: fertile, azospermic and oligospermic male to see if a correlation existed between the concentration of individual PGs in these three clinical groups and human male fertility. (Perry *et al,* 1979; Perry *et al,* 1978) An SIM trace is shown in Figure 7 for ions



monitored from the PGE_1 and PGE_2 and their 19-OH analogs. Ion currents were monitored for structurally significant ions of high relative abundance and at a

FIG. 7. *SIM traces for ions monitored from PGE_1 and PGE_2 and their 19-OH analogs.*

mass as close to $M^{+\cdot}$ as possible. The following m/z values were chosen for the five compounds quantified:

m/z 595	$(M-90)^{+\cdot}$	190H PGE_2
582	$(M-15)^+$	PGE_2
597	$(M-90)^{+\cdot}$	190H PGE_1
584	$(M-15)^+$	PGE_1
586	$(M-15)^+$	d_4-PGE_2

Relative response factors for these four prostaglandins had to be determined before quantifying endogenous levels of these compounds. Relative response factors are shown in Table 2. Concentrations of individual prostaglandins determined by SIM are given in Table 3. These data are more recent than, and do not qualitatively differ from, data previously published. (Perry *et al*, 1978) It can be readily seen that standard deviations for these measurements are quite high. However, they are not due to the analytical method, which has an error of approximately $\pm$ 7%, but instead to biological variations.

TABLE 2: *Relative Response Factors.*

PGE_2	0.96±0.07	n=12
PGE_1	0.74±0.07	n=12
190H PGE_2	0.73±0.05	n=21
190H PGE_2	0.73 (assumed value—no synthetic compound available)	

TABLE 3: *Concentration (μg/ml) of individual PGs determined in human seminal fluid of three clinical groups.*

	E_1	E_2	190H E_1	190H E_2	# of Samples
Fertile	84.0±63.2	58.0±38.8	138±100	389±275	10
Azospermic	51.3±55.4	61.6±59.1	67.1±32.2	199±136	4
Oligospermic	24.4±12.6	35.5±22.7	29.5±19.2	133±48.7	10

This preliminary study employed a small number of samples (four in the azospermic group, ten in fertile and oligospermic groups) but nevertheless provides sufficient data to arrive at preliminary conclusions. Table 4 contains a statistical evaluation of the differences of the average values of the data in Table 3.

TABLE 4: *Statistical evaluation of the differences of average values (t-test).*

	PGE	190H PGE	Degrees of Freedom
F/A	0.501 44%	1.337 88%	12
F/O	2.645 99%	3.082 99+%	18
A/O	1.387 84%	1.739 89%	12

Discussion

Even though the distribution of PG values within each category (e.g. fertile, E_1) is large, it is instructive (Table 4) to perform a t-test on the differences with a matrix defined by the three clinical groups and two types of PG families (PGE and 190H PGE). If no differences existed in this comparison, no incentive exists for further study. However, a very interesting fact emerges when fertile and oligospermic data are compared (F/O). A t-test value of 2.645 is found and indicates a highly significant difference between the two clinical groups. Additionally, 190H PGE values have even a greater statistically significant difference. It is clear that these two differences may be of great clinical importance. While no fertility problems exist for fertile men and it is obvious azospermic men are infertile, no clinical explanation can be offered at this time for infertility of oligospermic males. It becomes of crucial importance to correlate, in an expanded study, data similar to those in Table 3 (Conte *et al.* 1979; Taylor, 1979) with circulating gonadotropin hormone and androgenic steroid levels.

Acknowledgments

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SOME OBSERVATIONS ON THE LIFE HISTORY OF THE FLY *MEGASELIA SCALARIS* LOEW (PHORIDAE) WITH SPECIAL REFERENCE TO THE ECLOSION PATTERN

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Abstract

Males of a laboratory maintained population of the dipteran *Megaselia scalaris* were observed to eclose approximately four days prior to the females. The peak male eclosion occurred on day three while the female peak occurred on day seven. The smaller male larvae pupated approximately two days earlier than female larvae, and it appeared that male pupae eclosed earlier than female pupae. An excess of male progeny was recovered. The early eclosion of males coupled with delayed mating suggests mechanisms which might reduce the incidence of inbreeding. We also confirm observations that females have greater longevity than do males. The life history traits of larger size, longer developmental time and greater longevity may reflect a greater reproductive burden carried by the females.

Introduction

The dipteran fly *Megaselia scalaris* is geographically widespread, being reported in 58 countries (James, 1947), and is found in a wide diversity of habitats. It is characterized by a hunch-back appearance and rapid jerky movements (Patton, 1922). There is marked sexual dimorphism, females being approximately twice as large as males (Semenza, 1953). They are known to breed on decaying material (Brunetti, 1912; Patton, 1922), and Patton (1922) suggested they might be a significant medical and veterinary problem in India because they produce myiasis. Priyanond *et al* (1973) have reported a case of urethal obstruction caused by larval infection. Patton (1922), Semenza (1953) and Tumrasvin *et al* (1977) have recorded a number of observations on the basic biology of the fly and Mainx (1964) has reviewed its genetics, but little is known of its natural history. The following study was undertaken to examine the unusual eclosion pattern reported by Semenza (1953) in which the males eclose earlier than the females.

Methods and Materials

A laboratory stock of *Megaselia scalaris* was established from pupae collected from the sides of a container used to store anatomical specimens in the basement of the science building at East Tennessee State University. Subsequent generations have been maintained on a yeast, corn meal, sorghum-syrup, agar *Drosophila* medium in one-half pint (232 ml) bottles.

Adult flies were placed on fresh food (approximately 85ml/bottle) at 25°C and allowed to lay eggs for one to four days. The eclosion pattern was determined by collecting newly emerged flies twice daily, morning and afternoon, and recording the number of each sex at each collection. In order to establish the relation of the order of pupation to eclosion the position and date of pupation of individual pupae were marked on the side of the culture bottle, then the date of eclosion was recorded for each pupa as the flies emerged. Additional information on eclosion times and patterns was obtained by collecting white pupal cases and transferring them to fresh food in 8-dram shell vials. White pupal cases were selected because their age was known within four hours as it takes approximately four hours for the pupal case to darken. Female containing pupal cases are larger than male containing cases (Semenza, 1953), but the pupal cases were taken at random without reference to size. The eclosion period was measured from the day the first fly emerged.