

The identification of individual prostaglandins using a LKB 9000 gas chromatograph-mass spectrometer

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The two qualities that make combined gas chromatography-mass spectrometry (GC-MS) such a powerful analytical tool are the absolute identification of individual compounds coupled with high sensitivity. It has proved invaluable in prostaglandin research in identifying one or several particularly prostaglandins, since prostaglandins are a group of closely related, polar fatty acids and conclusive identification by other methods is not possible.

Suitable derivatives of the prostaglandins have to be formed for them to chromatograph on GC, and the methyl esters trimethylsilyl ethers (Me-TMS) are commonly prepared (Thompson, Los and Horton, 1970). For prostaglandins E (PGE) where an oxo group exists, the methoxime is also formed. However this derivatization is not essential for the chromatographing of prostaglandins A (PGA) or B (PGB), though they do contain an oxo group.

The basis of GC-MS relies on the fact that compounds have different mass spectra and/or retention times on GC. For example, PGF_{2α} and PGB₂ (Me-TMS derivatives) have different mass spectra and different retention times. PGF_{2α} and PGF_{2β} have identical mass spectra but their retention times are different. PGA₂ and PGB₂ are isomers therefore their mass spectra contain the same m/e peaks. However, the relative abundance of the peaks is different making each spectrum unique. Also PGA₂ and PGB₂ have different retention times. The difference between a prostaglandin of the one series and its two series counterpart is that, although the mass spectra are similar, the m/e peaks of the former are two units higher than those of the latter (though several common peaks at the lower m/e values may exist). Also the retention time of a one series prostaglandin is longer.

A full mass spectrum of PGF_{2α} (Me-TMS) can be achieved with 10 ng, though 50-100 ng is usually required to detect it in a biological extract even after purification by solvent extraction and chromatography. This quantity is often in the

range of the amounts of prostaglandins isolated from biological sources, though pooling of samples is sometimes required.

In this laboratory the use of GC/MS in prostaglandin research has resulted in the following achievements: (1) The identification of PGF_{2α} as the uterine luteolytic hormone (see Poyser, 1973 for references). (2) The identification of PGF_{2α} in sheep uterine venous blood around the time of parturition (Challis, Harrison, Heap, Horton and Poyser, 1972). (3) The identification of PGE₂ and PGF_{2α} in rabbit renal venous blood (Davis and Horton, 1972). (4) The identification of PGC₂ as the product of the isomerization of PGA₂ by the enzyme prostaglandin A isomerase (Jones, 1972). (5) The identification of PGF_{2α} as the product of the reduction of PGE₂ by an enzyme present in sheep blood (Hensby, 1974). (6) The identification of PGF_{2α} and PGE₂ in human endometrial tissue (Downie, Poyser and Wunderlich, 1974).

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