MASS SPECTROMETRIC DETERMINATION OF PICOMOLE AMOUNTS OF PROSTAGLANDINS E_2 AND $F_{2\alpha}$ USING SYNTHETIC DEUTERIUM LABELED CARRIERS

U. Axen, K. Green, D. Hörlin and B. Samuelsson

Department of Experimental Chemistry, The Upjohn Company, Kalamazoo, Michigan 49001

and

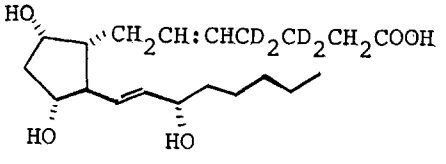
Department of Medical Chemistry, Royal Veterinary College, S-104 05 Stockholm, Sweden.

Received August 19, 1971

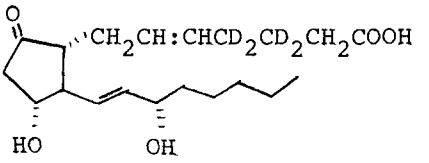
SUMMARY: [3,3,4,4-D_4]-prostaglandin E_2 and [3,3,4,4-D_4]-prostaglandin $F_{2\alpha}$ were prepared and used as carriers in quantitative gas chromatography-mass spectrometry. Ions of the protium form and the tetradeuterio form produced by electron impact on the effluent were monitored. Prostaglandin E_2 was analyzed as the diacetate of the O-methyl oxime of the methyl ester and prostaglandin $F_{2\alpha}$ as the triacetate of the methyl ester. The method allowed determination of picomole amounts of the prostaglandins.

Recently a mass spectrometric method was described which allows quantitative determination of prostaglandin E_1 (PGE_1) at the nanogram level by adding [D_3]-methoxime derivative of PGE_1 to protium-derivatized PGE_1 (1). The deuterated compound was used as carrier in the gas chromatographic separation and introduction into the mass spectrometer and served as internal standard in the final measurement.

Carriers which already contain deuterium in the parent compound would have distinct advantages: the deuterated standard could be added before any isolation and derivatization takes place and the choice of suitable derivatives would become broader. The present paper describes the preparation of [3,3,4,4-D_4]-$PGF_{2\alpha}$ (I) and [3,3,4,4-D_4]-PGE_2 (II) and their use in quantitative mass spectrometry with about 10 times higher sensitivity than reported earlier (c.f. 1).



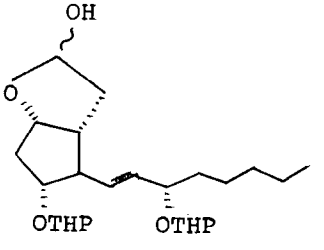
I



II

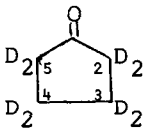
Several synthetic routes are now available which may be used for the preparation of deuterium labeled prostaglandins (2,3). Incorporation of deuterium in the carboxy side chain was chosen since the deuterated moiety is introduced at a very late state of the synthesis and since this part of the prostaglandin molecule is not lost in the formation of ions suitable for measurements. The positions C-3 and C-4 were selected because they exclude the possibility of proton exchange when handling the compounds.

In Corey's synthesis of $PGF_{2\alpha}$ and PGE_2 the Wittig reagent derived from the phosphonium salt of ω-bromo-valeric acid is reacted with lactol III to give $PGF_{2\alpha}$-ditetrahydropyranyl ether, which is converted to $PGF_{2\alpha}$ and PGE_2 (4,5).

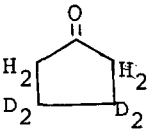


III

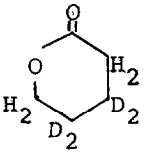
[3,3,4,4-D_4]-ω-bromo-valeric acid (VII) was prepared from commercially available [D_8]-cyclopentanone (IV) (Merck, Sharp & Dohme, Canada, min. 98 atom % D). This involved base catalyzed exchange of IV at C-2 and C-5, Baeyer-Villiger oxidation of the resulting [3,3,4,4-D_4]-cyclopentanone (V) to [3,3,4,4-D_4]-δ-valerolactone (VI) (6,7) and treatment of the lactone with HBr to give VII (8).



IV



V

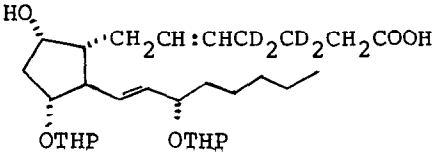


VI

$BrCH_2CD_2CD_2CH_2COOH$

VII

Wittig reaction of the triphenyl-phosphonium salt of VII with optically active lactol III (4,5) gave compound VIII which was partly hydrolyzed to $[3,3,4,4\text{-}D_4]$-$PGF_{2\alpha}$ (I) and partly oxidized with Jones reagent and then hydrolyzed to $[3,3,4,4\text{-}D_4]$-PGE_2 (II).



VIII

NMR and mass spectra of the deuterated compounds described above were in total agreement with the assigned structures.

The mass spectrometric analysis of deuterium content gave for I <0.1% D_0, <0.1% D_1, 0.6% D_2, 4.8% D_3 and 94.5% D_4 and for II <0.1% D_0, <0.1% D_1, 0.8% D_2, 7.2% D_3 and 91.9% D_4.

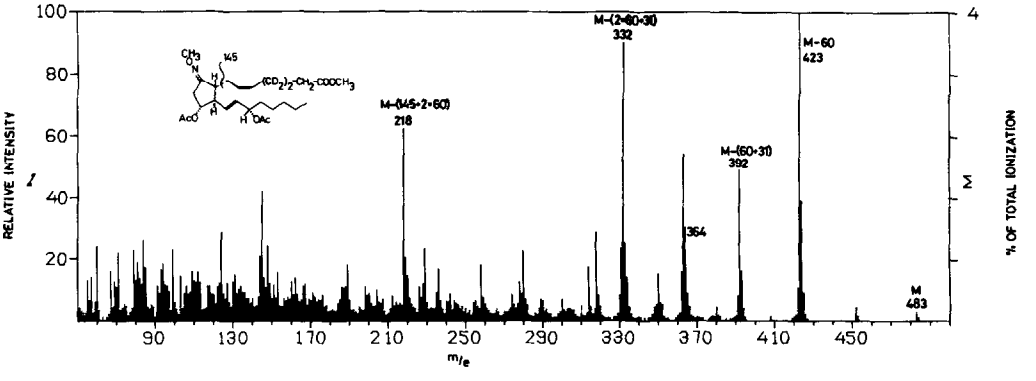


Fig. 1 Mass spectrum of second isomer (syn/anti) of acetylated methoxime derivative of methyl ester of $[3,3,4,4\text{-}D_4]$-PGE_2 (c.f. ref. 9).

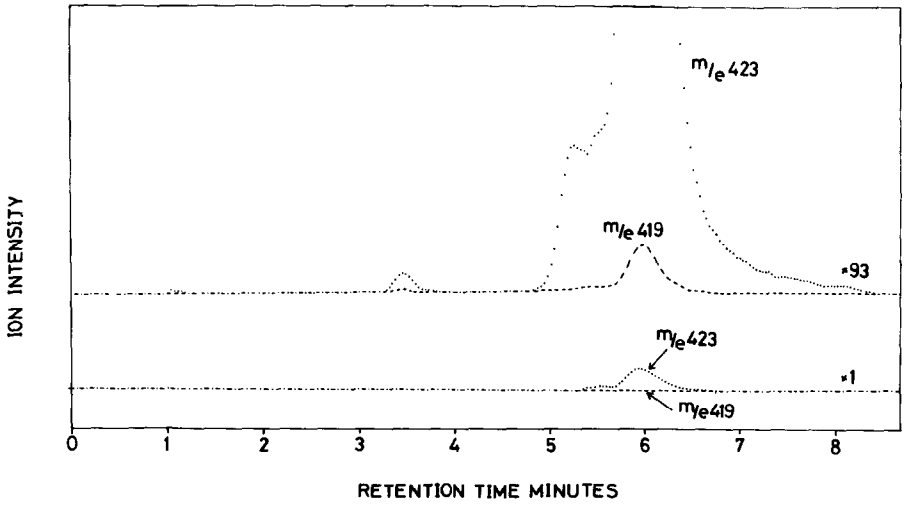


Fig. 2 Recording of ions m/e 419 and m/e 423 using LKB 9000 gas chromatograph-mass spectrometer equipped with accelerating voltage alternator unit. Column, 3 m x 2.5 mm i.d. 1% SE-30 on silanized gas chrom P. Carrier gas helium. Colum temperature, 250°. Electron energy, 30 eV. 250 nanog $PGE_2/[D_4]$-PGE_2 derivative (ratio 8:1000) was injected.

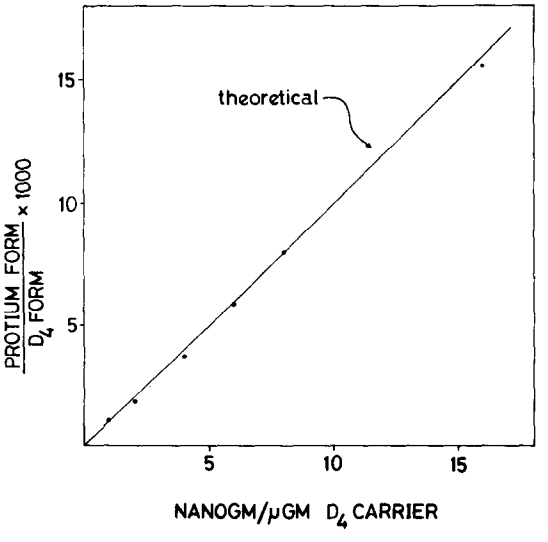


Fig. 3 Ratios of peak areas of PGE_2 and $[D_4]$-PGE_2 derivative versus composition of injected material.

PGE_2 was added to $[D_4]$-PGE_2 in ratios from 1:1000 to 16:1000. After treatment with diazomethane, O-methyl oxime (methoxime) derivatives were prepared and acetylated as previously described (9). The mass spectrum of this derivative of the carrier $[D_4]$-PGE_2 is shown in fig. 1. The base peak, M-60 was selected for measurement on the basis of response and background. Aliquots of the mixtures were analyzed with an LKB 9000 gas chromatograph-mass spectrometer equipped with the accelerating voltage alternating unit. A recording of intensities of ions m/e 419 and m/e 423 after injection of about 250 nanog PGE_2/$[D_4]$-PGE_2 derivative (ratio 8:1000) is shown in fig. 2. The ratios of the areas of each ion were calculated and the ratio obtained for the $[D_4]$-form was subtracted. A plot of these corrected ratios versus composition of the analyzed material is shown in fig. 3. The experimental data follow closely the theoretical line with a slope coefficient of 1. Since the injected amount of the carrier was 250 nanog the lower limit for quantitative determination of PGE_2 with the present equipment was 250 picog (0.7 picomole). The precision of the method determined by repetitive determinations was ±5.9% (S.D., n=10) for 2.8 picomoles and ±1.4% (n=10) for 11.2 picomoles of PGE_2.

$PGF_{2\alpha}$ was analyzed in a similar way as methyl ester and triacetyl derivative using $[D_4]$-$PGF_{2\alpha}$ as carrier. The mass spectrum of the carrier is shown in fig. 4. The intensities of ions m/e 314/318 were recorded (fig. 5).

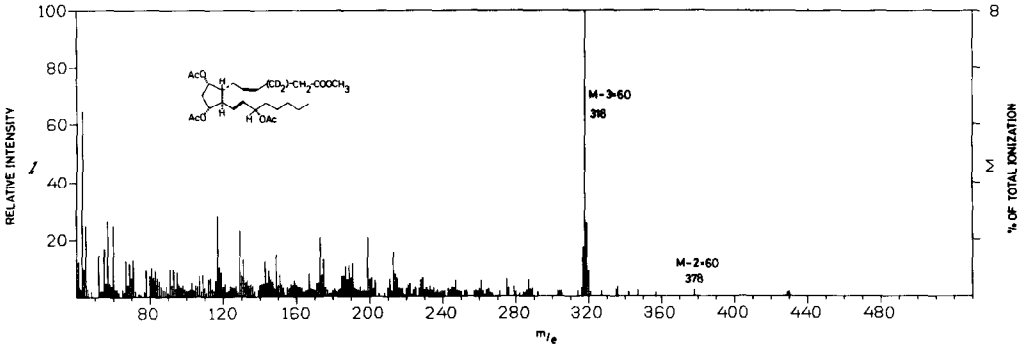


Fig. 4 Mass spectrum of triacetate of methyl ester of $[3,3,4,4\text{-}D_4]$-$PGF_{2\alpha}$.

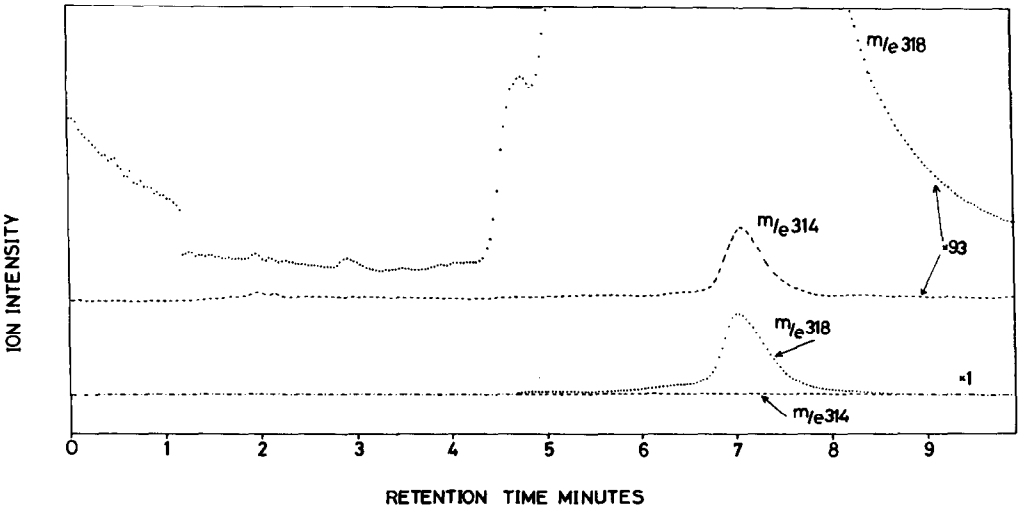


Fig. 5 Recording of ions m/e 314 and m/e 318. Conditions: see fig. 2. 250 nanog $PGF_{2\alpha}/[D_4]$-$PGF_{2\alpha}$ derivative (ratio 4:1000) was injected.

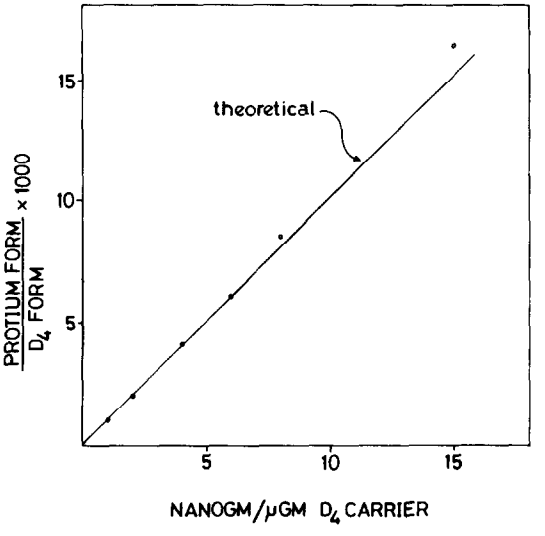


Fig. 6 Ratios of peak areas of $PGF_{2\alpha}$ and $[D_4]$-$PGF_{2\alpha}$ derivative versus composition of injected material.

The data obtained with 250 nanog carrier is shown in fig. 6. The precision was ±7.2% (S.D., n=10) for 1.4 picomoles and ±1.9% (S.D., n=10) for 5.6 picomoles of $PGF_{2\alpha}$.

The results demonstrate that the mass spectrometric method described using synthetic $[D_4]$-prostaglandins

allows determination of PGE_2 and $PGF_{2\alpha}$ at the picomole level with adequate precision. As will be reported elsewhere, the method has sufficient sensitivity and specificity for analysis of plasma prostaglandins and for studies of enzymatic synthesis of prostaglandins *in vitro* from endogenous precursors.

REFERENCES

1. Samuelsson, B., M. Hamberg, C.C. Sweeley, Analytical Biochemistry, 38, 301 (1970).
2. Axen, U., J.E. Pike, W.P. Schneider, Prostaglandins in the Total Synthesis of Natural Products, Vol. III. Ed. J.W. ApSimon; John Wiley and Sons, Inc. (in press).
3. Corey, E.J., Annals of the New York Academy of Sciences, 180, 24 (1971).
4. Corey, E.J., N.M. Weinshenker, T.K. Schaaf, W. Huber, J. Amer. Chem. Soc. 91, 5675 (1969).
5. Corey, E.J., T.K. Schaaf, W. Huber, U. Koelliker, N.M. Weinshenker, J. Amer. Chem. Soc. 92, 397 (1970).
6. Fries, S.L., J. Amer. Chem. Soc. 71, 2571 (1949).
7. Sager, W.F., A. Duckworth, J. Amer. Chem. Soc. 77, 188 (1955).
8. Boorman, E.J., R.P. Linstead, H.N. Rydon, J. Chem. Soc. 1933, 568.
9. Gréen, K., Chem. Phys. Lipids 3, 254 (1969).