Journal of Chromatography, 153 (1978) 83–90

CHROM. 10,699

USE OF NEW SILYLATING AGENTS FOR SEPARATION AND IDENTIFICATION OF PROSTAGLANDINS BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

HIROSHI MIYAZAKI, MASATAKA ISHIBASHI and KOUWA YAMASHITA

Research Laboratories, Pharmaceutical Division, Nippon Kayaku Co., 3-31 Shimo, Kita-ku, Tokyo 115 (Japan)

and

MAKOTO KATORI

Department of Pharmacology, Kitazato University School of Medicine, 1 Asamizodai, Sagamihara, Kanagawa 228 (Japan)

(Received October 17th, 1977)

SUMMARY

A method for the complete separation and selective ion monitoring of five kinds of primary prostaglandins was developed involving gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) using dimethylethylsilyl and dimethyl-*n*-propylsilyl imidazoles as silylating agents. Of these derivatives of prostaglandins, the dimethylethylsilyl ether derivative provided the most suitable properties in GC and GC–MS. The method described will be useful for the profile analysis of biologically important prostaglandins.

INTRODUCTION

In order to clarify the physiological role of individual prostaglandins (PGs), it is necessary to develop a microanalytical method that permits their determination simultaneously in biological specimens because primary prostaglandins play diverse roles in different tissues.

Thus, various PGs have been detected and determined by gas chromatography with electron-capture detection[1–3], radioimmunoassay[4] and gas chromatography–mass spectrometry (GC–MS)[5,6]. GC–MS is the most powerful technique in this area because it provides information that might otherwise be unobtainable.

Trimethylsilylation has been widely used as a derivatization procedure for the protection of labile hydroxyl groups in PGs as it affords more volatile and thermally stable derivatives. As PGs have similar chemical structures, it has been thought that the use of GC separation in determining their metabolic profile is very difficult when analyses are based on the use of trimethylsilyl (TMS) ether derivatives[7].

Although Vane and Horning[8] developed a GC method for determining the metabolic profile of PGs by use of their persilyl derivatives, it is difficult to apply this method to the quantitation of each component in a mixture of PGs. Consequently,

it is normally necessary to separate them by means of other tedious chromatographic steps prior to GC–MS[9].

In earlier work[10,11], the dimethylethylsilyl (DMES) ether derivatives of hydroxysteroids, which were derivatized with DMES-imidazole (DMES-I), showed excellent GC–MS properties. Recently, we have found these derivatives to be useful for the GC separation of bile acids[12] and biogenic amines[13].

This paper deals with the GC–MS properties and stability of DMES ether derivatives of PG methyl esters in relation to the determination of metabolic profiles.

EXPERIMENTAL

Gas chromatography

A Shimadzu GC-5AP gas chromatograph equipped with a flame-ionization detector was employed. The glass column had dimensions of 3 m × 2.5 mm I.D. and was packed with 1% OV-101 (Ohio Valley Co., Marietta, Ohio, U.S.A.) on Gas-Chrom Q (80–100 mesh) (Applied Science Labs., State College, Pa., U.S.A.). The temperature of the column oven was maintained at 240°, except that in profile analysis it was programmed from 180° to 300° at the rate of 2°/min. The flow-rate of the carrier gas (helium) was 40 ml/min. The temperature of the injection port and detector was 290°.

Mass spectrometry

An LKB 9000B GC–MS system equipped with a data-processing system was employed. The column was as above. The temperature of the column oven was maintained at 240° and the flow-rate of the carrier gas (helium) was 30 ml/min. The temperature of the injection port and separator was 280° and the ionization source was kept at 290°. The accelerating energy and trap current were 70 eV and 60 μA, respectively.

Samples and reagents

The PGs used (PGD_2, PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$) were kindly supplied by Ono Pharmaceutical Co. (Osaka, Japan).

TMS-imidazole (TSIM) and methoxylamine hydrochloride were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). DMES-I and dimethyl-*n*-propylsilylimidazole (DMPS-I) were synthesized in our laboratory by the method described previously[10].

Derivatization

The PG methyl ester and methoxime methyl ester derivatives were prepared according to the procedure of Gréen[14]. To 0.1–0.2 mg of the above derivatives, 100 μl of silylating agent were added and the mixture was allowed to stand at room temperature for 2 h. The DMES ether derivatives were chromatographed over silica gel with *n*-hexane and eluted with 20 ml of benzene–diethyl ether (95:5). After evaporation of the solvent, the residue was dissolved in *n*-hexane (100 μl) and used for GC and GC–MS analysis.

In order to examine the stability of the DMES ether derivative of $PGF_{2\alpha}$ methyl ester on storage, a dilute solution of the DMES ether of $PGF_{2\alpha}$ methyl ester (10

ng/ml) containing cholestane as an internal standard was prepared. The peak area ratio of this derivative to an internal standard was determined at intervals during 2 weeks using selective ion detection.

RESULTS AND DISCUSSION

PGD_2, PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were used as model compounds in order to investigate the derivatization and GC separation conditions.

$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were converted smoothly into the methyl ester-O-silyl ether derivatives by treatment with diazomethane and then with TSIM and DMES-I. When PGD_2, PGE_1 and PGE_2 methyl esters, which contain a carbonyl group, were treated directly with silylating agents, the desired derivatives could not be obtained because of their conversion into compounds of the PGB series and into enol ethers, owing to dehydration and enolization in the silylation process. Ketonic samples were accordingly converted into the methoxime derivatives prior to silylation in order to prevent the above conversions. However, GC revealed that each of the silyl ether derivatives of oxime methyl esters gave two well resolved peaks due to *syn*- and *anti*-forms in their gas chromatograms, as reported by Gréen[14].

The ratio of *syn*- and *anti*-isomers of the methoxime, benzyloxime and silyloxime derivatives in the gas chromatograms was investigated. The ratio of the isomers in the methoxime derivative was about 3:1–4:1, while the ratios of the isomers in the other two oximes were almost 1:1.

The silyl ether methyl esters of the PGF series and the silyl ether methoxime methyl esters of the PGD and PGE series gave well shaped peaks when non-polar liquid stationary phases were used, whereas with polar liquid stationary phases they appeared as poorly shaped peaks on the gas chromatograms.

Table I shows the GC results for the above mentioned derivatives obtained using OV-101.

TABLE I

GAS CHROMATOGRAPHIC DATA OF THE TMS, DMES AND DMPS ETHER DERIVATIVES OF FIVE REPRESENTATIVE PGs

PG = prostaglandins, MU = methylene unit, $\Delta\,[Um]_{E(\text{or P})} = MU_{DMES(\text{or DMPS})} - MU_{TMS}$.

PG	*MU values*			*Δ [Um] values*	
	TMS	*DMES*	*DMPS*	*Δ [Um]$_E$*	*Δ [Um]$_P$*
PGD_2	27.15	29.12	30.13	1.97	2.98
PGE_1	27.60	29.52	30.53	1.92	2.93
PGE_2	27.43	29.33	30.33	1.90	2.90
$PGF_{1\alpha}$	27.36	30.24	31.70	2.88	4.34
$PGF_{2\alpha}$	27.06	29.87	31.36	2.81	4.30

The *Δ[Um]* values, which are defined as the difference between the methylene unit values of a TMS and a DMES ether of each PG, were in agreement with those of di- and trihydroxysteroids described previously[10,11]. The retention times of the DMES ether derivatives were longer than those of the corresponding TMS ether derivatives.

As shown in Table I, the retention times of the TMS ether derivatives of these

PGs increased in the order of $PGF_{2\alpha} < PGD_2 < PGF_{1\alpha} < PGE_2 < PGE_1$, but these PGs could not be separated by this method. On the other hand, the retention times of the DMES and the DMPS ether derivatives increased in the order of $PGD_2 < PGE_2 < PGE_1 < PGF_{2\alpha} < PGF_{1\alpha}$. The separation of PGE_1, $PGF_{2\alpha}$ and $PGF_{1\alpha}$ was enhanced with an increase in the number of carbon atoms in the silyl ether groups, whereas the separation of PGE_1 and PGE_2 gradually became incomplete.

Fig. 1 shows a GC separation of the TMS and DMES ethers of the above derivatives. Of these, the DMES ether derivatives were the most suitable for the complete separation of PGs.

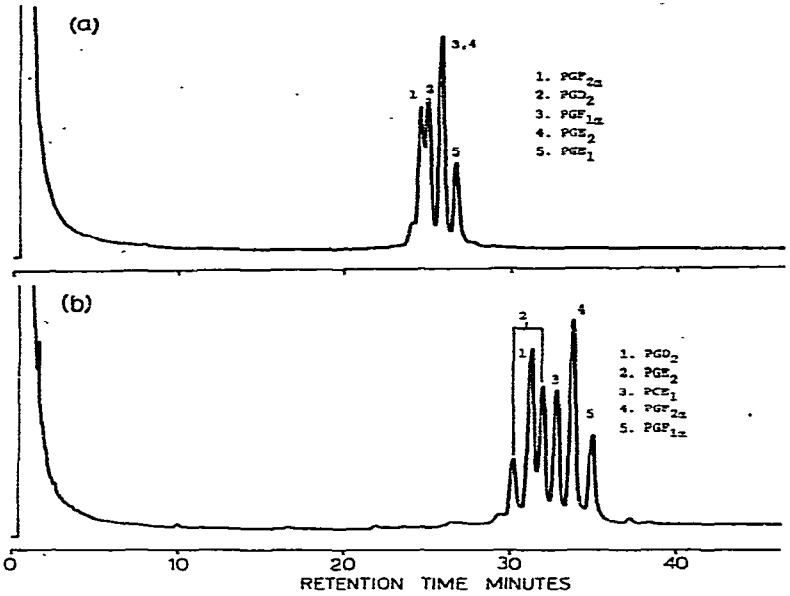

Fig. 1. Gas chromatograms of (a) the TMS and (b) the DMES ether derivatives of an authentic mixture of PG methyl esters and methoxime methyl esters.

Fig. 2 shows the mass spectra of the TMS and DMES ether derivatives of $PGF_{2\alpha}$ methyl esters. The appearance of the molecular ion, although in low abundance, was sufficient to confirm the structure of the expected derivative and the shift of the molecular ion from *m/e* 584 to 626 indicates the incorporation of the DMES group into $PGF_{2\alpha}$. The DMES ether was characterized by the molecular ion clusters M^+, $[M - 15]^+$ and $[M - 29]^+$, as described previously[10,11].

A comparison of the mass spectrum of the DMES ether with that of the TMS ether indicated that the fragment ion at *m/e* 555 was formed by the elimination of C_5H_{11} (the side-chain of C_{16}–C_{20}) from the molecular ion. The ions at *m/e* 522 and 451 resulted from the elimination of dimethylethylsilanol (DMESOH) from the molecular ion and from the ion at *m/e* 522, respectively.

The relative intensities of the characteristic ions from the DMES ether appearing in the high mass region, such as *m/e* 555, 522 and 451, were enhanced in comparison with the corresponding ions from the TMS ether.

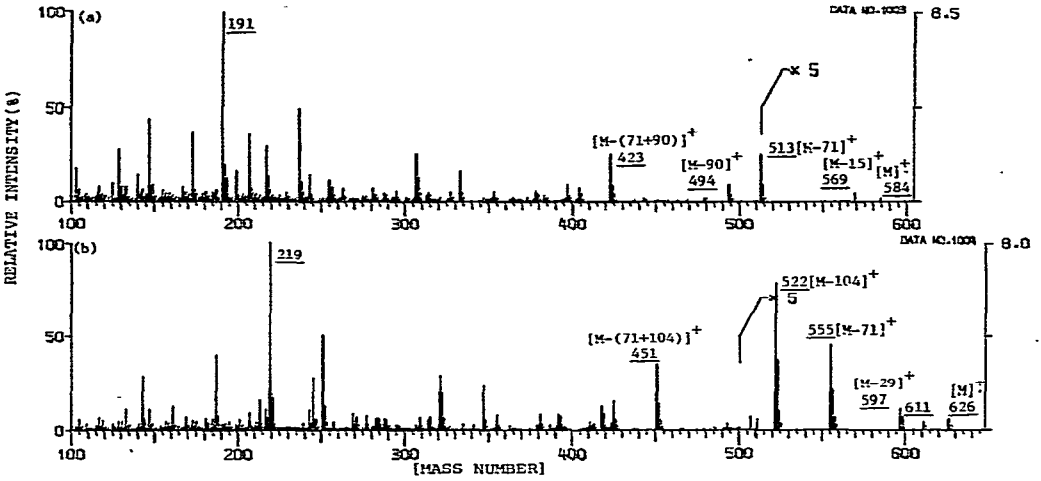

Fig. 2. Mass spectra of (a) the TMS and (b) the DMES ether derivatives of $PGF_{2\alpha}$ methyl esters.

The mass fragmentation of the DMES ether derivatives was in agreement with that of the TMS ether, except that the *m/e* values of the ion retaining the dimethylethylsilyloxy group in it were shifted by 14 *n* mass units, where *n* denotes the number of hydroxyl groups in the PGs. The above results suggest that the $\Delta[Um]$ values and the shift of 14 *n* mass units might be useful in estimating the number of hydroxyl groups in PGs and their metabolites.

Fig. 3 shows the mass spectra of the major isomers of methoxime DMES ethers of PGE_1, PGE_2 and PGD_2 methyl esters.

The fragmentations of these derivatives were in agreement with those of the corresponding TMS ethers, except for the 28 mass unit shift due to the presence of two hydroxyl groups. In the mass spectra of the isomers of the methoxime DMES ether derivatives of PGE_2 methyl esters, the relative intensities of the ions in the major isomer were widely different from those in the minor isomer; in the former, the base peak was at *m/e* 239, but that of the latter was the ion at *m/e* 496, which was formed by the elimination of the C_5H_{11} group from the molecular ion. However, in both isomers of methoxime DMES ether derivatives of PGD_2 methyl esters, the ion at *m/e* 496 was observed as the base peak in their mass spectra. The mass spectral pattern of the major isomer of the methoxime DMES ether derivative of PGD_2 methyl ester was similar to that of the minor isomer of the methoxime DMES ether derivative of PGE_2 methyl ester. These fragmentations were very similar to those of the corresponding TMS ether derivatives.

To investigate the stability of the DMES ether derivative, the DMES ether derivative of $PGF_{2\alpha}$ methyl ester was used as a model compound. The stability of this derivative on storage is shown in Fig. 4. This DMES ether derivative was unaltered after 2 weeks, while the corresponding TMS ether derivative was decomposed to the extent of more than 30%. This observation confirms the relative stability of the DMPS ether derivatives of catecholamines[13].

Further, the recovery of the DMES ether derivative in the eluate from a silica gel column was more than 95%. DMES ether derivatives may thus be convenient for

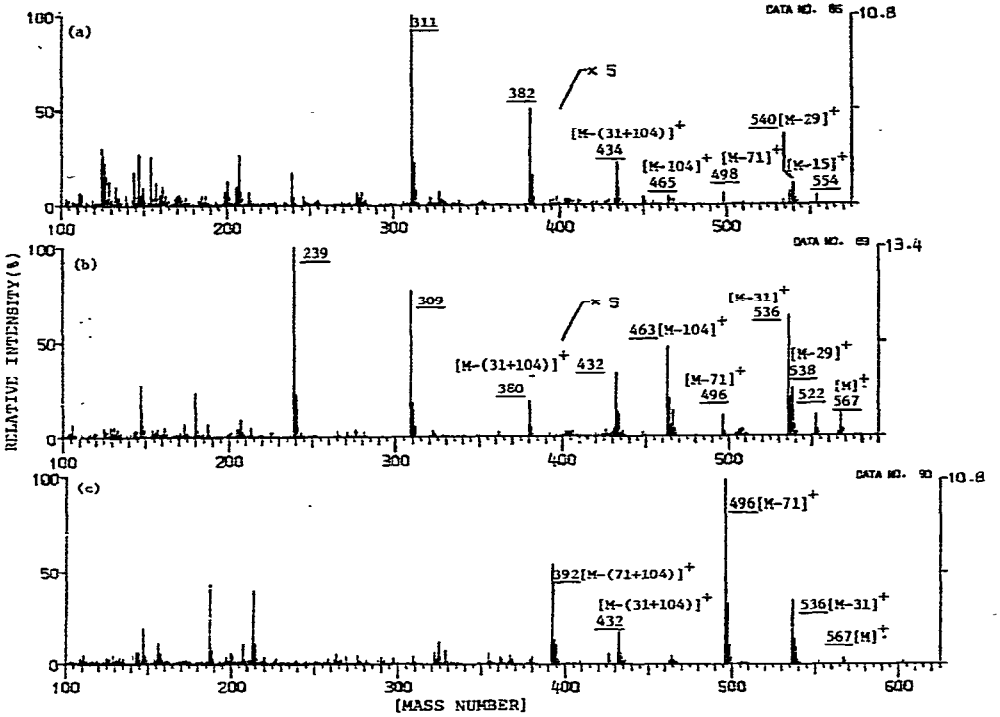

Fig. 3. Mass spectra of the DMES ether derivatives of (a) PGE_1, (b) PGE_2 and (c) PGD_2 methoxime methyl esters.

use with silica gel column chromatography in methods for the purification of PG extracts from biological samples.

Fig. 5 shows conventional mass chromatograms of the above derivatives of five representative PGs using characteristic fragment ions: *m/e* 496 (PGD_2), *m/e* 453

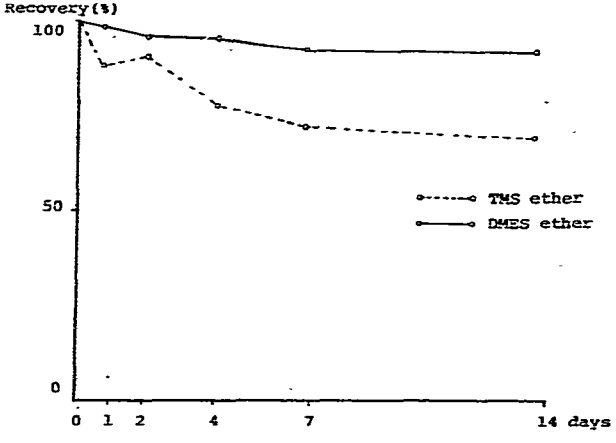

Fig. 4. Comparison of the storage stability of the TMS and DMES ether derivatives of $PGF_{2\alpha}$ methyl esters at room temperature.

($PGF_{1\alpha}$), *m/e* 451 ($PGF_{2\alpha}$), *m/e* 311 (PGE_1) and *m/e* 309 (PGE_2). These PGs were separated completely, as shown.

In conclusion, dimethylethylsilylation of PGs makes it possible to enhance their GC separation and stability. These derivatives may be useful for determining the metabolic profiles on prostaglandins. Biomedical applications of the method will be published elsewhere.

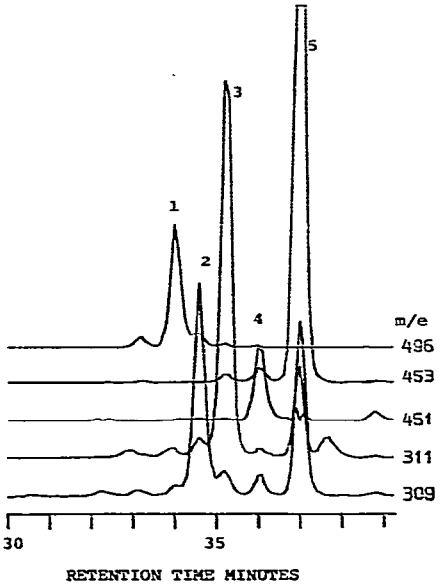

Fig. 5. Mass chromatographic separation of the DMES ether derivatives of PG methyl esters and methoxime methyl esters: (1) PGD_2; (2) PGE_2; (3) PGE_1; (4) $PGF_{2\alpha}$; (5) $PGF_{1\alpha}$.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. W. Tanaka, Research Laboratories, Nippon Kayaku Co., for his encouragement throughout this work. They express their sincere thanks to Dr. M. Hayashi, Ono Pharmaceutical Co., for the generous gift of samples and to Prof. C. J. W. Brooks, Chemistry Department, University of Glasgow, for his valuable advice and suggestions.

REFERENCES

1 G. H. Jouvenaz, D. H. Nugteren, R. K. Beerthuis and D. A. van Dorp, *Biochim. Biophys. Acta*, 202 (1970) 231.
2 G. H. Jouvenaz, D. H. Nugteren and D. A. van Dorp, *Prostaglandins*, 3 (1973) 175.
3 B. S. Middledich and D. M. Desiderio, *Prostaglandins*, 2 (1972) 195.
4 R. M. Gutierrez-Cernosek, L. Levin and H. Gujika, *Methods Enzymol.*, 35 (1975) 287.
5 P. F. Crain, D. M. Desiderio and J. A. McClosky, *Methods Enzymol.*, 35 (1975) 359.
6 B. Samuelsson and R. Paoletti, *Advances in Prostaglandin and Thromboxane*, Raven Press, New York, 1976.
7 J. Rosello, J. Tusell and E. Gelpi, *J. Chromatogr.*, 130 (1977) 65.
8 F. Vane and M. G. Horning, *Anal. Lett.*, 2 (1969) 357.

9 K. Gréen, E. Granström, B. Samuelsson and U. Axen, *Anal. Biochem.*, 54 (1973) 434.
10 H. Miyazaki, M. Ishibashi, M. Itoh and T. Nambara, *Biomed. Mass Spectrom.*, 4 (1977) 23.
11 H. Miyazaki, M. Ishibashi, M. Itoh, K. Yamashita and T. Nambara, *J. Chromatogr.*, 133 (1977) 311.
12 H. Miyazaki, M. Ishibashi and K. Yamashita, *Biomed. Mass Spectrom.*, submitted for publication.
13 H. Miyazaki, M. Ishibashi, K. Yamashita, M. Yakushiji and M. Yasutake, *97th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, Japan, April 1977.*
14 K. Gréen, *Chem. Phys. Lipids*, 3 (1969) 254.