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MICRODETERMINATION OF PROSTAGLANDINS AND THROMBOXANE B_2 BY GAS CHROMATOGRAPHY USING AN ELECTRON-CAPTURE DETECTOR

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SUMMARY

The separation of the dimethylethylsilyl, dimethyl-*n*-propylsilyl (DMnPS) and dimethylisopropylsilyl ether derivatives of pentafluorobenzyl (PFB) esters of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ and methoxime-PFB esters of PGE_1, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 was investigated by gas chromatography using an electron-capture detector. Of these silyl ether derivatives, the DMnPS ether derivatives gave the best separation when analysed with the use of a high-performance fused silica capillary column coated with OV-101.

The stability of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was compared with that of the trimethylsilyl (TMS) ether derivative. The recovery of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester in the eluate from a silica gel column was more than 95%, whereas the corresponding TMS ether derivative was partially decomposed and adsorbed on the silica gel column. In addition, the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was stable for at least 1 week during storage in *n*-hexane solution (10 ng ml^{-1}) at room temperature.

The method was applied to the quantitation of PGs in extracts obtained from the urine of spontaneous hypertensive rats. The amounts of PGE_2 and $PGF_{2\alpha}$ in the urine were calculated to be 92 ± 30 and 29 ± 7 ng ml^{-1}, respectively, when analysed using the orthogonal polynomial equation.

INTRODUCTION

Much attention has been focused on the elucidation of the physiological role of prostaglandins (PGs), which exhibit diverse physiological activity because of the different positions of the functional groups in the molecule. A number of methods for the microdetermination of PGs have been investigated in order to elucidate the rela-

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tionship between the pharmacological activity and metabolic profile of PGs and thromboxanes (TXs).

As gas chromatography with electron-capture detection (GC–ECD) has a sensitivity comparable to those of radioimmunoassay[1,2] and gas chromatography–mass spectrometry (GC–MS)[3–5], it has been widely used for the analysis of PGs in biological fluids[6,7]. On the other hand, the capillary column technique makes it possible to perform the profile analysis of biologically important substances in a complicated mixture obtained from biological fluids as a result of a great improvement in the GC separation[8–10].

In conventional methods, the trimethylsilyl (TMS) ether derivatives of pentafluorobenzyl (PFB) esters of PGs have been used to enhance their sensitivities in GC–ECD[11–13]. However, it has been suggested that the profile analysis of PGs using the TMS ether derivatives of PFB esters might be difficult owing to their incomplete separation even if an open-tubular glass capillary column is used.

In previous work, it was found that the dimethylethylsilyl (DMES), dimethyl-*n*-propylsilyl (DMnPS) and dimethylisopropylsilyl (DMiPs) ether derivatives gave a better GC separations than the corresponding TMS ether derivatives[14–16]. This paper describes the GC separation of these silyl ether derivatives of PG PFB esters or MO-PFB esters suitable for GC–ECD and a biomedical application of the method to the quantitation of PGE_2 and $PGF_{2\alpha}$ in rat urine.

EXPERIMENTAL

Gas chromatography

A Shimadzu GC-7A gas chromatograph equipped with a 10-mCi ^{63}Ni electron-capture detector was employed. A thermostable open-tubular fused silica capillary column coated with OV-101 (25 m × 0.25 mm I.D.) was prepared in our laboratories using a dynamic coating method as described by Schomburg and Hasman[17]. An all-glass solventless injector constructed according to the method of Van den Berg and Cox[18] was mounted horizontally on the heated injector block of the gas chromatograph. Helium was used as the carrier gas and nitrogen make-up gas was introduced at the end of the column to keep the flow-rate through the detector at 40 ml min^{-1}. An inlet pressure of 0.4 kg cm^{-1} produced a linear gas velocity of 25 cm sec^{-1}. The gas inlet pressure was adjusted to obtain maximum column efficiency with respect to the TMS ether derivative of $PGF_{2\alpha}$ PFB ester as a representative sample. The temperature of the column oven was maintained at 270–280°C. The temperature of the injection heating block and detector was 290°C.

Gas chromatography–mass spectrometry

An LKB 2091 gas chromatograph–mass spectrometer equipped with a data processing system was employed. The column was a 2 m × 2.5 mm I.D. glass coil packed with 1.5% OV-101 (Ohio Valley Co., Marietta, OH, U.S.A.) on Chromosorb W HP (80–100 mesh) (Applied Science, U.S.A.). The temperature of the column oven was maintained at 250–270°C. The flow-rate of the carrier gas (helium) was 20 ml min^{-1}. The temperature of the injection heating block and separator was kept at 290°C and that of the ionization source at 280°C. The ionization energy and trap current were 22.5 eV and 100 μA, respectively. The accelerating voltage was 2.33 kV.

Materials

PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were purchased from Fuji Chemical Industry (Takaoka, Japan). TXB_2 and 6-keto-$PGF_{1\alpha}$ were kindly supplied by Ono Pharmaceutical Co. (Osaka, Japan). TMS-imidazole, DMES-imidazole, DMnPS-imidazole, methoxyamine hydrochloride and pentafluorobenzyl (PFB) bromide were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMiPS-imidazole was synthesized in our laboratories as previously reported[19].

Sephadex LH-20 (25–100 μm) and silica gel (Kieselgel 60, 70–230 mesh) were obtained from Pharmacia (Uppsala, Sweden) and Merk (Darmstadt, G.F.R.), respectively. Extube® 1003 was purchased from Analytichem International (CA, U.S.A.). Other reagents were of the highest purity available.

Extraction of PGs from rat urine

After PGE_1 (100 ng) and $PGF_{1\alpha}$ (50 ng) had been added to the rat urine (1.0 ml) as internal standards, the urine was acidified to pH 3.0 with 0.2 *N* hydrochloric acid and the resulting solution was transfered on to Extube 1003. After being allowed to stand for 5 min, PGs were eluted with 30 ml of benzene–ethyl acetate (90:10). The organic layer was then evaporated under reduced pressure below 40°C and the residue was treated with methoxyamine hydrochloride and PFB bromide, followed by reaction with silylating agents.

Derivatization

Authentic $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were converted directly into their PFB esters by treatment with PFB bromide and diisopropylethylamine in acetonitrile at 40°C for 1 h according to the method of Wickramasinghe and Shaw[20]. The resulting mixture was diluted with benzene and extracted. The organic layer was washed with water, dried over anhydrous sodium sulphate and then introduced into a microcolumn of silica gel (5 cm × 0.8 cm I.D.). PG PFB esters were eluted with ethyl acetate–methanol (99:1) (25 ml). The solution of the eluate was evaporated and the resulting PFB ester was treated with TMS-imidazole, DMES-imidazole, DMnPS-imidazole and DMiPS-imidazole (100 μl) at room temperature for 30 min. Ketonic PGs were converted into methoxime derivative by treatment with a saturated solution of methoxyamine hydrochloride in dry pyridine at 60°C for 1 h prior to esterification.

In order remove the excess of silylating reagents, the reaction mixture was chromatographed over Sephadex LH-20 (5 cm × 0.8 cm I.D.) with chloroform–*n*-hexane–methanol (10:10:1). After evaporation of the solvent the residue was dissolved in *n*-hexane containing 1% (v/v) pyridine and used for GC analysis. The extract obtained from rat urine was treated in the same manner as ketonic PGs.

RESULTS AND DISCUSSION

The DMES, DMnPS and DMiPS ether derivatives of PGs and thromboxane B_2 (TXB_2) PFB esters or methoxime(MO)–PFB esters were prepared and used for the investigation of derivatization and GC separation conditions.

$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were smoothly converted into their PFB esters by treatment with PFB bromide in acetonitrile in the presence of diisopropylethylamine according to the procedure of Wickramasinghe and Shaw[20]. On being treated under the above

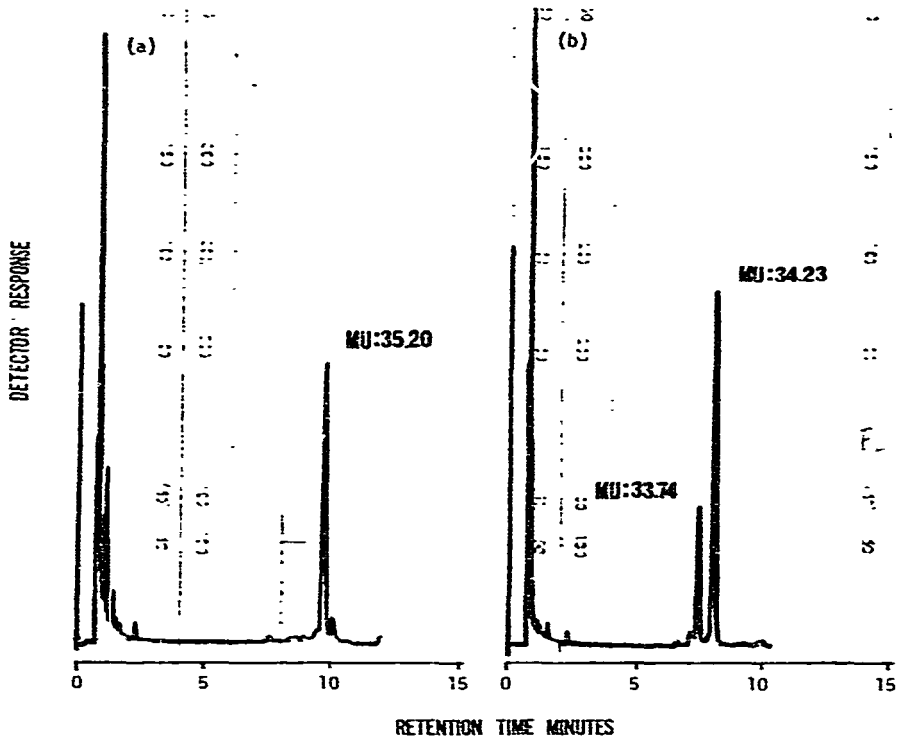

Fig. 1. ECD gas chromatograms of the reaction products of (a) $PGF_{2\alpha}$ PFB ester and (b) PGE_2 MO-PFB ester with DMnPS-imidazole by the use of a fused silica capillary column coated with OV-101 (25 m × 0.25 mm I.D.) at 280°C.

esterification conditions using a basic catalyst, PGE_1 and PGE_2, which contain a β-ketol system, were partially dehydrated and converted into α,β-unsaturated ketones such as the PGA or PGB series. Ketonic samples should initially be converted into the MO derivatives prior to esterification in order to prevent the above undesirable side-reaction. The PG and TXB_2 PFB esters or MO–PFB esters were then silylated with trimethylsilyl(TMS)-, DMES-, DMnPS- or DMiPS-imidazole at room temperature and the resulting derivatives gave well shaped GC peaks, suggesting that the stepwise derivatization proceeded smoothly and quantitatively.

Fig. 1 shows the gas chromatograms with electron-capture detection of the reaction products of $PGF_{2\alpha}$ PFB ester (a) and PGE_2 MO-PFB ester (b) with DMnPS-imidazole obtained with the use of an open-tubular fused silica capillary column coated with OV-101 (25 m × 0.25 mm I.D.). Each of the silyl ether derivatives of PG MO-PFB esters except TXB_2 provided two well resolved peaks owing to the formation of *syn*- and *anti*-isomers, as in the case of PG MO-methyl ester (ME) reported by Gréen[21].

Table I lists the methylene unit (*MU*) values of the resulting TMS, DMES, DMnPS and DMiPS ether derivatives when analysed using the fused silica capillary column. Although the TMS ether derivatives were eluted in the order $PGF_{2\alpha}$, $PGF_{1\alpha}$, PGE_2, PGE_1, TXB_2 and 6-keto-$PGF_{1\alpha}$, the individual PGs could not be separated

TABLE I

GC DATA FOR THE TMS, DMES, DMnPS AND DMiPS ETHER DERIVATIVES OF PGs AND TXB_2 PFB ESTERS OR MO-PFB ESTERS

Compound	*MU values*				*Δ[Um] values*		
	TMS	*DMES*	*DMnPS*	*DMiPS*	$\Delta[Um]_E$	$\Delta[Um]_{nP}$	$\Delta[Um]_{iP}$
PGE_1	31.66*	33.62	34.03	34.89	1.96	2.37	3.23
	32.15**	34.10	34.59	35.39	1.95	2.44	3.24
PGE_2	31.40*	33.32	33.74	34.61	1.92	2.34	3.21
	31.86**	33.79	34.23	35.06	1.93	2.44	3.20
$PGF_{1\alpha}$	31.70	34.79	35.70	36.67	3.09	4.00	4.97
$PGF_{2\alpha}$	31.34	34.28	35.20	36.18	2.94	3.86	4.84
6-Keto-$PGF_{1\alpha}$	32.35	35.21	36.28	37.00*	2.86	3.93	4.65
				37.10**			4.75
TXB_2	32.11	34.92	35.89	36.95	2.81	3.78	4.84

* The minor isomer of the methoxime derivative.
** The major isomer of the methoxime derivative.

completely with these TMS ether derivatives, as shown in Table I. On the other hand, the DMES ether derivatives were eluted in the order PGE_2, PGE_1, $PGF_{2\alpha}$, $PGF_{1\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$. The separation of the DMES ether derivatives of PGs and TXB_2 PFB esters or MO-PFB esters was better than that of the TMS ether derivatives. However, the major isomer of PGE_1 and the peak of $PGF_{2\alpha}$ overlapped completely and were observed as a single peak.

The DMES, DMnPS and DMiPS ether derivatives of hydroxysteroids gave larger *MU* values than the TMS ether derivatives, and this was multiplied in proportion to the number of hydroxyl group in the molecule[22–25]. The separation of PGE and PGF series was improved with an increase in the carbon number in the silyl ether derivatives[14,19]. Particularly when PGs and TXB_2 were analysed as their DMiPS and DMnPS ether derivatives the peaks of the PGF series were well separated from those of the PGE series.

Table I also shows the *Δ[Um]* values, which are defined as the difference between the *MU* values of the TMS ether and the DMES ($\Delta[Um]_E$), DMnPS ($\Delta[Um]_{nP}$) or DMiPS ($\Delta[Um]_{iP}$) ether derivatives. The average and standard deviations of the $\Delta[Um]_E$ values were 1.94 ± 0.02 for dihydroxy compounds and 2.93 ± 0.12 for trihydroxy compounds, and those of the $\Delta[Um]_{nP}$ values were 2.40 ± 0.05 and 3.89 ± 0.09, respectively. The $\Delta[Um]_{iP}$ values of these compounds were larger than the corresponding $\Delta[Um]_{nP}$ values. The *Δ[Um]* values of the silyl ether derivatives of PGs and TXB_2 PFB esters or MO-PFB esters were in agreement with those of di- and trihydroxysteroids[22,23] bile acids[15,24] and PG ME derivatives[14,19].

The DMES, DMnPS and DMiPS ether derivatives permitted the PGs and TXB_2 to be classified into two distinct groups of di- and trihydroxy compounds and greatly enhanced the separation of the individual PGs and TXB_2 in comparison with the corresponding TMS ether derivatives.

Fig. 2 shows the GC separation of the standard mixture of PGs and TXB_2 as their DMnPS ether derivatives of PFB esters or MO-PFB esters by the use of the

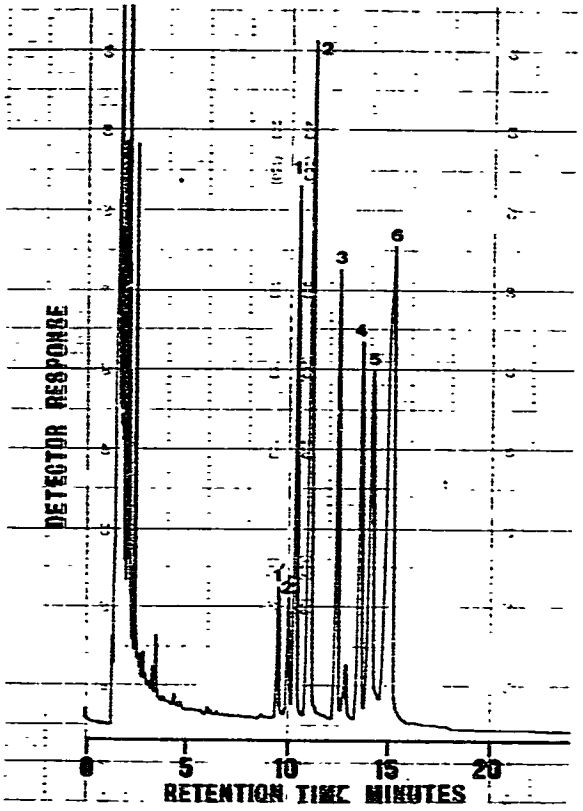

Fig. 2. GC–ECD separation of the DMnPS ether derivatives of five kinds of PGs and TXB_2 PFB esters or MO-PFB esters by the use of fused silica capillary column at 280°C: (1', 1) PGE_2; (2', 2) PGE_1; (3) $PGF_{2\alpha}$; (4) $PGF_{1\alpha}$; (5) TXB_2; (6) 6-keto-$PGF_{1\alpha}$.

fused silica capillary column as described above. Complete separation of the DMnPS of PFB esters or MO-PFB esters could be achieved within 20 min.

Fig. 3 shows the mass spectra of the TMS and DMnPS ether derivatives of $PGF_{2\alpha}$ PFB ester. The appearance of the molecular ion, although in low abundance, was sufficient to confirm the structure of the expected derivative. The shift of the molecular ion from m/z 750 to 834 indicated the incorporation of the DMnPS group into the PFB ester. When the mass spectrum of the DMnPS ether derivative was compared with that of the TMS ether derivative, the mass spectral fragmentation patterns were closely related except for 28 mass unit shift per silanoxy group. For instance, the [M−71] ion produced by the cleavage of the C_{15}–C_{16} bond implied the presence of three silanoxy groups by the shift from m/z 679 in the TMS ether derivative to m/z 763 (679 + 28 × 3) in the DMnPS ether derivative.

With hydroxysteroids, the mass spectra of the DMES and DMnPS ether derivatives were characterized by their inherent [M−alkyl] ion. Contrary to our expectation, the intensity of this inherent ion in the DMES and DMnPS ether derivatives of PGs and TXB_2 PFB ester or MO-PFB ester was not enhanced to that of the correspond-

ing TMS ether derivative as in the ME or MO-ME derivatives of PGs previously reported[14,19].

The ion of *m/z* 763 produced by the loss of 71 mass units (C_5H_{11}) from the molecular ion was typical of a β-chain. Successive elimination of three dimethyl-*n*-propylsilanol (DMnPSOH) groups from the molecule gave rise to ions of *m/z* 716, 598 and 480 from the molecular ion and the ions of *m/z* 645, 527 and 409 from the [M−71] ion. The ion of *m/z* 247 was a constituent with an F prostaglandin ring system, which was assigned as the structure corresponding to the ion of *m/z* 191 in the TMS ether derivative of $PGF_{2\alpha}$ ME. The ion of *m/z* 265 which was observed as a base peak was characterized and assigned as the ion corresponding to the ion of *m/z* 237 in the TMS ether derivative.

Fig. 4 shows the mass spectrum of the DMnPS ether derivative of the major isomer of PGE_2 MO-PFB ester. The ions of $[M]^{+\cdot}$ and $[M-43]^+$ were observed in low abundance, but the appearance of the molecular ion was sufficient to confirm the structure of the expected derivative. The ion of *m/z* 688 which was produced by the loss of C_5H_{11} from the molecular ion was typical of a β-chain as in the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester. The mass spectrum of the major isomer of the DMnPS ether derivative of PGE_2 MO-PFB ester was dominated by the ions of *m/z* 489 and 253. The ion of *m/z* 489 was considered to be formed by the cleavage of the E prostaglandin ring system, which was assigned as the structure corresponding to the analogous ion of *m/z* 323 in the DMnPS ether derivative of MO-ME. The shift of the ion of *m/z* 323 to *m/z* 489 indicated the incorporation of a PFB moiety in the DMnPS ether of PGE_2 MO. The ion of *m/z* 253 was produced by the cleavage of the C_{10}–C_{11} bond, as deduced from a comparison with the mass spectra of the corresponding TMS and DMnPS ether derivative of PGE_2 MO-ME[14,19].

Table II summarizes the mass spectral data of PGs and TXB_2 as their DMnPS ether derivatives of the PFB esters or MO-PFB esters. The appearance of the molecular ion was sufficient to confirm the structural elucidation of the expected derivatives. The elimination of the appropriate silanol and production of the subsequent characteristic fragment ion were observed in all PGs except TXB_2.

The storage stability of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester in *n*-hexane solution was compared with that of the corresponding TMS ether derivative. The DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was diluted with *n*-hexane to 10 ng/ml and the residual amount of the derivative in the solution was determined by GC–ECD using the PFB ester of 5β-cholanic acid as an internal standard. The results are shown in Fig. 5. The DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was stable in *n*-hexane solution for at least 7 days at room temperature, whereas the corresponding TMS ether derivative was decomposed to the extent of more than 30%. This observation was in good agreement with the storage stability of the DMnPS ether derivatives of catecholamine trifluoroacetamides[16] and the DMES ether derivative of $PGF_{2\alpha}$ ME[14].

Further, the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was chromatographed over a silica gel column and eluted with benzene–diethyl ether (95:5). When [^{14}C]$PGF_{2\alpha}$ was used as a marker, the recovery of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester (10 ng) in the eluate from the column was more than 95%. On the other hand, the recovery of the corresponding TMS ether derivative was found to be 75% owing to decomposition and adsorption onto the column. The DMnPS ether

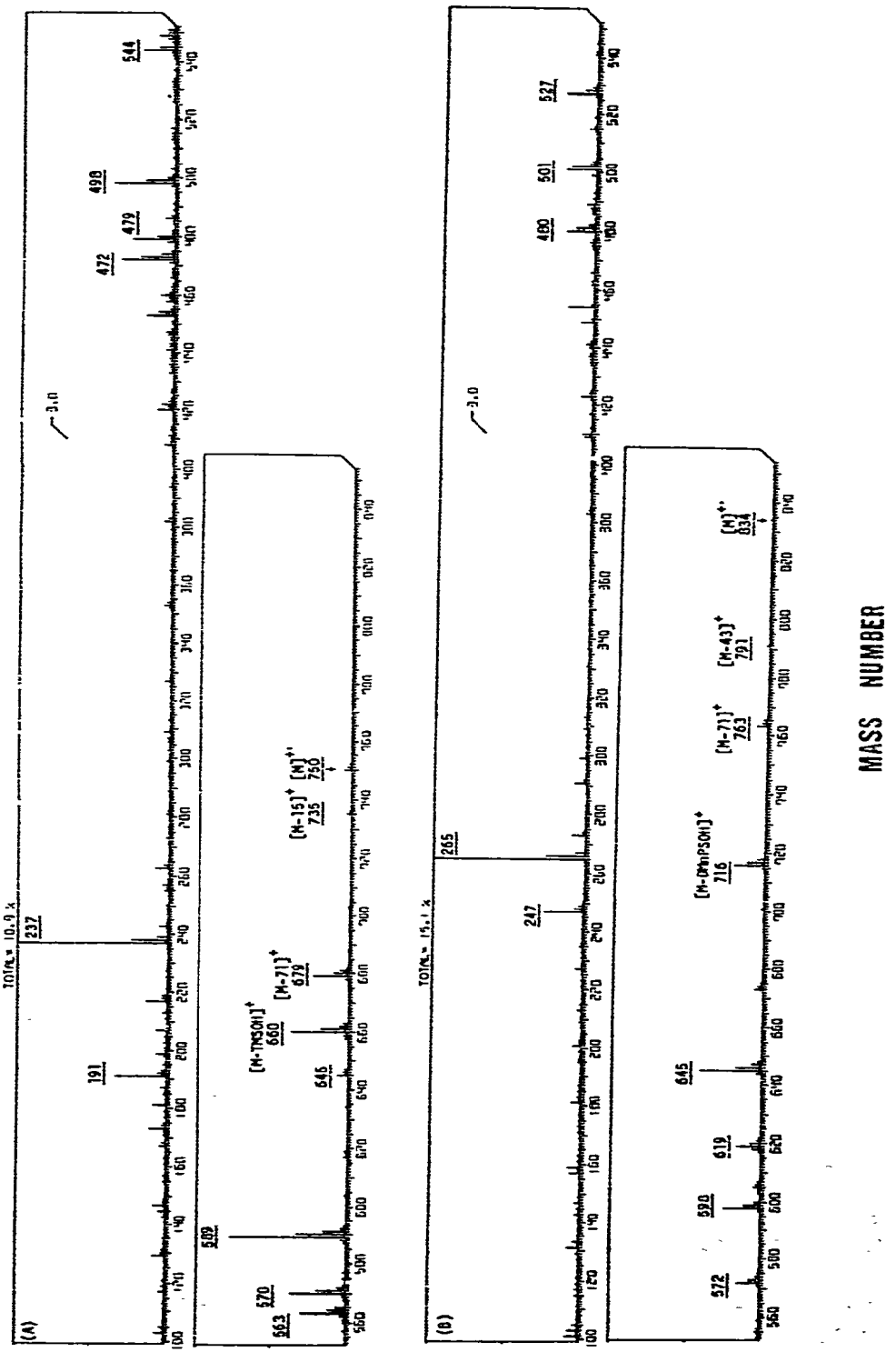

Fig. 3. Mass spectra of (A) TMS and (B) DMnPS ether derivative of PGF_2, PFB ester.

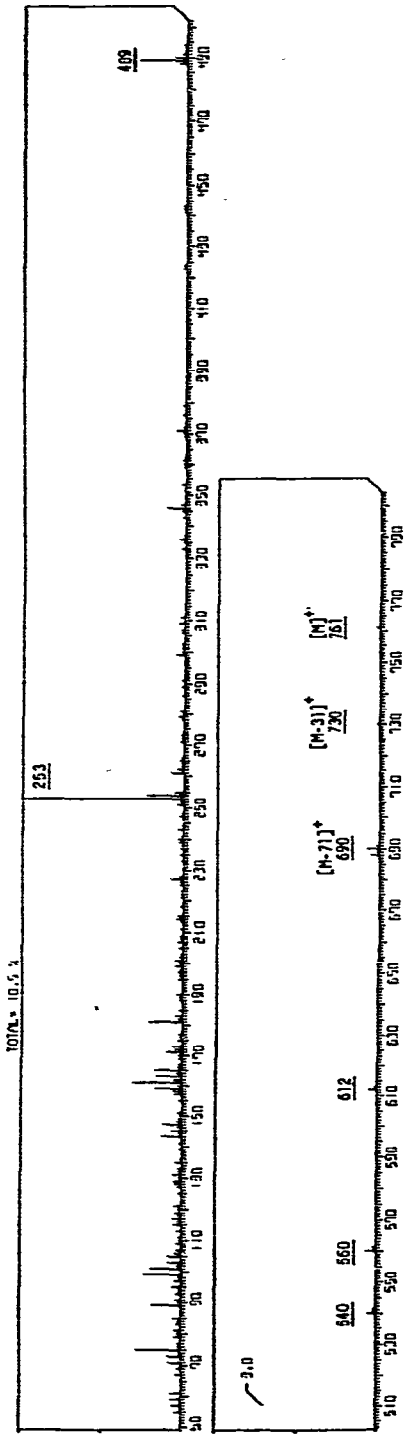

Fig. 4. Mass spectrum of the DMnPS ether derivative of the major isomer of PGE_2 MO-PFB ester.

TABLE II

MASS SPECTRAL DATA FOR THE DMnPS ETHER DERIVATIVES OF PGs AND TXB_2 PFB OR MO-PFB ESTERS

Compound	*Mol. wt.*	*Relative intensity (%)*								
		$M^{+\cdot}$	$M-31$	$M-43$	$M-71$	$M-118$	*Other ions*			
PGE_1	763	1.2*	22.5	8.8	100	10.5	614 (34)***	574 (90)‡	491 (23)	227 (98)
		1.0**	2.5	2.0	5.0	2.3	614 (3)***	562 (24)	491 (100)	253 (25)
PGE_2	761	1.0*	15.0	7.0	43.0	10.0	612 (26)***	572 (54)‡	489 (10)	75 (100)
		0.8**	1.0	0.5	7.5	0.2	612 (2)***	560 (2)	489 (29)	253 (100)
$PGF_{1\alpha}$	836	0.2	–	3.8	20.1	28.8	647 (98)‡	600 (16)‡‡	265 (35)	247 (100)
$PGF_{2\alpha}$	834	0.4	–	1.0	2.5	7.1	645 (14)‡	598 (9)‡‡	265 (100)	247 (26)
6-Keto-$PGF_{1\alpha}$	879	5.0	40.1	17.5	12.5	10.0	730 (63)***	690 (25)	612 (70)‡‡‡	572 (100)†
TXB_2	879	0.2	2.0	0.5	0.2	0.3	730 (5)***	690 (4)	612 (6)‡‡‡	308 (100)

* The minor isomer of the methoxime derivative.
** The major isomer of the methoxime derivative.
*** $[M-(31+118(DMnPSOH))]^+$.
‡ $[M-(71+118)]^+$.
‡‡ $[M-(2\times 118)]^+$.
‡‡‡ $[M-(31+(2\times 118))]^+$.
† $[M-(71+(2\times 118))]^+$.

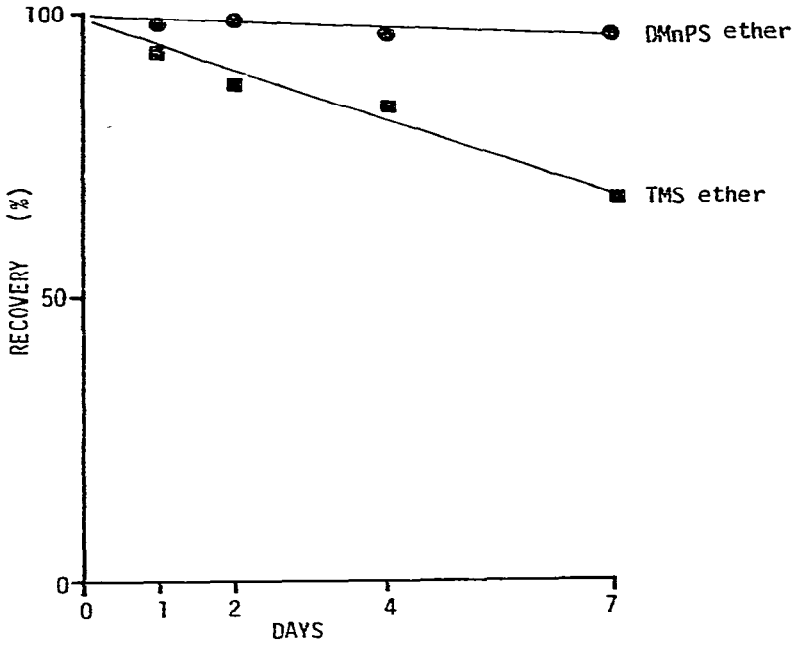

Fig. 5. Storage stability of the TMS and DMnPS ether derivatives of $PGF_{2\alpha}$ PFB ester in *n*-hexane solution.

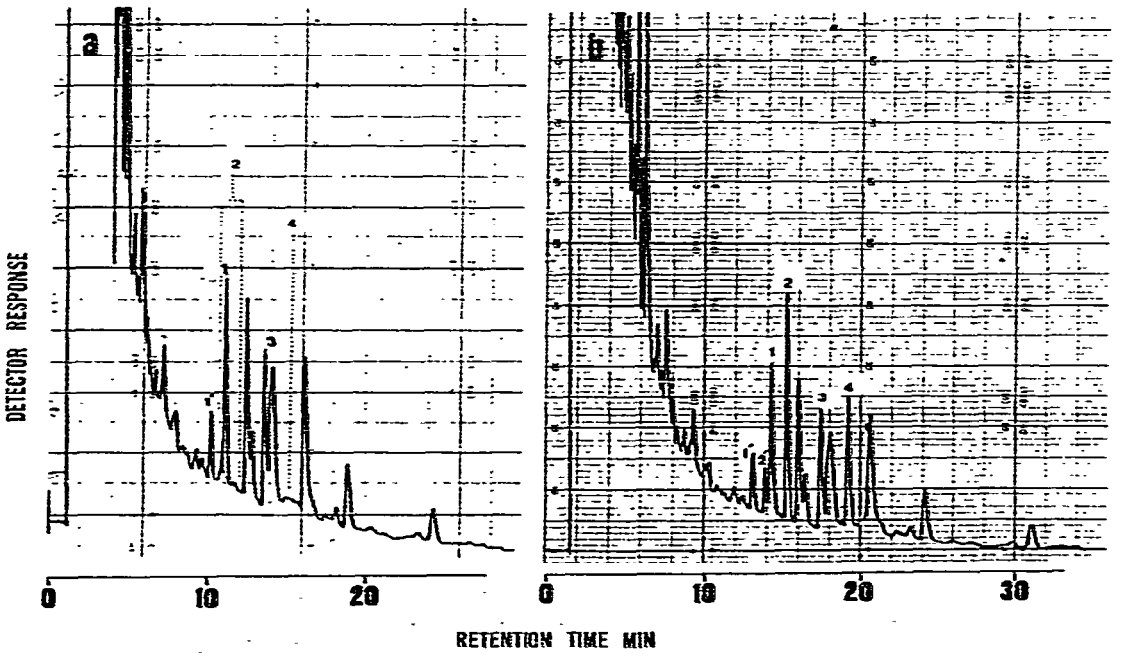

Fig. 6. ECD gas chromatograms of the DMnPS ether derivatives of PG PFB esters and MO-PFB esters (a) in the extracts from the urine of SH rats and (b) after addition of PGE_1 and $PGF_{1\alpha}$ as internal standards by the use of a fused silica capillary column at (a) 285°C and (b) 280°C: (1′, 1) PGE_2; (2′, 2) PGE_1; (3) $PGF_{2\alpha}$; (4) $PGF_{1\alpha}$.

derivative may be very useful for the further purification of PGs and TXB_2 in extracts of biological samples with a silica gel column.

The present method, based on the combination of the DMnPS ether derivatives and fused silica capillary GC–ECD, was applied to the determination of primary PGs in the urine of spontaneous hypertensive (SH) rats. Fig. 6 shows typical ECD gas chromatograms of the DMnPS ether derivatives of PGs PFB esters or MO-PFB esters in the extract of urine. As shown in Fig. 6a, the peaks with *MU* values of 34.23 and 35.20 were identified as PGE_2 and $PGF_{2\alpha}$, by comparison with the mass spectra of the authentic compounds. Several unidentified peaks were observed in addition to PGE_2 and $PGF_{2\alpha}$. Fortunately, the *MU* values of these unidentified peaks did not overlap those of the peaks corresponding to the DMnPS ether derivatives of PGE_2, PGE_1, $PGF_{2\alpha}$ and $PGF_{1\alpha}$. This fact indicated that the interfering substances coexisting in the urine could be eliminated completely by this sample preparation using Extube® and silica gel column chromatography.

It was essential to add an internal standard to the urine prior to extraction in order to compensate for the losses during the extraction and derivatization process. PGE_1 and $PGF_{1\alpha}$ could be utilized as internal standards for the quantitation of PGE_2 and $PGF_{2\alpha}$ in the urine of SH rats.

Fig. 7. shows the calibration graphs for PGE_2 and $PGF_{2\alpha}$ as their DMnPS ether derivatives using PGE_1 and $PGF_{1\alpha}$ as internal standards. Good linearity was

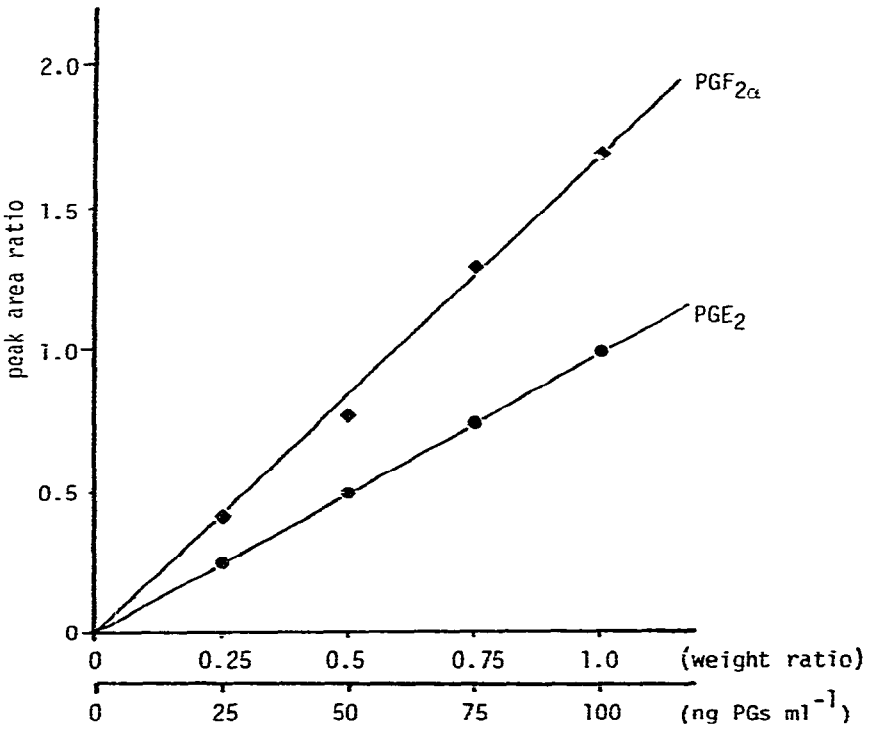

Fig. 7. Calibration graphs for PGE_2 and $PGF_{2\alpha}$ obtained by plotting the wright ratio (PGE_2/PGE_1 or $PGF_{2\alpha}/PGF_{1\alpha}$) *versus* the peak-area ratio.

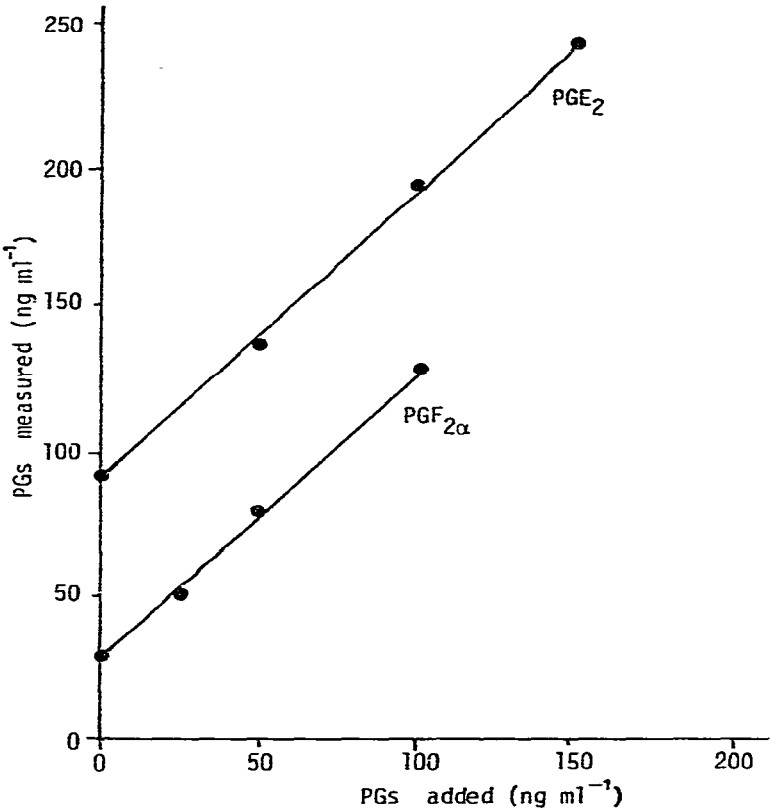

Fig. 8. Determination of PGE_2 and $PGF_{2\alpha}$ in the extracts obtained from the urine of SH rats by statistical analysis using the orthogonal polynomial equation after addition of known amounts of PGE_2 and $PGF_{2\alpha}$ to the urine.

obtained for concentrations of PGE_2 and $PGF_{2\alpha}$ in the range from 25–100 ng ml^{-1} in urine.

To obtain a more accurate determination of PGE_2 and $PGF_{2\alpha}$ in the urine of SH rats, we applied the orthogonal polynomial equation[26] with addition of known amounts of authentic PGE_2 and $PGF_{2\alpha}$. Fig. 8 shows the plots of amounts of PGs measured *versus* known amounts of PGs added before extraction. The exact amounts of PGE_2 and $PGF_{2\alpha}$ in the extracts obtained from the urine of SH rats were calculated to be 92 ± 30 and 29 ± 7 ng ml^{-1} respectively, from the orthogonal polynomial equation.

In conclusion, the microdetermination based on the capillary GC–ECD using the DMnPS ether derivatives was very useful for the analysis of PGs and TXB_2 in biological fluids without any interference from endogeneous substances. This method may be useful for the elucidation of the relationship between the pharmacological activity and metabolic profile of PGs and TXB_2 in biological fluids.

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