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PROSTAGLANDINS AND THROMBOXANE: *tert.*-BUTYLDIMETHYLSILYL ETHERS AS DERIVATIVES FOR THEIR ANALYSES BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY–SELECTED ION MONITORING

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SUMMARY

tert.-Butyldimethylsilyl ethers of eighteen prostaglandins (PGs) and thromboxane have been analyzed by combined packed-column or fused-silica capillary gas chromatography–mass spectrometry (GC–MS). Except for thromboxane B_2 and PGs of the 3 series the [M − 57] ion, the loss from the molecular ion of the *tert.*-butyl radical, is generally the major peak in the spectra of PGs. This high abundance of large fragments provides suitable criteria for the employment of these derivatives in GC–MS selected ion monitoring for rapid quantity estimation of PGs from biological samples. PGs from rat stomach fundus were rapidly quantitated in a single chromatogram using this procedure.

INTRODUCTION

Corey and Venkateswarlu[1] first described the favorable chromatographic properties of the *tert.*-butyldimethylsilyl (*t*-BDMS) group for the analysis of prostaglandins (PGs). Subsequently, a variety of hydroxylated compounds (carbohydrates[2], fatty acids[3], nucleosides[4], phosphoglycerol dialkyl ethers[5], cannabinoids[6], steroids[7], salbutanol[8], terbutaline[9], prostaglandins[10–14] and metabolites of $PGF_{1\alpha}$ and $PGF_{2\alpha}$[15–17] were determined as their *t*-BDMS ether derivatives.

The principal advantage of *t*-BDMS ethers are their simplified mass spectra and abundance of large mass fragments owing to the greater stability of the derivatives as compared to those of trimethylsilyl (TMS) ethers. Generally $[M - 57]$, $[M - 132]$ and $[M - (57 + 132)]$ (for compounds containing more than one hydroxyl group) are the most characteristic peaks found in mass spectra of *t*-BDMS ether derivatives. This derivatization though less structurally diagnostic than the TMS derivatives[7] therefore provides a more accurate estimate where mass abundances are used to assess compound quantities, for example in selected ion monitoring applications. Another advantage is that *t*-BDMS ethers are some 10,000 times more stable to hydrolysis[18,19]. Thus, protection against atmospheric moisture during the preparation, isolation and storage of *t*-BDMS ethers is usually not necessary. Finally, compared to the TMS

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ethers, *t*-BDMS ethers, derivatives of PGs have longer retention times and are better resolved on gas-chromatographic columns.

In this study, we examined the mass spectra of eighteen *t*-BDMS ether derivatives of PGs and thromboxane (TX) using both packed and fused-silica capillary gas chromatography–mass spectrometry (GC–MS). A simple computer program was developed to facilitate identification of the source of fragments from selected ion monitoring (SIM) analyses. Examples of the use of this technique for the identification and quantification of naturally occurring prostaglandins are discussed.

MATERIALS AND METHODS

Gas chromatography–mass spectrometry

A Hewlett-Packard 5995A GC–MS system was used to obtain the mass spectra of the *t*-BDMS derivatives of PGs. Either a packed 3% OV-101 (or 100–120 mesh Supelcoport) (0.6 m × 3 mm) column or a 12 m × 0.2 mm fused-silica capillary column (Hewlett-Packard) coated with methyl silicone was used to separate the PG derivatives. For the packed-column analyses the temperature was programmed from 200 to 280°C at 15°C/min and the flow-rate of helium carrier gas was set at 20 ml/min. A Hewlett-Packard capillary injection system was employed and the splitless mode was used. The capillary column was coupled to the mass spectrometer by direct insertion. For the capillary analysis, the temperature at injection was kept at 90°C and the purge time was set at 0.3 min. After purging, the oven was programmed at a maximum rate (*ca.* 30°C/min) to 210°C. Then the temperature was programmed at 15°C/min to 280°C until all the compounds of interest were eluted. The helium flow-rate from the chromatograph was 0.8 ml/min, the purge flow-rate $\geqslant$ 40 ml/min. The electron energy was 70 eV, the ion source temperature 240°C, transfer line temperature 280°C and the analyzer temperature 270°C.

For quantitative purposes a SIM program was used, and the GC–MS conditions were the same as for the qualitative analyses. The performance of the quadrupole mass spectrometer was constantly checked by injecting standard methyl ester *t*-BDMS ether of $PGF_{1\alpha}$ and comparing the relative abundance of *m/z* 655 [$M - 57$] and 523 [$M - (57 + 132)$].

Interpretation of GC–MS spectra

To aid in the interpretation of mass spectral data a computer program was developed to identify the important characteristic ion fragments derived from some reasonable losses of fragments from the molecular ion of the *t*-BDMS derivatives.

The algorithm was designed to output the mass combinations making up specific losses from the molecular ion using the input list of the ion fragments from a single spectrum and the specific fragments of interest.

The program itself simply sequentially computes all possible subsets from the set of mass fragments. It simultaneously identifies and outputs those individuals or combinations which when subtracted from the molecular ion yield the characteristic ion fragments of interest.

The program and a typical application used in the interpretation of ion fragments of *t*-BDMS ether of PGs is available.

Prostaglandins and thromboxane

Prostaglandins A_1, A_2, B_1, B_2, D_2, E_1, E_2, E_3, 20-methyl esters (MEs) E_1, $F_{1\alpha}$, $F_{2\alpha}$, dihomo-$F_{2\alpha}$, 15-keto-$F_{2\alpha}$, 6-keto-$F_{1\alpha}$, 13,14-dihydro-15-keto-$F_{2\alpha}$, 19-OH-$F_{2\alpha}$ and thromboxane B_2 were gifts from Dr. John Pike of the Upjohn Company. $PGF_{3\alpha}$ was prepared from PGE_3 by reduction with sodium borohydride ($NaBH_4$). PGE_3 methyl ester was dissolved in 1 ml of methanol and stirred on ice with sodium borohydride (2 mg) for 5 min. The reaction was continued for another 25 min at room temperature. Water (4 ml) and 0.1 *M* hydrochloric acid were added to adjust the pH to 4.0, the reduced PGF_3 (F_α + F_β) were extracted into diethyl ether and concentrated. Over 95% conversion was obtained.

For quantitative studies, 3,4,4,4-d_4-$PGF_{2\alpha}$ (Merck, St. Louis, MO, U.S.A.) was used. Depending on the concentration of PGs present in the samples, 250–1000 ng of deuterated $PGF_{2\alpha}$ was added to the tissue sample before extraction which was done 10 min after equilibration.

Preparation of methyl ester

The methyl esters of PGs were prepared by treating the PGs with diazomethane[21].

Preparation of methoxime

For ketonic PGs the keto group was converted to a methoxime (MO). Methyl hydroxylamine hydrochloride (Applied Science, State College, PA, U.S.A.) (10 mg/ml pyridine) (50 μl) was added to the methyl esters of PGs and held at 25°C for 12 h. The excess reagent was removed using a stream of nitrogen. The methoximation of PGB_1 and PGB_2 under these conditions did not proceed as usual and these two compounds were chromatographed as the unprotected ketones (see Results).

Preparation of tert.-butyldimethylsilyl ether

After methylation and methoximation, the PGs were further silylated using *tert.*-butyldimethylchlorosilane–imidazole (60 μl) (Applied Science). The reaction mixture was heated at 60°C for 1 h. After removal of the excess reagent using a stream of nitrogen, 0.8% saline (1 ml) was added to the residue and 3 × 2 ml hexane was used to extract the *t*-BDMS ether derivatives of PGs. No interference was encountered during the GC–MS analysis of these derivatives, hence no further purification was necessary.

Extraction of prostaglandins

For the analysis of PGs from biological samples, PGs were first extracted by homogenizing tissue in 0.8% saline (1:4). Deuterated $PGF_{2\alpha}$ (200–1000 ng) was added as an internal standard before homogenization. The homogenate was further incubated at 25°C for 15 min to maximize PG production. The pH was then adjusted to 4.0–4.5 using formic acid. The PGs were extracted from the homogenate by twice extracting with 1.5 volumes of ethyl acetate[22]. The extract was then further purified by thin-layer chromatography (TLC)[22].

For the purification of PGs, pre-coated silica gel G plates (20 × 20 cm) were used. The PGs extracted from tissue were applied 1.5 cm above the bottom edge of the plate and the plates were placed in a filter paper-lined developing tank containing

the solvent system chloroform–isopropanol–ethanol–formic acid (45:5:0.5:0.3)[23]. The solvent was allowed to rise 16 cm from the origin. The plate was removed, dried by using a stream of air redeveloped and extracted from the TLC plates[22] and then derivatized as described above.

RESULTS AND DISCUSSION

A typical capillary chromatogram of methyl ester methoxime *t*-BDMS ether of PGE_2 is shown in Fig. 1. The upper tracing (Fig. 1A) is a single ion chromatogram

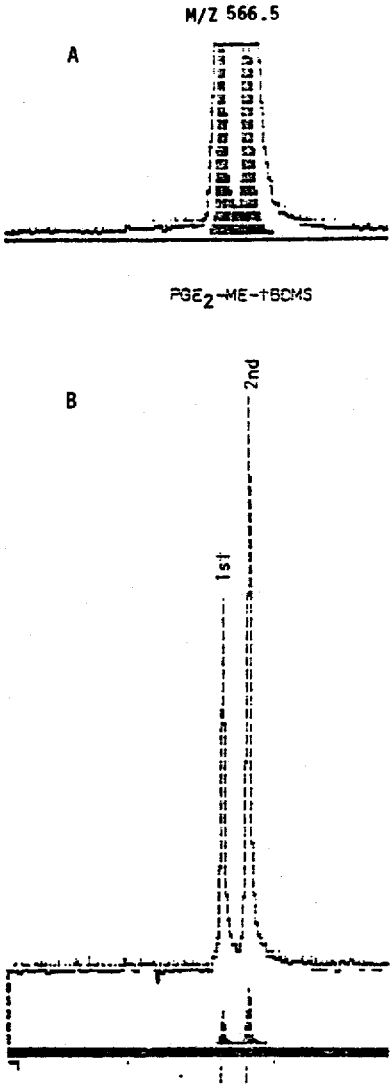

Fig. 1. A typical reconstructed capillary chromatogram of methyl ester methoxime *t*-BDMS ether of PGE_2. A, Selected ion monitored at m/z 566 [$M - 57$]. B, Total ion chromatogram of the first and second isomers PGE_2.

monitored at m/z 566 [M − 57] and the lower tracing (Fig. 1B) represents the total ion chromatogram recorded from mass 400 to 800. Some representative mass spectra of t-BDMS derivatives of PGs are shown in Fig. 2A–D.

The derivatization of ketonic PGs to their methoxime derivatives proceeded quantitatively in all cases except PGB_1 and PGB_2. Examination of their chromatograms and mass spectra indicated that the C-9 keto group in both PGB_1 and PGB_2 was still intact.

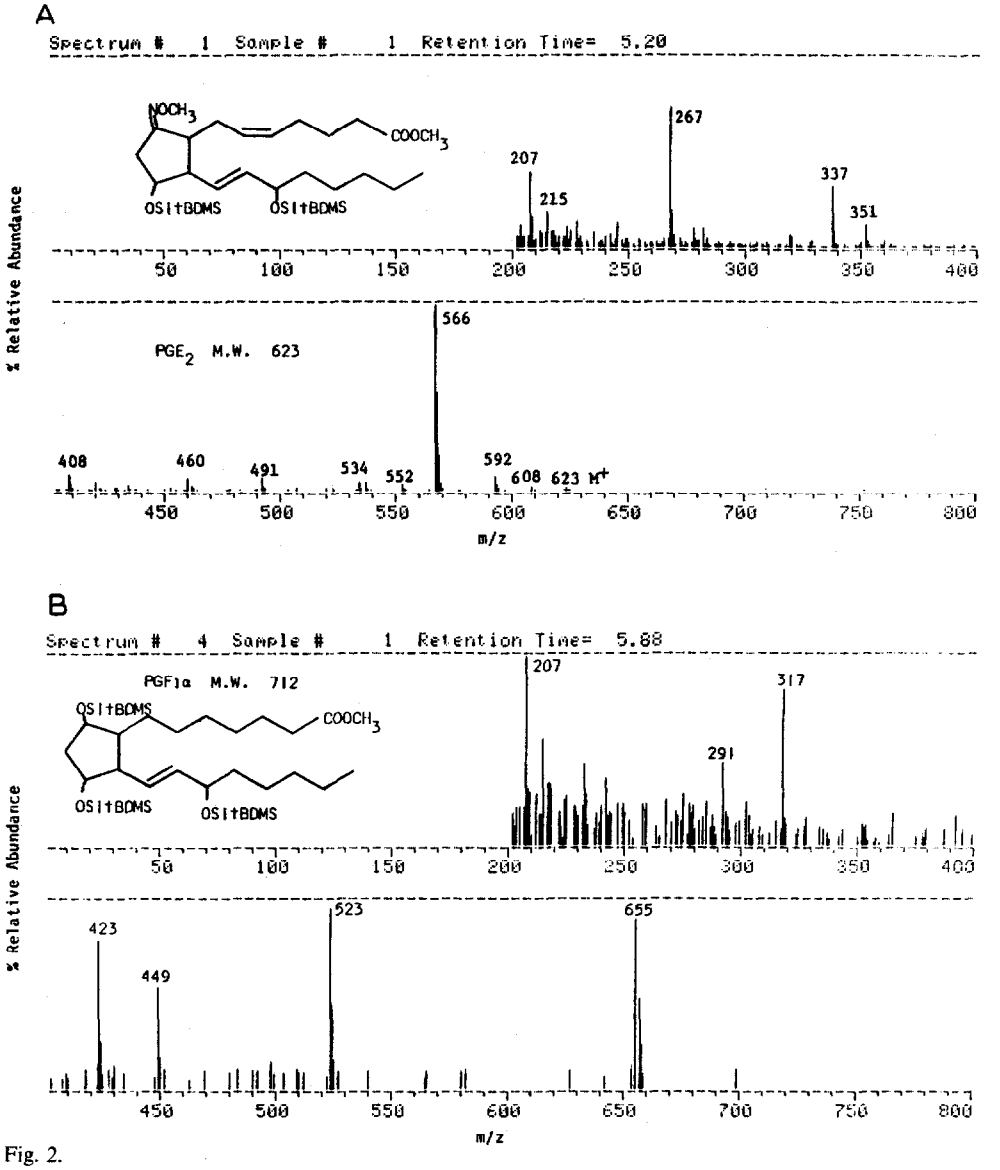

Fig. 2.

(Continued on p. 96)

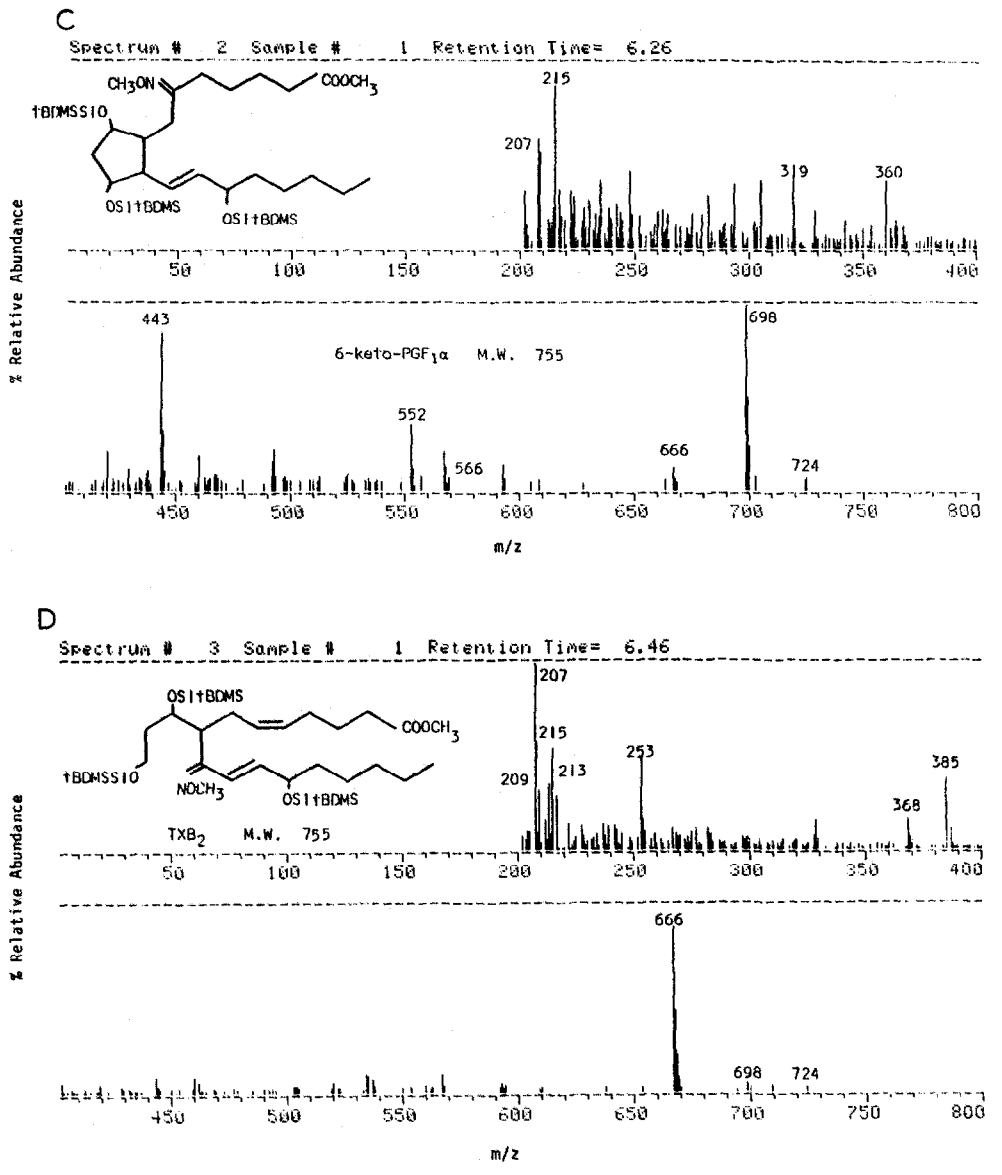

Fig. 2. Representative mass spectra of *t*-BDMS derivatives of prostaglandins PGE_2, $PGF_{1\alpha}$, 6-keto-$F_{1\alpha}$, thromboxane.

The *m/z* [*M* − 57] and/or the *m/z* [*M* − (57 + 132)] as well as other abundant ion fragments in each mass spectrum, of the *t*-BDMS ether derivatives, of PGs are reported in Table I. Only ions above *m/z* 200 were tabulated in order to avoid citation of trivial ions of low mass. Where ions of lower mass or of low abundance have been deemed to be of diagnostic importance, these are discussed separately in the text. For those methoxime derivatives, giving *syn*- and *anti*-isomers, only data for the major peaks are presented. The *t*-BDMS ether derivatives of $PGF_{2\alpha}$, 19-OH-$PGF_{2\alpha}$, $PGF_{1\alpha}$,

15-keto-$PGF_{2\alpha}$, $PGF_{3\alpha}$, PGD_2 and dihomo-$PGF_{2\alpha}$ gave both the m/z [M − 57] and m/z [M − (57 + 132)] to a significant extent (among the ten most significant ions) PGB_1, PGB_2, PGE_1, PGE_2, PGE_3, 13,14-diH-15-keto-$PGF_{2\alpha}$, PGA_2, PGA_1, 6-keto-$PGF_{1\alpha}$ and 20-ME PGE_1 gave only m/z [M − 57] ion; however, TXB_2 gave neither m/z [M − 57] nor m/z [M − (57 + 132)] ion, but the major ion fragment of m/z 666 [M − (57 + 32)]. More detailed discussions of the individual mass spectra of the t-BDMS ether derivatives of PGs are given in the following sections. (The mass spectrum of each individual PG t-BDMS ether derivative is available.)

Generally [M − 57], [M − 132] and [M − (57 + 132)], reflecting the elimination of the *tert.*-butyl radical, the loss of *tert.*-butyldimethylsilanol and the loss of both the *tert.*-butyl and *tert.*-butyldimethylsilanol groups, respectively, were the most characteristic and abundant mass fragments found in the mass spectra of t-BDMS ether derivatives. Other fragments characterized with the aid of the computer analyses also proved of diagnostic value for particular PG derivatives and are discussed below where relevant.

Prostaglandin A_1

In addition to the m/z 436 [M − 57] characteristic peak, the important ion fragments occurring in the spectrum of methyl-ester methoxime t-BDMS ether of PGA_1 are m/z 493, [M^+], 478 [M − 15], 462 [M − 31], 422 [M − 71] [loss of C(16)–C(20)], 330 [M − (31 + 132)] (loss of $^{\cdot}OCH_3$ and t-BDMS), 207 [M − (45 + 241)] [loss of $^{\cdot}NOCH_3$ and the lower side chain C(13)–C(20)].

Prostaglandin A_2

The m/z 434 [M − 57] ion is the base peak present in the mass spectrum of the methyl ester methoxime t-BDMS ether of PGA_2. Other ions of importance are m/z 491 [M^+], 460 [M − 31], 420 [M − 71], 328 [M − (31 + 132)], 359 [M − 132], 328 [M − (31 + 132)] loss of $^{\cdot}OCH_3$ and t-BDMS OH), 262 [M − (31 + 57 + 141)] [loss of $^{\cdot}OCH_3$, *tert.*-butyl radical and C(1)–C(7) upper side chain], 207 [M − (15 + 57 + 71 + 141)] [loss of $^{\cdot}CH_3$, *tert.*-butyl radical, C(16)–C(20) and C(1)–C(7)] or [M − (45 + 239)] [loss of $^{\cdot}NOCH_3$ and the lower side chain C(13)–C(20)].

Prostaglandin B_1

The spectra of the B series t-BDMS ether derivative prostaglandins in spite of the unprotected ketone group on carbon 9 showed abundant molecular ions. Nevertheless, the M − 57 fragment was most abundant. Also evident in the PGB_1 spectrum are the m/z 433 [M − 31], m/z 301 [M − (31 + 132)], m/z 393 [M − 71], loss of C(16)–C(20), m/z 375 [M − (32 + 57)], m/z 365 [M − 99], and m/z 207 [M − 257] ion fragments; losses similarly observed from PGA_1.

Prostaglandin B_2

This PG was again not derivatized by the methoxime reagent and gave an abundant molecular ion. The M − 57 fragment was also most abundant in the spectrum, and as with PGB_1 the fragments with losses of 31: (m/z 431); 31 + 132: (m/z 299); 89: (m/z 373); and 99 (m/z 363) were prominant.

TABLE I

MASS SPECTROMETRIC DATA FOR PROSTAGLANDIN METHYL ESTER (METHOXIME) *tert.*-BUTYLDIMETHYLSILYL ETHERS (70 eV)

Relative abundances in parentheses. Data obtained from a 0.6 m × 3 mm 3% OV-101 column.

PG derivatives	*MW*	*[M − 57]*	*[M − (57 + 132)]*	*Other abundant ion fragments*							
A_1-ME-MO-*t*-BDMS	493	436 (100)	–	462, (7)	422, (10)	372, (12)	330, (12)	298, (10)	215, (16)	207 (22)	
A_2-ME-MO-*t*-BDMS	491	434 (100)	–	420, (16)	359, (37)	328, (27)	281, (40)	262, (25)	209, (54)	207 (35)	
B_1-ME-*t*-BDMS	464	407 (82)	–	393, (31)	375, (100)	365, (46)	301, (35)	207 (89)			
B_2-ME-*t*-BDMS	462	405 (100)	–	373, (18)	363, (39)	299, (24)	247, (41)	215, (71)	203 (29)		
D_2-ME-MO-*t*-BDMS	623	566 (100)	434 (17)	592, (10)	579, (11)	552, (17)	460, (19)	420 (14)			
E_1-ME-MO-*t*-BDMS	625	568 (100)	–	538, (11)	536, (19)	410, (22)	353, (29)	339, (45)	321, (31)	279 (27)	
E_2-ME-MO-*t*-BDMS	623	566 (100)	–	592, (8)	351, (12)	337, (34)	267, (74)	215, (19)	207 (40)		
E_3-ME-MO-*t*-BDMS	621	564 (25)	–	552, (11)	420, (7)	337, (100)	207, (27)	205 (14)			
20-ME E_1-ME-MO-*t*-BDMS	639	582 (100)	–	476, (6)	424, (19)	367, (23)	339, (35)	335, (19)	293 (15)		
$F_{1\alpha}$-ME-*t*-BDMS	712	655 (90)	523 (96)	449, (54)	423, (79)	317, (82)	291, (43)	207 (100)			
$F_{2\alpha}$-ME-*t*-BDMS	710	653 (100)	521 (72)	447, (38)	421, (40)	315, (97)	281, (53)	215 (99)			
$F_{3\alpha}$-ME-*t*-BDMS	708	651 (45)	519 (22)	467, (37)	455, (100)	445, (57)	401 (30)				
19-OH-$F_{2\alpha}$-ME-*t*-BDMS	840	783 (78)	651 (25)	519, (30)	445, (31)	313, (100)	287, (47)	281 (54)			
dihomo-$F_{2\alpha}$-ME-*t*-BDMS	738	681 (100)	549 (80)	523, (16)	475, (86)	449 (51)					
6-keto-$F_{1\alpha}$-ME-MO-*t*-BDMS	755	698 (100)	–	552, (35)	443, (84)	360, (37)	319, (39)	215, (87)	207 (59)		
15-keto-$F_{2\alpha}$-ME-MO-*t*-BDMS	623	566 (100)	434 (22)	592, (20)	460, (22)	328, (29)	324, (78)	226, (34)	207 (64)		
13,14-diH-15-keto-$F_{2\alpha}$-ME-MO-*t*-BDMS	625	568 (100)	–	536, (18)	462, (32)	362, (63)	349, (40)	339, (28)	330, (54)	233 (72)	
TXB_2-ME-MO-*t*-BDMS	755	–	–	666, (88)	385, (38)	368, (18)	253, (49)	215, (54)	213, (35)	209, (32)	207 (100)

Prostaglandin D_2

Both the *m/z* 566 [*M* − 57] and 434 [*M* − (57 + 132)] characteristic ions were present in the mass spectrum of the methyl ester methoxime *t*-BDMS ether of PGD_2. Other ions, *m/z* 623 [M^+], 608 [*M* − 15], 592 [*M* − 31], 552 [*M* − 71] [loss of C(16)–C(20)], 460 [*M* − (71 + 132)], and 420 [*M* − (71 + 132)], were also of significance.

Prostaglandin E_1

Except for the *m/z* 568 [*M* − 57] characteristic ion, the mass spectrum of the methyl ester methoxime *t*-BDMS ether of PGE_1 contained the following important ions: *m/z* 594 [*M* − 31], 554 [*M* − 71], 462 [*M* − (31 + 132)], 436 [*M* − (57 + 132)] and 422 [*M* − (71 + 132)]. The *m/z* 536 [*M* − (18 + 71)] might have been derived from the loss of the C(16)–C(20) lower side chain and a water molecule from the molecular ion.

Prostaglandin E_2

The characteristic [*M* − 57] ion for the methyl ester methoxime *t*-BDMS ether was *m/z* 566. Other ions of diagnostic importance were: *m/z* 623 [M^+], 608 [*M* − 15], 592 [*M* − 31], 552 [*M* − 71], 534 [*M* − (18 + 71)], 460 [*M* − (31 + 132)], 434 [*M* − (57 + 132)] and 420 [*M* − (71 + 132)].

Prostaglandin E_3

Except for the *m/z* 564 [*M* − 57] ion, other diagnostically important ions present in the mass spectrum of the methyl ester methoxime *t*-BDMS ether of PGE_3 were *m/z* 621 [M^+], 606 [*M* − 15], 590 [*M* − 31], 552 [*M* − 69] [loss of C(16)–C(20)], 420 [*M* − (69 + 122)][loss of C(16)–C(20) and *t*-BDMSOH), 337 [*M* − (45 + 239)] [loss of $^{\cdot}NOCH_3$ and C(13)–C(20)] and 205 [*M* − (15 + 31 + 131 + 239)] or [*M* − (45 + 132 + 239)] [loss of $^{\cdot}CH_3$, $^{\cdot}OCH_3$, *t*-BDMSO and C(13)–C(20) or loss of $^{\cdot}NOCH_3$, *t*-BDMSOH and C(13)–C(20)].

20-Methyl ester prostaglandin E_1

The *m/z* 582 [*M* − 57] ion was the base peak present in the mass spectrum of methyl ester methoxime *t*-BDMS ether of 20-ME PGE_1. Other ions of importance were *m/z* 639 [M^+], 624 [*M* − 15], 608 [*M* − 31], 476 [*M* − (31 + 132)], 424 [*M* − (15 + 57 + 143)] [loss of $^{\cdot}CH_3$, *tert.*-butyl radical and C(1)–C(7)], 339 [*M* − (15 + 57 + 85 + 143)] [loss of C(16)–C(20) from *m/z* 424], 335 [*M* − (29 + 132 + 143)] or [*M* − (31 + 57 + 85 + 131)][loss of $^{\cdot}CH_2CH_3$, *t*-BDMSOH and C(1)–C(7) or loss of $^{\cdot}OCH_3$, *tert.*-butyl radical, C(16)–C(20) and *t*-BDMSO] and 293 [*M* − (15 + 45 + 229)] [loss of $^{\cdot}CH_3$, $^{\cdot}NOCH_3$ and C(15)–C(20)].

Prostaglandin $F_{1\alpha}$

Prostaglandin $F_{1\alpha}$ methyl ester *t*-BDMS ether gave a base peak at *m/z* 655 [*M* − 57]. This was in agreement with the previous reports[12,17]. Other ions of interpretational importance were *m/z* 523 [*M* − (57 + 132)], 509 [*M* − (71 + 132)], 497 [*M* − 215], 449 [*M* − (131 + 132)] and 423 [*M* − 289]. The mechanism for the formation of the [*M* − 289] fragment was unknown to us. However, this ion fragment was common to 19-OH-$PGF_{2\alpha}$, $PGF_{1\alpha}$, $PGF_{2\alpha}$ and dihomo-$PGF_{2\alpha}$ as discussed in the following section. Brash and Baillie[17] reported the same ion fragment in $PGF_{2\alpha}$

and $PGF_{1\alpha}$ as well as in urinary metabolites of PGFs. No interpretation was given for these [$M - 289$] ion fragments.

Prostaglandin $F_{2\alpha}$

The ion fragment patterns derived from the methyl ester *t*-BDMS ether of $PGF_{2\alpha}$ were similar to those of $PGF_{1\alpha}$ by a difference of 2 mass units. The major ion fragments were *m/z* 695 [$M - 15$], 653 [$M - 57$], 521 [$M - (57 + 132)$], 507 [$M - (71 + 132)$], 495 [$M - 215$], 447 [$M - (131 + 132)$] and 421 [$M - 289$].

Prostaglandin $F_{3\alpha}$

The relative abundance of *m/z* 651 [$M - 57$] ion of the methyl ester *t*-BDMS ether of $PGF_{3\alpha}$ was much weaker than those from $PGF_{1\alpha}$ and $PGF_{2\alpha}$. The other ions of importance were *m/z* 519 [$M - (57 + 132)$], 467 [$M - (31 + 69 + 141)$], 445 [$M - (131 + 132)$] and 401 [$M - \{69 + (2 \times 132)\}$].

Dihomo-prostaglandin $F_{2\alpha}$

The diagnostically important ions *m/z* 681 [$M - 57$] and 549 [$M - (57 + 132)$] were the most abundant ions present in the mass spectrum of the methyl ester *t*-BDMS ether of dihomo-$PGF_{2\alpha}$. The other important ions were *m/z* 523 [$M - 215$] [loss of C(17)–C(22)], 475 [$M - (131 + 132)$] and 449 [$M - 289$], 417 [$M - \{57 + (2 \times 132)\}$] and 403 [$M - \{71 + (2 \times 132)\}$].

19-Hydroxy-prostaglandin $F_{2\alpha}$

The *m/z* 783 [$M - 57$], as reported by Taylor and Kelly[11], was the base peak for the methyl ester *t*-BDMS ether of 19-OH-$PGF_{2\alpha}$. The *m/z* 651 [$M - (57 + 132)$] peak was also present to a significant extent. The other diagnostically important ions were *m/z* 708 [$M - 132$], 577 [$M - (131 + 132)$], 551 [$M - 289$], 519 [$M - \{57 + (2 \times 132)\}$], 445 [$M - (57 + 59 + 132 + 147)$] [loss of *tert.*-butyl radical, $^{\cdot}COOCH_3$, *t*-BDMSOH and C(19)–C(20)], 313 [$M - \{131 + (3 \times 132)\}$] (loss of *t*-BDMSO and three *t*-BDMSOH groups) and 287 [$M - \{57 + 59 + 147 + (2 \times 132)\}$].

6-Keto-prostaglandin $F_{1\alpha}$

The mass spectrum of the methyl ester methoxime *t*-BDMS ether of 6-keto-$PGF_{1\alpha}$ gave a significant *m/z* 698 [$M - 57$] characteristic ion. The *m/z* 566 [$M - (57 + 132)$] ion was also present but in less abundance. Ions *m/z* 724 [$M - 31$], 666 [$M - (57 + 32)$] and 552 [$M - (15 + 57 + 131)$] were of diagnostic importance. The *m/z* 443 was the second most abundant ion next to *m/z* 698.

15-Keto-prostaglandin $F_{2\alpha}$

Both the *m/z* 566 [$M - 57$] and 434 [$M - (57 + 132)$] were present in the mass spectrum of the methyl ester methoxime *t*-BDMS ether of 15-keto-$PGF_{2\alpha}$. The molecular ion *m/z* 623 was also present. The other important ions were *m/z* 608 [$M - 15$], 592 [$M - 31$], 460 [$M - (31 + 132)$], 328 [$M - \{31 + (132 \times 2)\}$] or [$M - (141 + 154)$] [loss of methoxy group and two *t*-BDMSOH or loss of two side chains C(1)–C(7) and C(13)–C(20)], 226 [$M - \{31 + 45 + 57 + (2 \times 132)\}$] (loss of methoxy group at the ester end, $^{\cdot}NOCH_3$, *tert.*-butyl radical and two *t*-BDMSOH groups) and 207 [$M - (15 + 57 + 71 + 132 + 141)$] [loss of $^{\cdot}CH_3$, *tert.*-butyl radical, C(16)–C(20), *t*-BDMSOH and the upper side chain C(1)–C(7)].

13,14-Dihydro-15-keto-prostaglandin $F_{2\alpha}$

The *m/z* 568 [*M* − 57] peak was the most abundant ion fragment present in the mass spectrum of the methyl ester methoxime *t*-BDMS ether of 13,14-diH-15-keto-$PGF_{2\alpha}$. Several ion fragments of distinctive importance were *m/z* 610 [*M* − 15], 594 [*M* − 31], *m/z* 536 [*M* − (57 + 32)], 462 [*M* − (32 + 132)] [loss of CH_3OH and *t*-BDMSOH) and 362 [*M* − (131 + 132)] or [*M* − (15 + 48 + 71 + 132)]. Some other ions, such as *m/z* 339, 330 and 233, of significant abundance could not be assigned with reasonable loss.

Thromboxane B_2

The mass spectrum of the methyl ester methoxime *t*-BDMS ether of TXB_2 was one of those few spectra which gave no significant [*M* − 57] characteristic ion fragment. Instead *m/z* 666 [*M* − (32 + 57)] (loss of CH_3OH and *tert.*-butyl radical) was the most characteristic ion fragment at the high mass range. The other important ions were *m/z* 724 [*M* − 31], 534 [*M* − (31 + 59 + 131)] (loss of $CH_3O^{\cdot}$, $^{\cdot}CO_2CH_3$ and *t*-BDMSO$^{\cdot}$), 460 [*M* − (32 + 131 + 132)] and 443 [*M* − (15 + 31 + 45 + 57 + 132)] (loss of $^{\cdot}CH_3$, $^{\cdot}NOCH_3$, *tert.*-butyl group and *t*-BDMS ≃ OH).

GC–MS–SIM

After establishing the specific MS data for each *t*-BDMS ether derivative of PGs, we proceeded with the GC–MS–SIM for both the characterization and quantification of PGs. For quantitative purposes, d_4-$PGF_{2\alpha}$ was used as an internal standard for determining all the PGs.

To assess the resolution we analyzed the authentic standard PGs of both the 1 and 2 series, separately and mixtures thereof. The ion fragments monitored were *m/z* 698 [6-keto-$PGF_{1\alpha}$], 582 [20-ME-PGE_1], 436 [PGA_1], 568 [PGE_1], 464 [PGB_1], 655 [$PGF_{1\alpha}$], 462 [PGB_2], 666 [TXB_2], 653 [$PGF_{2\alpha}$], 566 [PGE_2] and 434 [PGA_2]. All the PGs monitored were satisfactorily separated by packed column for quantification (Fig. 3A–C).

Because of the complexity of naturally occurring PGs we evaluated the separation and quantification of PGs from biological tissues using a fused-silica capillary column (12 m × 0.2 mm, methyl silicone).

The PGs present in the extract from rat stomach fundus were characterized by the same GC–MS–SIM procedure. Each individual PG was identified by the selected characteristic ions given in Table I. The 6-keto-prostaglandin $F_{1\alpha}$ was the major PG found in rat fundus, the other PGs of smaller concentrations were $PGF_{1\alpha}$, PGE_2, PGE_1 and TXB_2 (Fig. 4). For quantification, the *m/z* 525 (d_4-$PGF_{2\alpha}$) was monitored together with the characteristic ion of the PGs of interest. A sample standard curve for 6-keto-$PGF_{1\alpha}$ (*m/z* 698) GC–MS–SIM is shown in Fig. 5A and B. Both the *syn*- and *anti*-isomers of 6-keto-$PGF_{1\alpha}$ were partially resolved by the 12 m × 0.2 mm fused-silica capillary column. This indicated the superiority of the capillary column to the conventional packed column for the separation of *syn*- and *anti*-isomers of ketonic PGs. The sensitivity limit with capillary column was *ca.* 1 ng for each PG component injected. The average coefficient of variance at the range tested (0.8–13 ng) was 7.8%.


A
M/Z 698
M/Z 582
M/Z 436
M/Z 568
M/Z 464
M/Z 655.5
20 ME PGE1
PGA1
PGE1
PGB1
PGF1α
B
M/Z 462
M/Z 666
M/Z 653.6
M/Z 566
M/Z 434
M/Z 698
PGB2
TXB2
PGF2α
2nd
PGE2
1st
1ST
PGA2
2nd
6-keto-
PGF1α

Fig. 3.

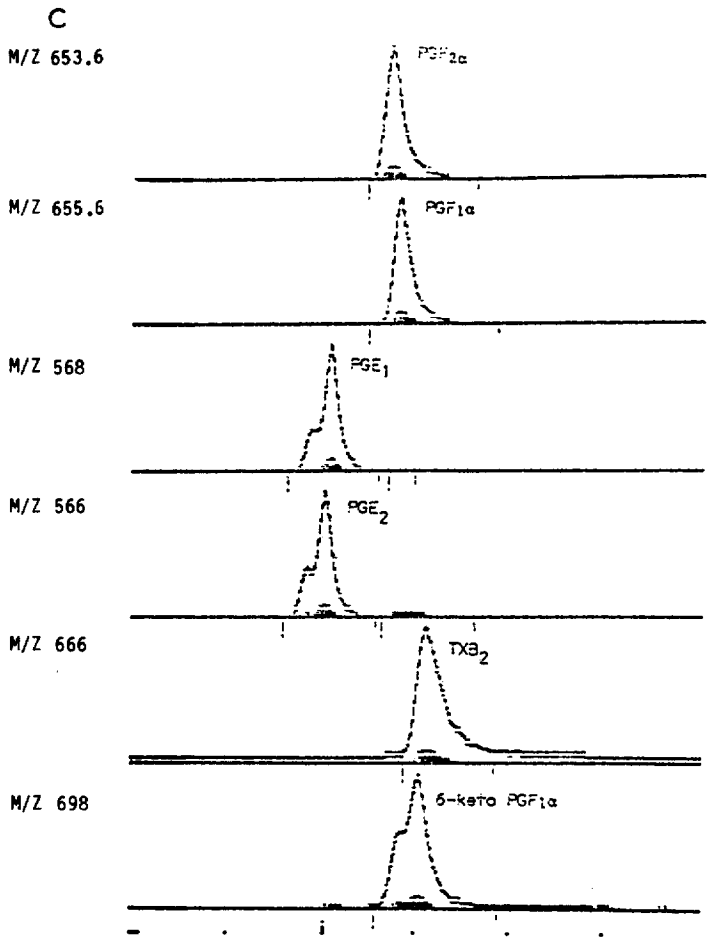

Fig. 3. Separation of standard PG *t*-BDMS ethers using GC–MS–SIM technique. A, Mixture of different 1 series PGs. B, Mixture of 2 series PGs and 6-keto-$PGF_{1\alpha}$. C, Mixture of both 1 and 2 series PGs. A 0.6 m × 3 mm 3% OV-101 packed column was used for the separation. See text for detailed conditions.

DISCUSSION

Although some of the *t*-BDMS ether derivatives of PG have been used for both qualitative[7,11,13,14,17] and quantitative[7,16] studies, this study reports a systematic analysis of the *t*-BDMS ether derivatives of the 1, 2 and 3 series PGs by GC–MS. The data provide the fragmentation patterns of PG *t*-BDMS ether derivatives. These should aid researchers using GC–MS–SIM for both qualitative and quantitative (in nanogram quantities) monitoring of PGs extracted from biological tissues. The high mass fragments given by *t*-BDMS ether are not only more unique to the compound of interest but also the high relative abundance of these fragments significantly increase the detection sensitivity.

The stepwise conversion of PGs to their *t*-BDMS ether methyl ester proceeds

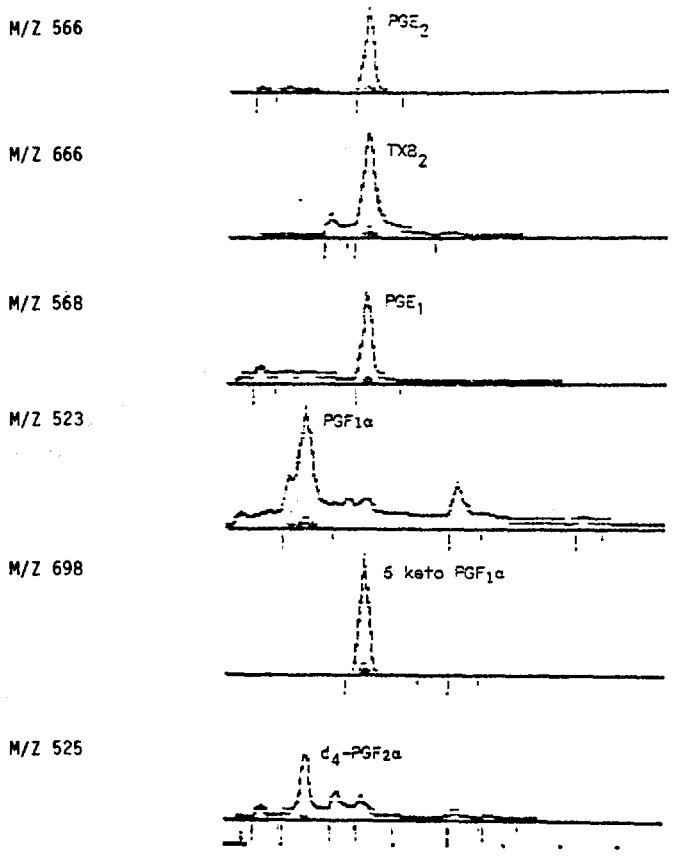

Fig. 4. A typical GC–MS–SIM profile of prostaglandins from rat stomach fundus.

smoothly and in high yield (>85% based on radioactive study) and affords derivatives with good GC properties. Because of the stability of *t*-BDMS ether derivatives[18,19] especially to hydrolysis, further purification of the derivatives is possible. In the present study it was not necessary to further purify the products after the formation of *t*-BDMS ether derivatives. Even though *t*-BDMS ethers have considerably higher retention indices than the corresponding TMS derivatives, the *t*-BDMS ether derivatives with up to four *t*-BDMS groups, *i.e.* 19-OH-$PGF_{2\alpha}$ were successfully analyzed using either the 3% OV-101 (0.6 m × 3 mm) packed column or a short fused-silica capillary column (12 m × 0.2 mm) coated with high-temperature-stable methyl silicone. All the *t*-BDMS ether derivatives of PGs were eluted within 10 min. However, for metabolites bearing six or more hydroxy groups the *t*-BDMS ether derivatives may well limit the usefulness of this type of derivative for the vapor phase analysis of such compounds. MS problems associated with the accompanying increment in molecular weight have been discussed[7].

The *t*-BDMS ether derivatives of PGs provide simple mass spectra which have considerable advantages. The *t*-BDMS ethers are particularly useful in the PG field, where they provide good derivatives for GC–MS–SIM and are very useful aids in

qualitative analysis of unknown compounds. Importantly, the increase in higher mass peaks monitored in GC–MS–SIM reduces the chance of interference from other compounds. For this reason, the *t*-BDMS ethers are almost ideal derivatives for PG analyses.

Except for TXB_2, all the *t*-BDMS ether derivatives of PG studies gave predominant $[M - 57]$ ion or both the $[M - 57]$ and $[M - (57 + 132)]$ ions. The $[M - 57]$ ion represents the loss of the *tert.*-butyl group as an odd electron species from the charged molecule. The ion remaining is identical to the $[M - 15]$ ion of a TMS ether derivative, which is not usually particularly prominent. It is possible that the mechanism accounting for the $[M - 57]$ ion reflects the ease of elimination of the *tert.*-butyl radical[7]. When PGB_2 *t*-BDMS ether methyl ester was chromatographed with the keto group unprotected, the $[M - 57]$ ion was still the most abundant ion in the spectrum. In a study of the *t*-BDMS ether methyl ester of $PGF_{2\alpha}$, Kelly and Taylor[12] found that on the MS 12 instrument only the $[M - (132 + 57)]$ ion was observed, but when the same derivative was examined by a DuPont 490 mass spectrometer, the $[M - 57]$ ion was the base peak. They concluded that instrumental fac-

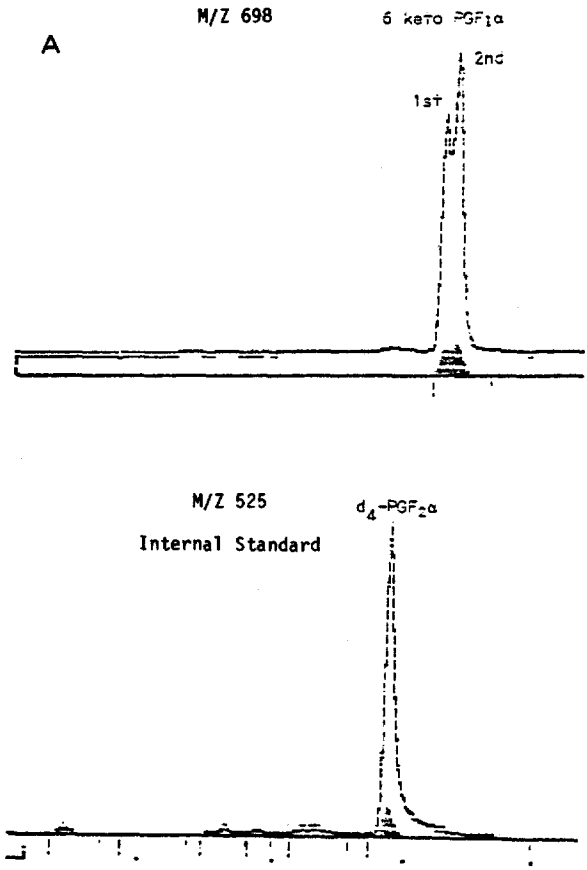

Fig. 5.

(Continued on p. 106)

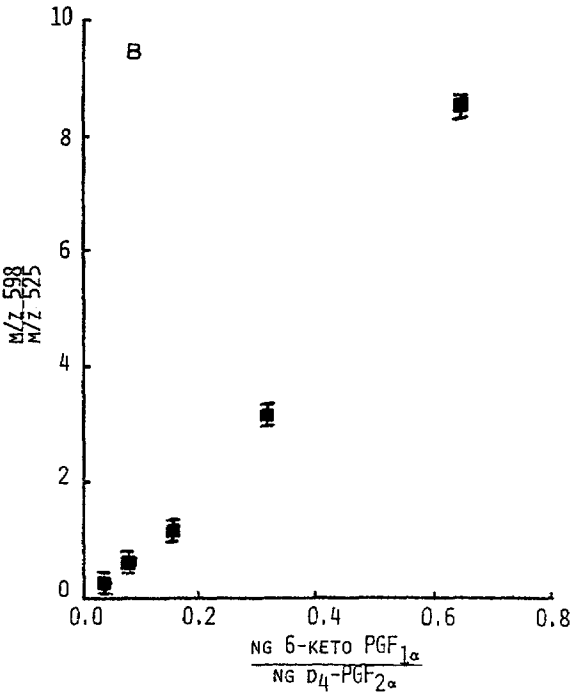

Fig. 5. Quantification of PGs using GC–MS–SIM technique. A, Single ion monitoring 6-keto-$PGF_{1\alpha}$ at m/z 698 and for internal standard d_4-$PGF_{4\alpha}$ at m/z 525. B, A calibration curve for 6-keto-$PGF_{1\alpha}$ from the ratio of m/z 598 and 525.

tors could affect the mass spectrum of *t*-BDMS ether derivative and this should be kept in mind when inter-laboratory comparisons are being made.

The degree of unsaturation affects the mass fragmentation of *t*-BDMS ether to some extent. As the degree of unsaturation increases, the *t*-BDMS ether moiety is less stable to direct the fragmentational mode of the molecule and accordingly the proportion of low-molecular-weight hydrocarbon ions increases. The $[M - 57]$ ion in the spectrum of *t*-BDMS ether of PGE_3 accounted for only 25% as compared to 100% given in the spectra of PGE_1 and PGE_2. This has also occurred in the spectra of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and $PGF_{3\alpha}$. The same phenomena were observed in the spectra of *t*-BDMS fatty acid esters[3]. The $[M - 57]$ ion in the mass spectrum of *t*-BDMS ester of arachidonic acid gives only 23% relative abundance as compared to 100% from less unsaturated fatty acids[3]. This may partly explain the non-linearity observed (Fig. 5B); *i.e.*, the relative increase in fragility of the more unsaturated PG, $PGF_{2\alpha}$ compared to $PGF_{1\alpha}$ could result in non-linearity.

For the separation of structurally closely related compounds differing only by degree of unsaturation or geometric configuration, the liquid phase used in this study was not totally satisfactory. More polar phases, such as Silar 10C, SP-2340 or OV-275 phases, are superior for that purpose.

Using a glass capillary column coated with SP-2340, Heckers *et al.*[23] successfully separated a series of different geometric and configurational isomers of fatty acids. Using a SP-2340 packed column, Ferretti *et al.*[24] developed a GC–MS–SIM

procedure for the quantification of PGE_2 and PGE_3 as their PGB_2 and PGB_3 derivatives, respectively. According to the authors, baseline separation of PGB methyl ester TMS ethers was obtained. However, in the field of capillary column chromatography successfully coating polar phases to fused-silica capillary column is still a challenge.

In conclusion, despite relatively few mass fragmentation peaks the *t*-BDMS ether derivatives of PGs provided an excellent tool for the determination of the molecular weight and the quantitative analyses of PGs from biological tissues by GC–MS–SIM. The procedures in this paper should facilitate research in this important area, where routine qualitative and quantitative methods for the analyses of PGs from biological tissues are needed.

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