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DIETHYLHYDROGENSILYL-CYCLIC DIETHYLSILYLENE DERIVATIVES IN GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF PROSTANOIDS

III. F_α-PROSTAGLANDINS AND THROMBOXANE B_2 DERIVATIVES

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SUMMARY

The gas chromatographic (GC) and GC–mass spectrometric properties of the diethylhydrogensilyl-cyclic diethylsilylene (DEHS-DES) derivatives of prostaglandin (PG) $F_{1\alpha}$ methyl ester, $PGF_{2\alpha}$ methyl ester, 6-keto-$PGF_{1\alpha}$ methyl ester-alkyloxime and thromboxane (TX) B_2 methyl ester-alkyloxime and the DES derivative of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ methyl ester-alkyloxime were studied. When the ketonic PGs and TXB_2 were converted into their methyloxime derivatives, the methylene unit values of these five prostanoid derivatives were slightly greater than those of the corresponding dimethylethylsilyl ether derivatives. When the ketonic PGs were converted into their corresponding ethyloxime derivatives, baseline separation was achieved in 20 min by use of a methylsilicone cross-linked fused-silica capillary column. The mass spectra of these derivatives were characterized by the ion at *m/z* 157 for F_α prostaglandins and *m/z* 269 for TXB_2. The major fragmentations were directed by the DES group, and other fragmentations common to the prostanoid derivatives were losses of an ethyl radical at the silicon atom, C_5H_{11} hydrocarbon fragment, diethylhydrogensilanol and C_{15}–C_{20} hydrocarbon fragment. The mass fragmentations of these prostanoid derivatives are briefly discussed. GC with high-resolution selected-ion monitoring was carried out for the TXB_2 derivative at a resolution of 8000 by monitoring the ion at *m/z* 269.1573. A 25-pg amount of this derivative showed a well shaped doublet with a signal-to-noise ratio of more than 300:1.

INTRODUCTION

Gas chromatography with selected-ion monitoring (GC-SIM) has been widely

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used as the most specific method for microanalysis of PGs (prostaglandins) and TXB_2 (thromboxane B_2) and to investigate their occurrence, distribution and/or physiological role in biological fluids and tissues. It furnishes both the structural identity and GC retention time in a single analysis. In the biochemical and biomedical fields, however, GC-SIM may be affected by unexpexted impurities having quite similar physical and/or chemical properties to the compounds of interest. In order to improve the reliability of the analytical results, a number of intensive investigations have focused on the purification, derivatization, GC separation and instrumentation employed.

Derivatization plays an important role in microanalysis by GC-SIM. The GC performance is improved by selection of suitable derivatives to enhance compound reliability and thermal stability. Trimethylsilyl (TMS) ether derivatives of methyl esters and methyl ester-methyloximes of PGs and TXB_2 have been used extensively for GC and GC–mass spectrometric (MS) analysis of these compounds[1–3]. As the reliability in microanalysis using GC-SIM may be enhanced by choosing a derivative providing baseline separations, a number of new derivatives such as the dimethylethylsilyl (DMES)[4], dimethyl-*n*-propylsilyl[4], dimethylisopropylsilyl[5] and *tert.*-butyldimethylsilyl[6–9] ethers have been extensively used for quantification of PGs and TXB_2.

Use of the cyclization reaction for derivatization enabled preparation of specific derivatives which reflected more useful structural information on the compound of interest and allowed more efficient and easier separation in a mixture of isomers and/or homologues. In addition, the reaction product obtained provided a mass spectrum containing characteristic ions with prominent intensity. The F_α-prostaglandins (F_α-PGs), with 1,3-dihydroxyl groups suitable for the cyclization reaction, react with alkaneboronic acids to yield cyclic alkaneboronate derivatives. Cyclic *n*-butaneboronate derivatives of prostanoids were first demonstrated in the analysis of F_α-PGs[10] and alkaneboronate derivatives of $PGF_{2\alpha}$[11–13], 6-keto-$PGF_{1\alpha}$[14–16] and TXB_2[7,16] were investigated for GC–MS analysis. However, a disadvantage of these derivatives is that the remaining hydroxyl group requires an additional derivatization.

Lawson *et al.*[17] reported a novel approach to the extraction of TXB_2 from biological fluids, which was based on the ability of the tetrahedral anionium form of borate to condense with 1,3-diol originating from the hemiacetal ring of TXB_2, to form a six-membered covalent complex. This selective extraction of TXB_2 confers excellent analytical specificity on the microanalysis of TXB_2 by GC–SIM. In our previous papers, the cyclization reaction was applied to gain more useful information in the microanalysis of hydroxypregnanes with 17,20-diols and 17,20,21-triols and hydrocortisone. Their cyclic diethylsilylene (DES) derivatives were prepared by treating them with N,O-bis(diethylhydrogensilyl)trifluoroacetamide (DEHS-BSTFA) and used for the investigation of their GC and GC–MS properties. These DES derivatives gave mass spectra in which the molecular ion was prominent[18,19].

In order to use the dual properties of selective extraction and specific derivatization for the microanalysis of F_α-PGs and TXB_2, the DES derivative formation reaction was applied to TXB_2 as a model compound which yielded the 1,3-diol by opening the hemiacetal ring[20,21]. The GC and GC–MS properties of these novel DEHS-DES derivatives of TXB_2 methyl ester-alkyloximes are comparable to those of the corresponding TMS ether or other silyl ether derivatives[5,7,22,23]. Thus, this derivatization reaction may be extended to F_α-PGs which can be adsorbed onto a

phenylboronic acid column to enable their separation from the PGD, PGE and PGF_{β} series by utilizing the property of the 1,3-dihydroxyl function. This paper deals with the GC and GC–MS properties of DEHS-DES derivatives of F_{α}-PGs and of TXB_2.

EXPERIMENTAL

Gas chromatography (GC)

A GC-7A gas chromatograph (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame ionization detector and VandenBerg's solventless injector[24] was employed. The column was a 25 m × 0.32 mm fused-silica capillary cross-linked with methylsilicone (Ultra 1, Hewlett-Packard Avondale, PA, U.S.A.). The temperature of the column oven was maintained at 250–290°C. The carrier gas was helium at a linear velocity of 25 cm/s. The temperature of the injection port and detector was kept at 300°C.

Gas chromatography–mass spectrometry (GC–MS)

A VG Micromass ZAB-HF mass spectrometer (VG Analytical, Manchester, U.K.) interfaced to a Shimadzu GC-9A gas chromatograph with a solventless injector and a DS-2035 data-processing system (VG Analytical) was employed. The capillary column was introduced into the mass spectrometer source. The GC conditions were the same as above. The carrier gas was helium at a velocity of 30 cm/s. The temperature of the injector port and transfer line was kept at 290°C and that of the ion source at 200°C. The ionization energy and trap current were 70 eV and 200 μA, respectively. The accelerating voltage was 8 kV.

The mass spectrum of each prostanoid derivative was recorded by repeated scanning (2.0 s/decade) in the range of *m/z* 850–90 (cycle time 3 s) with a dynamic resolution of 2000. High-resolution (HR) MS was carried out under the same GC–MS conditions as used in the low resolution mode. The mass range *m/z* 800–90 was scanned at 3 s/decade (cycle time 4 s) with a dynamic resolution of 5000. The linked scanning was performed in daughter-ion scan mode at constant B/E with a total scan cycle of 5–6 s under data-system control.

Gas chromatography with selected-ion monitoring (GC–SIM)

GC–SIM was performed using a VG-70S mass spectrometer (VG Analytical) interfaced to a Hewlett-Packard 5890A gas chromatograph and a VG 11-250J+ data-processing system. The column was a 30 m × 0.25 mm fused-silica capillary cross-linked with 5% phenylmethylsilicone (DB-5; J & W Scientific, Rancho Cordova, CA, U.S.A.). The temperature of the column oven was initially set at 120°C, and 1 min after the injection it was increased to 250°C at 20°C/min and thereafter to 300°C at 10°C/min. The carrier gas was helium with a linear velocity of 30 cm/s. The temperature of the injection port and transfer line was kept at 290°C, and that of the ion source at 200°C. The ionization energy and trap current were 35 eV and 200 μA, respectively. The accelerating voltage was 8 kV. For GC-HRSIM, the ion at *m/z* 269.1573 was maintained in focus by reference to the lock-mass ion at *m/z* 268.9824 from perfluorokerosene, which was introduced independently into the ion source.

Samples and reagents

$PGF_{1\alpha}$, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and TXB_2 were obtained from Funakoshi Yakuhin (Tokyo, Japan). The hydrochloride salts of O-methylhydroxylamine, O-ethylhydroxylamine, O-isobutylhydroxylamine and *n*-butylhydroxylamine were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). DEHS-BSTFA was synthesized in our laboratory as previously reported[25]. Other reagents and solvents used were of the highest purity available.

Derivatization

The methyl esters of PGs and TXB_2 were prepared by treatment with diazomethane.

Alkyloximes of 13,14-dihydro-15-keto-$PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 methyl esters (50–100 μg) were prepared by treating the corresponding methyl esters with 5 mg of O-alkylhydroxylamine hydrochloride in pyridine at 60°C for 1 h. The pyridine was evaporated under a stream of nitrogen, and 0.3 ml of an aqueous saturated sodium chloride solution were added. The methyl ester-alkyloxime derivatives were extracted twice with 0.2 ml of ethyl acetate. The ethyl acetate solution was collected and dried under a stream of nitrogen.

After methylation and alkyloximation, the resulting PGs and TXB_2 derivatives were treated with DEHS-BSTFA in pyridine for 30 min at room temperature. These derivatives were used for GC and GC–MS analysis without removal of the excess of DEHS-BSTFA.

RESULTS AND DISCUSSION

Gas chromatography

For the study of the GC and GC–MS properties of cyclic diethylsilylene (DES) derivatives of prostanoids, four F_{α}-PGs and TXB_2 were examined. Fig. 1A and B

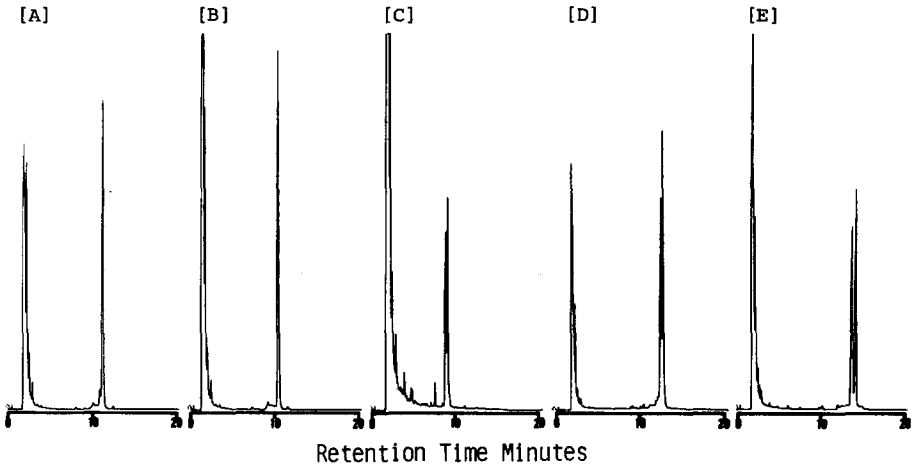


Fig. 1. Gas chromatograms of the reaction products of $PGF_{1\alpha}$ (A) and $PGF_{2\alpha}$ (B) with diazomethane and then with DEHS-BSTFA and of 13,14-dihydro-15-keto-$PGF_{2\alpha}$(C), 6-keto-$PGF_{1\alpha}$ (D) and TXB_2 (E) with diazomethane, O-ethylhydroxylamine hydrochloride and then with DEHS-BSTFA.

show the gas chromatograms of the products of reaction of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ obtained by treating them with diazomethane and then with DEHS-BSTFA. The $PGF_{1\alpha}$ and $PGF_{2\alpha}$ methyl esters were readily silylated at room temperature. These reaction products gave a single well shaped gas chromatographic peak, when analyzed using a methylsilicone cross-linked fused-silica capillary column. On the other hand, the products of reaction of 13,14-dihydro-15-keto-$PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 obtained by treating them with diazomethane, O-alkylhydroxylamine hydrochloride and then with DEHS-BSTFA exhibited a well resolved doublet which was considered to correspond to the *syn*- and *anti*-isomers. These results suggest that the stepwise derivatization proceeded smoothly and quantitatively.

Table I lists the methylene unit (MU) values of corresponding derivatives of PGs and TXB_2. All the *syn*- and *anti*-isomer pairs of DEHS-DES derivatives of ketonic PG methyl ester-alkyloximes were well resolved, except for that corresponding to the *n*-butyloxime derivative of TXB_2 which showed a single peak with a shoulder. When the ketonic PGs and TXB_2 were converted into their methyloxime derivatives, the prostanoids studied were eluted in the order of 13,14-dihydro-15-keto-$PGF_{2\alpha}$, $PGF_{2\alpha}$, $PGF_{1\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$. Their MU values were slightly greater than those of the corresponding DMES ether derivatives.

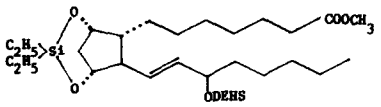
Judging from the separation patterns of DEHS-DES derivatives of 6-keto-$PGF_{1\alpha}$ methyl ester-alkyloximes, a difference between MU values of more than 0.15–0.20 seemed to be required for complete separation of each of the prostanoid derivatives. When the ketonic PGs were converted into their corresponding methyloxime derivatives, the peak of the TXB_2 derivative overlapped that of $PGF_{1\alpha}$. When the ethyloxime derivatives of ketonic PGs were prepared and analysed, their MU values were artificially increased with reasonable increments, indicating that $PGF_{1\alpha}$ and TXB_2 could be separated completely by changing the alkyl group in the alkyloxime moiety. In addition, from Table I, the *syn*- and *anti*-isomer pair of the TXB_2 isobutyloxime derivative surprisingly overlapped with $PGF_{1\alpha}$ and $PGF_{2\alpha}$ derivatives, suggesting that the corresponding isobutyloxime derivatives of ketonic PGs are not suitable for the profile analysis of F_α-prostaglandins and TXB_2. On the other hand, as the DEHS-DES derivatives of 6-keto-$PGF_{1\alpha}$ methyl ester-alkyloximes have the same molecular weight as the corresponding TXB_2 derivatives, complete GC separation is required in their microanalysis in the determination of plasma levels of TXB_2 by use of GC–SIM. The MU values of the first eluted component of DEHS-DES derivatives of 6-keto-$PGF_{1\alpha}$ methyl ester-ethyloximes were 0.34 larger than of the second eluted component of the corresponding TXB_2 derivative, enabling them to be discriminated. Therefore, when $PGF_{1\alpha}$ is present in the sample, the corresponding ethyloxime derivatives of ketonic PGs may be recommended for the profile analysis of F_α-PGs and TXB_2. If the $PGF_{1\alpha}$ levels are negligibly small, methyloxime derivatives of ketonic PGs may also be used.

These facts indicate that the DEHS-DES derivatives of ketonic PG methyl ester-alkyloximes may be used for deconvoluting the poorly resolved GC peaks produced by the methyloxime derivatives, as was already discussed by Horning and co-workers[26] and Brooks and co-workers[27].

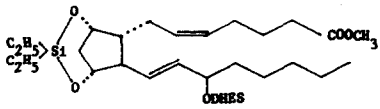
As mentioned above, the DEHS-DES derivatives of TXB_2 methyl ester-alkyloximes, except for *n*-butyloxime, gave a well resolved doublet. This was also observed for the reaction products obtained under other oximation conditions used by Smith

TABLE I

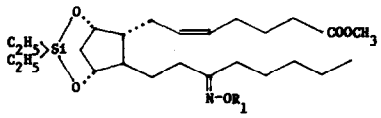
STRUCTURES AND GC DATA FOR THE DEHS-DES DERIVATIVES OF $PGF_{1\alpha}$ METHYL ESTER (A), $PGF_{2\alpha}$ METHYL ESTER (B), 6-KETO-$PGF_{1\alpha}$ METHYL ESTER-ALKYLOXIME (D) AND TXB_2 METHYL ESTER-ALKYLOXIME (E) AND THE DES DERIVATIVE OF 13,14-DIHYDRO-15-KETO-$PGF_{2\alpha}$ (C) METHYL ESTER-ALKYLOXIME



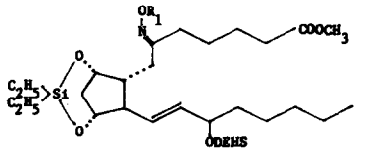
[A]



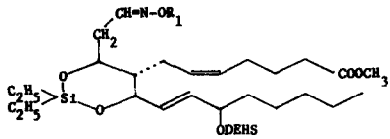
[B]



[C]



[D]



[E]

Compound	R_1	*MU value*		
			Ketonic PGs	
			*	**
$PGF_{1\alpha}$				
A		30.48		
$PGF_{2\alpha}$				
B		30.29		
13,14-Dihydro-15-keto-$PGF_{2\alpha}$				
C1a, C1b	CH_3		28.89	29.02
C2a, C2b	C_2H_5		29.21	29.34
C3a, C3b	*iso*-C_4H_9		30.27	30.49
C4a, C4b	*n*-C_4H_9		30.65	30.88
6-Keto-$PGF_{1\alpha}$				
D1a, D1b	CH_3		31.22	31.38
D2a, D2b	C_2H_5		31.50	31.68
D3a, D3b	*iso*-C_4H_9		32.48	32.60
D4a, D4b	*n*-C_4H_9		32.89	32.97
Thromboxane B_2				
E1a, E1b	CH_3		30.56	30.72
E2a, E2b	C_2H_5		31.05	31.16
E3a, E3b	*iso*-C_4H_9		32.30	32.40
E4a, E4b	*n*-C_4H_9		32.78	(shoulder)

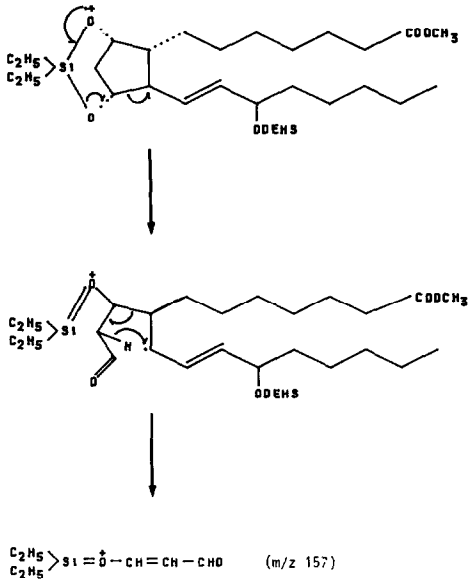
* First peak eluted.
** Second peak eluted.

et al.[7] (80°C, 3 h). This interesting finding may be explained as follows: rotation of the protected aldehydic chain (C_{10}–C_{11} moiety) seems to be very restricted by the formation of the rigid 9,11-DES ring, while in the TMS ether derivative of the methyl ester-methyloxime the protected aldehydic chain may rotate freely either individually or as a part of the bulky C_9–C_{12} side-chain substituted at C_8. As a result, structural differences between the *syn*- and *anti*-isomers appear to be reflected in their gas chromatogram as a well resolved doublet.

Mass spectrometry

Fragmentation

F_α-prostaglandins. The DEHS-DES derivatives of $PGF_{1\alpha}$ methyl ester, $PGF_{2\alpha}$ methyl ester and 6-keto-$PGF_{1\alpha}$ methyl ester-ethyloxime and the DES derivative of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ methyl ester showed mass spectra with the ion at *m/z* 157 as a base peak or a prominent peak. This suggests the formation of the 9,11-DES ring moiety for each of the F_α-PGs. Therefore, the accurate mass measurement indicates the elemental composition of this ion to be $C_7H_{13}O_2Si$, leading to the prediction that this ion was formed by fission of the F_α-PG ring initiated by cleavage of the C_{11}–C_{12} bond and loss of α- and β-side-chains. A plausible fragmentation pathway is shown in Scheme 1. Another characteristic fragmentation was fission of the DES ring with loss of the protected aldehydic chain followed by migration of diethylsilyloxy (ODEHS) group at C-15 to another silicon atom, giving the ion at *m/z* 205, $[(C_2H_5)_2Si(OH)–O=Si(C_2H_5)_2]^+$. The formation of this ion could be elucidated by the fragmentation pathway of the ion of the same mass found for the DEHS-DES derivatives of 5β-pregnane-3α,17α,20α,21-tetraol and its 20β-isomer[20]. In addition,



C_2H_5, C_2H_5 > Si = O – CH = CH – CHO (*m/z* 157)

Scheme 1.

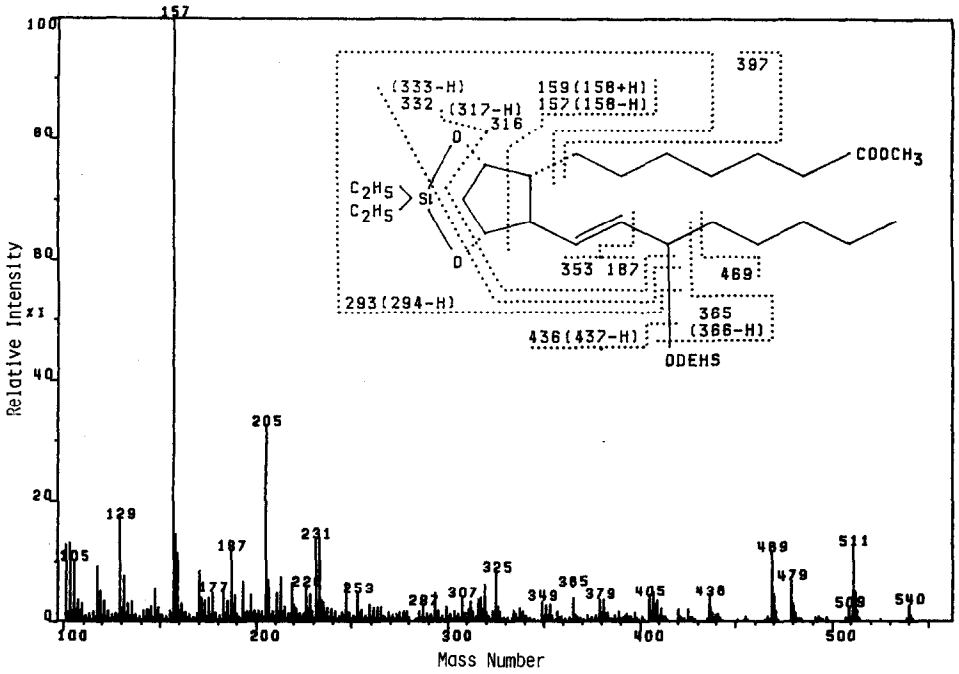


Fig. 2. Mass spectrum of the DEHS-DES derivative of $PGF_{1\alpha}$ methyl ester (A).

these derivatives were characterized by the ions $[M-C_2H_5]^+$,$[M-C_5H_{11}]^+$, $[M-$diethylhydrogensilanol(DEHSOH)$]^{+\cdot}$, $[M-C_2H_5-DEHSOH]^+$, $[M-CH(ODEHS)C_5H_{11}]^+$ and $[CH(ODEHS)C_5H_{11}]^+$ and $[M-OC_2H_5]^+$ from 13,14-dihydro-15-keto-$PGF_{2\alpha}$. The elimination of the appropriate silanol and production of subsequent characteristic fragment ions were also found for the prostanoids studied, except for 13,14-dihydro-15-keto-$PGF_{2\alpha}$.

Proslaglandin $F_{1\alpha}$. The mass spectrum of the DEHS-DES derivative of $PGF_{1\alpha}$ methyl ester is shown in Fig. 2. The molecular ion at *m/z* 540, which is 46 mass units lower than that of the $PGF_{1\alpha}$ methyl ester-tris-TMS ether derivative[28,29], was observed with very low intensity, and the ion $[M-C_2H_5]^+$ (*m/z* 511) with low intensity.

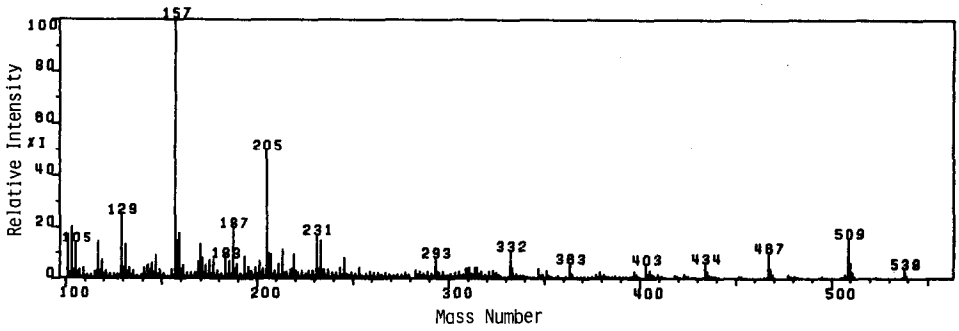


Fig. 3. Mass spectrum of the DEHS-DES derivative of $PGF_{2\alpha}$ methyl ester (B).

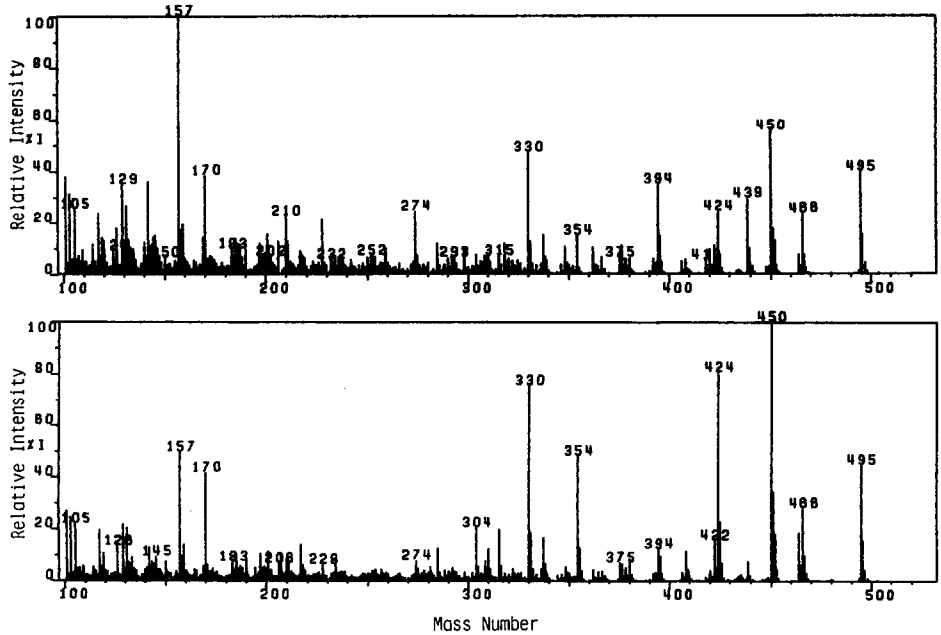


Fig. 4. Mass spectra of the structural isomers of the DES derivative of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ methyl ester-ethyloxime. Top: the first component eluted (C2a). Bottom: the second component eluted (C2b).

This indicates the incorporation of one DES and one DEHS group into the $PGF_{1\alpha}$ molecule to form the expected $PGF_{1\alpha}$ methyl ester-15-DEHS-9,11-DES derivative. The ion at *m/z* 469 produced by the loss of C_5H_{11} (71 a.m.u.) from the molecular ion was typical of a β-side-chain. Elimination of diethylhydrogensilanol (DEHSOH) from the molecular ion gave rise to the ion at *m/z* 436. The ion at *m/z* 187, corresponding to the fragment C_{15}–C_{20}, was observed with low intensity as a characteristic of prostanoid series one and two. From accurate mass measurement, the ions of *m/z* 316 and 332 were estimated to be of elemental composition $C_{21}H_{32}O_2$ and $C_{21}H_{32}O_3$. These ions were considered to be produced from the molecular ion by fission of the DES ring with the loss of DEHSOH. In addition, the elimination of the α-side chain from the molecular ion gave rise to the ion at *m/z* 397, and successive loss of DEHSOH from this ion produced the ion at *m/z* 293 with low intensity.

Prostaglandin $F_{2\alpha}$. The mass spectrum of the DEHS-DES derivative of $PGF_{2\alpha}$ was closely related to that of the corresponding $PGF_{1\alpha}$ derivative, except for the obvious shift produced by the existence of the C_5–C_6 double bond (Fig. 3).

13,14-Dihydro-15-keto-prostaglandin $F_{2\alpha}$. Fig. 4 shows the mass spectra of the *syn-* and *anti-*isomer pairs of the DEHS-DES derivative of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ methyl ester-ethyloxime (C2a, C2b). This derivative gave characteristic ions with prominent intensity in the high mass region. The appearance of the molecular ion at *m/z* 495 was sufficient to confirm the formation of the expected derivative. The ion at *m/z* 157 was observed as a base peak in the mass spectrum of the first component eluted (C2a) and as a prominent peak in that of the second component eluted

(C2b). The molecular ion lost an ethoxy radical from the ethyloxime moiety to give an ion $[M-OC_2H_5]^+$ at *m/z* 450 with prominent intensity for C2a and as the base peak for C2b. Losses of an ethyl radical at the silicon atom, a methoxy radical at the methoxycarbonyl group, the C_{16}–C_{20} fragment and α-side-chain (141 a.m.u.) from the molecular ion gave ions at *m/z* 466, 464, 424 and 354, respectively. In this case, the ion at *m/z* 354 was regarded as one of the inherent ions of C2b. The ion at *m/z* 439 was produced by loss of the C_{17}–C_{20} fragment with migration of the hydrogen atom from C-17 to the nitrogen atom, and the successive loss of the ethoxyl group at the ethyloxime moiety from this ion gave the ion at *m/z* 394. The ions of *m/z* 439 and 394 were characteristic in the mass spectrum of C2a. The ion at *m/z* 330 was prominent in both spectra. It was formed by the loss of diethyldihydroxysilane $[(C_2H_5)_2Si(OH)_2$, 120 a.m.u.] from the ion $[M-OC_2H_5]^+$. The ion at *m/z* 170 was a β-side-chain fragment formed by fission of the C_{12}–C_{13} bond.

When the mass spectral data of the DEHS-DES derivatives of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ methyl ester-alkyloximes were examined, the mass spectral patterns of these derivatives were found to be closely related to each other except for the obvious shift in alkyloxime-containing fragment ions. Elimination of the alkoxy radical from the molecular ion to form the subsequent characteristic fragment ion $[M-O\text{-alkyl}]^+$, observed as the base peak, was not an especially favoured process for each of the alkyloxime derivatives, as compared with the corresponding methyloxime derivative.

6-Keto-prostaglandin $F_{1\alpha}$. Structural isomers of the DEHS-DES derivative of 6-keto-$PGF_{1\alpha}$ methyl ester-ethyloxime (D2a, D2b) gave simplified mass spectra with

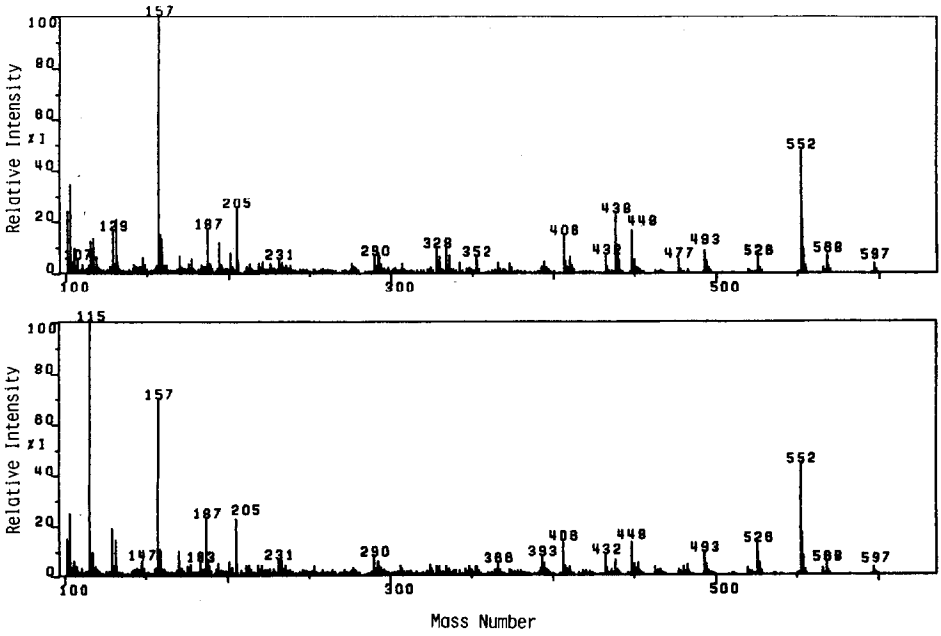


Fig. 5. Mass spectra of the structural isomers of the DEHS-DES derivative of 6-keto-$PGF_{1\alpha}$ methyl ester-ethyloxime. Top: the first component eluted (D2a). Bottom: the second component eluted (D2b).

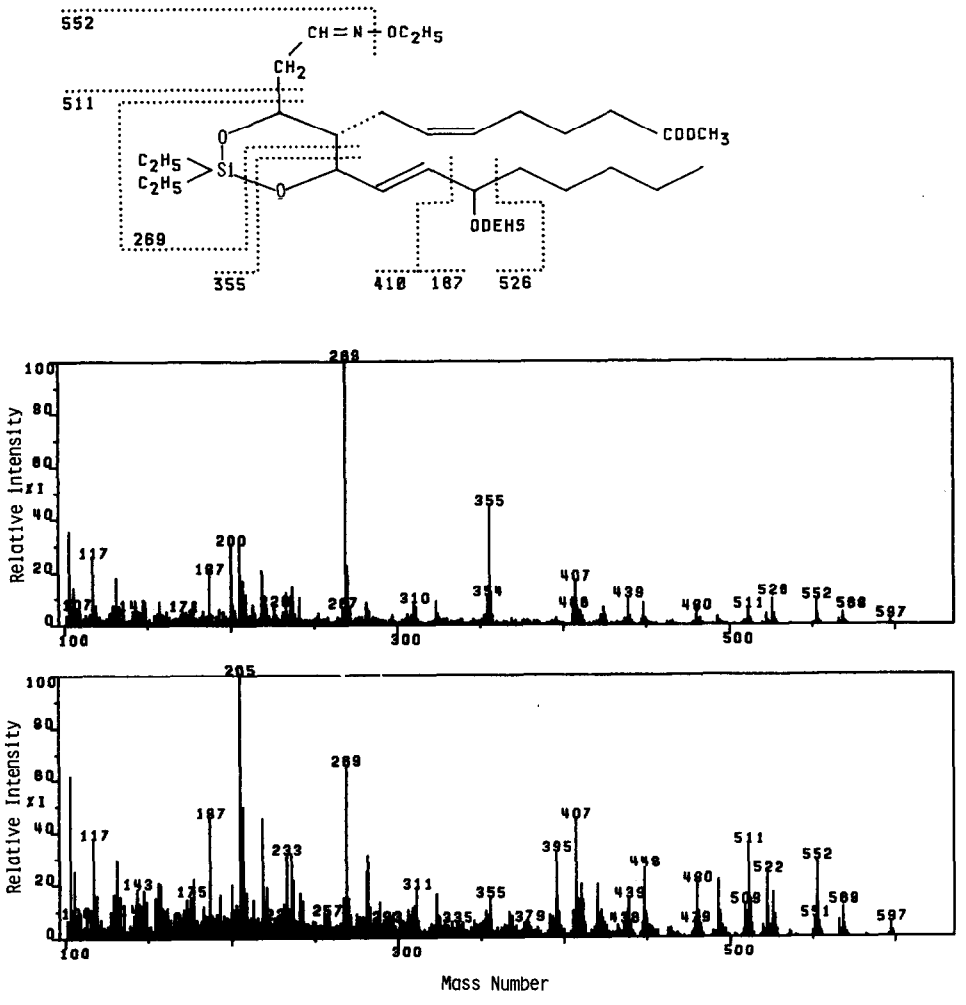


Fig. 6. Mass spectra of the structural isomers of the DEHS-DES derivative of TXB_2 methyl ester-ethyl-oxime. Top: the first component eluted (E2a). Bottom: the second component eluted (E2b).

the ion of *m/z* 157 as a base peak or prominent peak as shown in Fig. 5. The mass spectrum of the first component eluted D2a was considerably more complex than that of the second D2b. Molecular ions with very low intensity were found in the mass spectra of the *syn-* and *anti*-isomers. The ions $[M-C_2H_5]^+$ (*m/z* 568), $[M-OCH_3]^+$ (*m/z* 566) and $[M-C_5H_{11}]^+$ (*m/z* 526) appeared with low intensity and $[M-OC_2H_5]^+$ (*m/z* 552) was prominent. Loss of DEHSOH from the molecular ion and from $[M-OC_2H_5]^+$ gave ions of *m/z* 493 and 448. These six ions were also characteristic of the TXB_2 derivative, which has the same molecular weight.

Fission of the C_5–C_6 bond yielded the fragment ion at *m/z* 115 containing the methoxycarbonyl group, with moderate intensity for D2a and as a base peak for D2b. This ion is the fragment ion of the α-chain containing the methoxycarbonyl group. The characteristic fragment ion at *m/z* 201 observed with low intensity was

produced by fission of the C_7–C_8 bond with migration of the hydrogen atom at C-9 to the nitrogen atom. This ion corresponds to that at *m/z* 245 in the mass spectrum of the methyl ester-trimethylsilyloxime-TMS ether derivative, the fragmentation mechanism of which has been discussed in detail by Cockerill *et al.*[15].

Thromboxane B_2

Fig. 6 shows the mass spectra of the structural isomers of the DEHS-DES derivative of TXB_2 methyl ester-ethyloxime (E2a, E2b). These exhibited series of ions which were found to be characteristic of the expected derivative. Cleavage of the DES ring has been shown to be more complex than the single-bond fission mechanism found in the corresponding TMS ether derivatives. Many fragmentations were directed by the fission of the DES ring initiated by the loss of an aldehydic chain (CH_2–$CH=N$–OC_2H_5, 86 a.m.u.) at C-9 and/or fission of the β-side-chain and gave many silicon-containing characteristic ions because the C_9–C_{10} bond is particularly weak by virtue of its being α to the 9-silyloxyl group. This observation indicates that the DES ring takes a leading part in the formation of characteristic ions in the mass spectra of E2a and E2b.

The mass spectrum of E2b was considerably more complex than that of E2a. Almost all of the fragment ions in the mass spectrum of E2a were also observed as characteristic ions of E2b. The molecular ion could not be found in the mass spectrum of the first component eluted (E2a), but it was observed in that of the second component eluted (E2b). The ions $[M-C_2H_5]^+$ (*m/z* 568) and $[M-DEHSOH]^+$ (*m/z* 493) were found with low intensity. Each mass spectrum gave the ions $[M-OC_2H_5]^+$ (*m/z* 552) and $[M-C_5H_{11}]^+$ (*m/z* 526), which corresponded to fragment ions of *m/z* 598 and 558 in the mass spectrum of the TMS ether derivative of TXB_2 methyl ester-methyloxime[7,22,23]. The ion at *m/z* 511 arose from loss of the aldehydic side chain (CH_2–$CH=N$–OC_2H_5, 86 a.m.u.) by the C_9–C_{11} bond cleavage characteristic of the protected aldehydic moiety of the TXB_2 hemiacetal ring system. Loss of the C_{15}–C_{20} fragment (187 a.m.u.) from the molecular ion gave the ion at *m/z* 410.

The ion of *m/z* 355 was produced by cleavage of the C_8–C_{12} bond initiated by fission of the DES ring system, followed by subsequent loss of the β-side-chain. Successive loss of the aldehydic side-chain from this ion gave rise to the ion of *m/z* 269 as a base peak for E2a and a prominent peak for E2b. Compound E2b may have another fragmentation pathway because the ion at *m/z* 355 appears with low intensity. A plausible mechanism for the formation of this ion was discussed in our previous papers[20,21]. The fission of the DES ring also initiated the migration of the ODEHS group at C-15 to another silicon atom, as was seen with the $PGF_{1\alpha}$ derivative. Consequently, the ion at *m/z* 205 was prominent for E2a and a base peak for E2b. This TXB_2 derivative had the same molecular weight as the corresponding 6-keto-$PGF_{1\alpha}$ derivative, but its mass spectra were considerably more complex and lacked the ion of *m/z* 157 typical of a F_α-PG ring system. It was clear from these GC–MS results that the reaction product was a mixture of *syn-* and *anti-*isomer pairs of the expected TXB_2 methyl ester-ethyloxime-15-DEHS-9,12-DES derivative. The detailed fragmentation pathways involved in the formation of these ions are now under investigation.

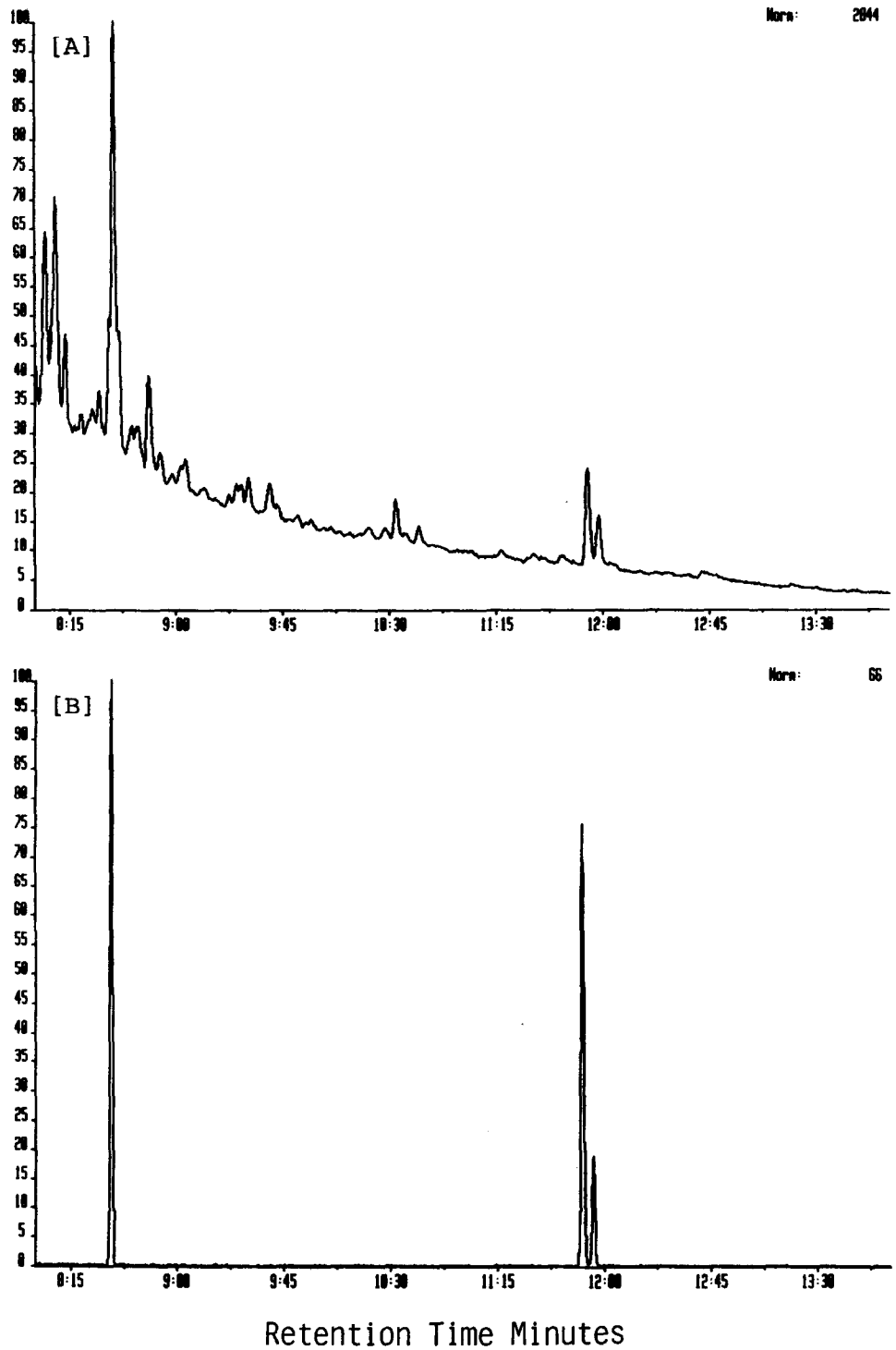


Fig. 7. SIM analyses of TXB_2 corresponding to injection of 25 pg of the DEHS-DES derivative of its methyl ester-methyloxime at resolutions of 1000 (A) and 8000 (B) over a GC retention time range of 8–14 min.

GC–SIM of TXB_2

Sensitive and selective detection with GC–LRSIM is hindered by the presence of ions derived from endogenous substances having the same nominal mass number as that of the compound under investigation. In response to this problem, a significant improvement has been achieved by increasing the resolving power of the mass spectrometer so as to detect only the ion of exact mass corresponding to the elemental composition of the compound itself or to a suitably characteristic fragment ion. GC–HRSIM has been found to be a versatile tool for the microanalysis of prostanoids in biological samples[30].

Fig. 7A shows the selected-ion recordings (SIRs) of the DEHS-DES derivative of TXB_2 methyl ester-methyloxime obtained by monitoring the characteristic fragment ion at *m/z* 269.1573 ($C_{14}H_{25}O_3Si$) specific for the structural integrity of TXB_2, with two different resolutions. 1000 and 8000 (10% valley). When 25 pg TXB_2 derivative were injected, LR-SIR at a resolution of 1000 showed a doublet with a signal-to-noise ratio (S/N) of more than 25:1 and a rather high background noise level. As this TXB_2 derivative gave the *syn-* and *anti*-isomer pairs with a well resolved doublet in its gas chromatogram and showed extensive mass fragmentation, the absolute ion intensity of this ion may not be so high in comparison with that of the prominent peak of the corresponding TMS and other silyl ether derivatives. Consequently, the detection limit of this TXB_2 derivative at low resolution was considered to be reasonable. On the other hand, the HR-SIR result illustrated in Fig. 7B gave a well resolved doublet with S/N of more than 300:1 calculated by magnifying the same result by 100 times to clarify the background noise level, since the latter was dramatically reduced by operation at high resolution. GC–HRSIM of high specificity and high sensitivity was less susceptible to interference by co-existing impurities, yielding, in this case, an high degree of confidence in the ability to detect 10^{-13} g of F_α-PGs and TXB_2.

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