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THE DIETHYLHYDROGENSILYL-CYCLIC DIETHYLSILYLENE DERIVATIVE IN THE GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF THROMBOXANE B_2

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The diethylhydrogensilyl-cyclic diethylsilylene (DEHS-DES) derivative of thromboxane B_2 (TXB_2) methyl ester-methyloxime was prepared by treating with diazomethane, O-methylhydroxylamine-HCl and then with N,O-bis(diethylhydrogensilyl)trifluoroacetamide (DEHS-BSTFA). The gas chromatogram of the reaction product showed a well-resolved doublet using a 5% phenylmethylsilicon cross-linked fused silica capillary column, corresponding to the syn- and anti-isomers. In their mass spectra, these components showed an ion of at 269 as a base or prominent peak. This ion and many other characteristic ions reflect the TXB_2 system with moderate intensity. The ion at m/z 269 consisted of a fragment of the DES ring moiety and the α-side chain. The mass spectrum of the second eluting component was considerably more complex than that of the first. Almost all of the fragment ions in the mass spectrum of the first eluting component were also observed in that of the second component. When gas chromatography/high resolution-selected ion monitoring (GC/HR-SIM) was carried out at a resolution of 8000 to monitor the ion of m/z 269.1753, specific for the structural integrity of TXB_2, the selected ion recording (SIR) showed a well-shaped doublet with signal to noise ratio (S/N) of greater than 300 after an injection of 25 pg of the derivative.

KEYWORDS——thromboxane B_2; derivatization; cyclic diethylsilylene derivative; mass spectrum; GC/MS; GC/HR-SIM; DEHS-BSTFA

The trimethylsilyl (TMS) ether derivative of thromboxane B_2 (TXB_2) methyl ester-methyloxime[1)] has been used exclusively as a derivative suitable for gas chromatography/mass spectrometry (GC/MS) and GC/SIM. Electron impact ionization GC/MS of this derivative gives a mass spectrum which comprises ions useful in identifying the compound, but the characteristic ions in the high mass region suitable for GC/SIM are of low abundance because of preferential fissioning of the C_8-C_{12} bond. In response to this problem, a number of new silyl ether derivatives have been developed to improve the GC and GC/MS properties.[2-7]

Use of a cyclization reaction for derivatization permits the preparation of specific derivatives reflecting a more useful structural information of the compound of interest and allows more efficient and easier separation from the compounds in a mixture of isomers and/or homologues. In addition, it is expected that the cyclization product will show the characteristic mass spectrum in which the inherent ions are given with prominent intensity in the high mass region. In an attempt to gain more useful information in the microanalysis of hydroxypregnanes with 17,20-diols and 17,20,21-triols, their cyclic diethylsilylene (DES) derivatives were prepared and used to investigate their GC and GC/MS properties. As a result these DES derivatives gave mass spectra in which the molecular ion was observed with prominent intensity.[8-10]

Lawson et al.[11] reported a novel approach to the extraction of TXB_2 from biological fluids 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 6-membered covalent complex. This selective extraction of TXB_2 confers excellent analytical specificity on the microanalysis of TXB_2 by GC/SIM. Thus, in order to use the dual properties of selective extraction and specific derivatization for the microanalysis of TXB_2, 6-membered DES derivative formation using DEHS-BSTFA was applied to TXB_2 yielding the 1,3-diol by opening the hemiacetal ring. This paper deals with the GC/MS properties of a novel TXB_2 methyl ester-methyloxime-15-DEHS-9,12-DES derivative.

The DEHS-DES derivative of TXB_2 methyl ester-methyloxime was prepared by treating with diazomethane, O-methylhydroxylamine-HCl and then with DEHS-BSTFA. This derivatization reaction is based on the hemiacetal form of TXB_2 which is in equilibrium with the hydroxyaldehyde and is susceptible to reaction of 9,12-diol with DEHS-BSTFA to form the DES ring. The gas chromatogram of the reaction product showed a well-resolved doublet when a 5% phenylmethylsilicon cross-linked fused silica capillary column was used whereas the corresponding tris-TMS ether deriatives gave a single peak.[6] The reaction product was thought to be the expected derivative of TXB_2 since the results showed that the GC peak height ratio did not change when the reaction was repeated at 60°C for one hour or more. This suggests that these peaks may be a pair of the syn- and anti-isomers. These two isomers of corresponding ethyloxime and isobutyloxime were also resolved under the same GC conditions, but the n-butyloxime homologue showed a single peak with a shoulder. The MU-values of the two components were 30.56 and 30.72. This was about 2.6 higher than that of the corresponding TMS ether derivative (MU-value 28.00). On the other hand, the syn- and anti-isomer pair of the DEHS-DES derivative of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) methyl ester-methyloxime, having the same molecular weight as the corresponding derivative of TXB_2, gave MU-values of 31.22 and 31.38, thus allowing complete discrimination by GC.

The mass spectra of the isomer pair of DEHS-DES derivative of methyl ester-methyloxime are shown in Fig. 1. The molecular ion was not found in the mass spectrum of the first eluting component, but the ion of $[M-C_2H_5]^+$ (m/z 554) appeared with low intensity. Loss of diethylhydrogensilanol from the molecular ion gave the ion at m/z 479. The mass spectrum of the first eluting component gave ions of $[M-OCH_3]^+$ (m/z 552) and $[M-C_5H_{11}]^+$ (m/z 512), by losses of the methoxy radical at the aldehydic chain or the methoxycarbonyl group and the C_{16}-C_{20} hydrocarbon at the β-side chain. These ions were assigned to be identical with the fragment ions of m/z 598 and 558 in the mass spectrum of the TMS ether derivative of TXB_2 methyl ester-methyloxime. The ion at m/z 511 arised from the fragment loss of the CH_2-CH=$NOCH_3$ radical because of the C_9-C_{11} bond cleavage characteristic of the protected aldehydic moiety of the TXB_2 hemiacetal ring system. The ion at m/z 341 is produced by the cleavage of the C_8-C_{12} bond initiated by the fission of the DES ring system followed by a 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 in the first eluting component and a prominent peak in the second eluting component. The mass spectrum of the second eluting component was considerably more complex than that of the first. Almost all of the fragment ions in the mass spectrum of the first eluting component were also observed as characteristic ions common in the second eluting component. It is clear from these GC/MS results that the reaction product was a mixture of the syn- and anti-isomers of the expected TXB_2 methyl ester-methyloxime-15-DEHS-9,12-DES derivative.

Figure 2 shows the SIR of this derivative of TXB_2 obtained by monitoring the ion of m/z 269.1573 at a resolution of 8000. When TXB_2 25 pg of the derivative was injected onto the GC column, the SIR showed a well-shaped doublet with an S/N of more

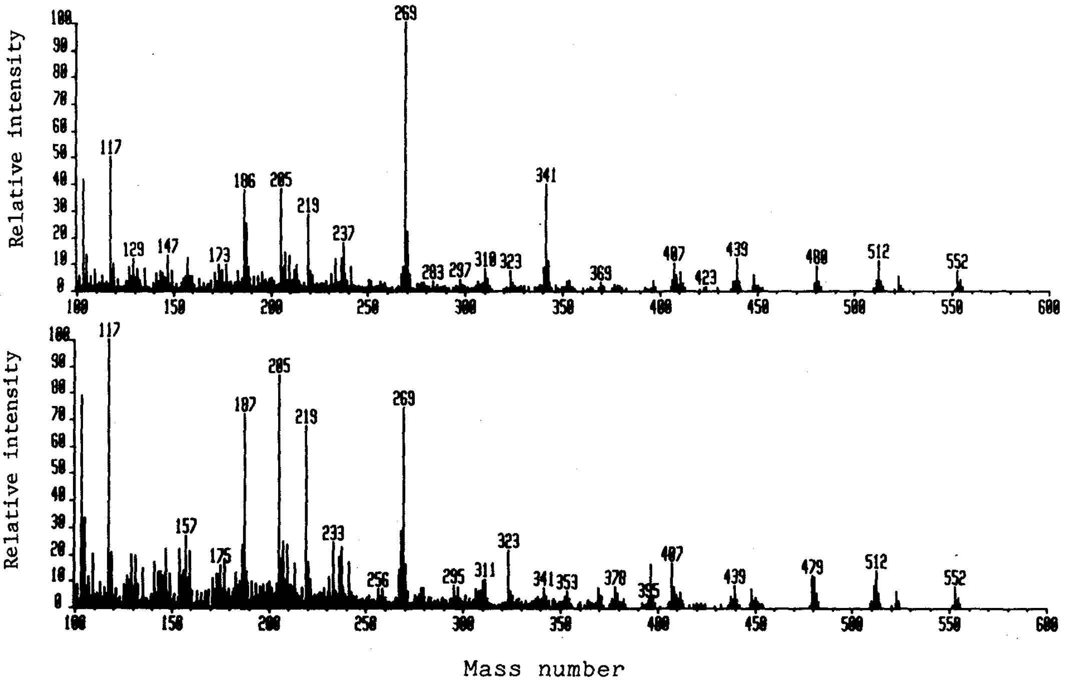

Fig. 1. Mass Spectra of the Structural Isomers of the DEHS-DES Derivative of TXB_2 Methyl Ester-Methyloxime Top: 1st eluting component, bottom: 2nd eluting component

GC/MS was performed using a VG-70S mass spectrometer (VG Analytical Ltd., Manchester, UK) interfaced to a Hewlett Packard 5890A gas chromatograph and a VG 11-250J+Data processing system. GC conditions: column, 30 m x 0.25 mm, a 5% phenyl-methylsilicon cross-linked fused silica capillary column (DB-5, J & W Scientific, Inc., CA., USA); column temp, 120°C (1 min), programmed to 250°C at 20°C/min and then to 300°C at 10°C/min; carrier gas, helium (linear gas velocity 30 cm/min). MS conditions: ion source temp, 200°C; ionization energy, 70 eV; trap current, 200 μA; acceleratiing voltage, 8 kV.

than 300. This suggests that the TXB_2 derivative makes it possible to detect picogram level of TXB_2 with a high degree of confidence. Moreover, it is very interesting that the ion at m/z 269, consisting of the fragment of the DES ring moiety and the α-side chain, makes it possible to utilize internal standards for GC/SIM, which have been synthesized with deuterium atom labels at the α-side chain.

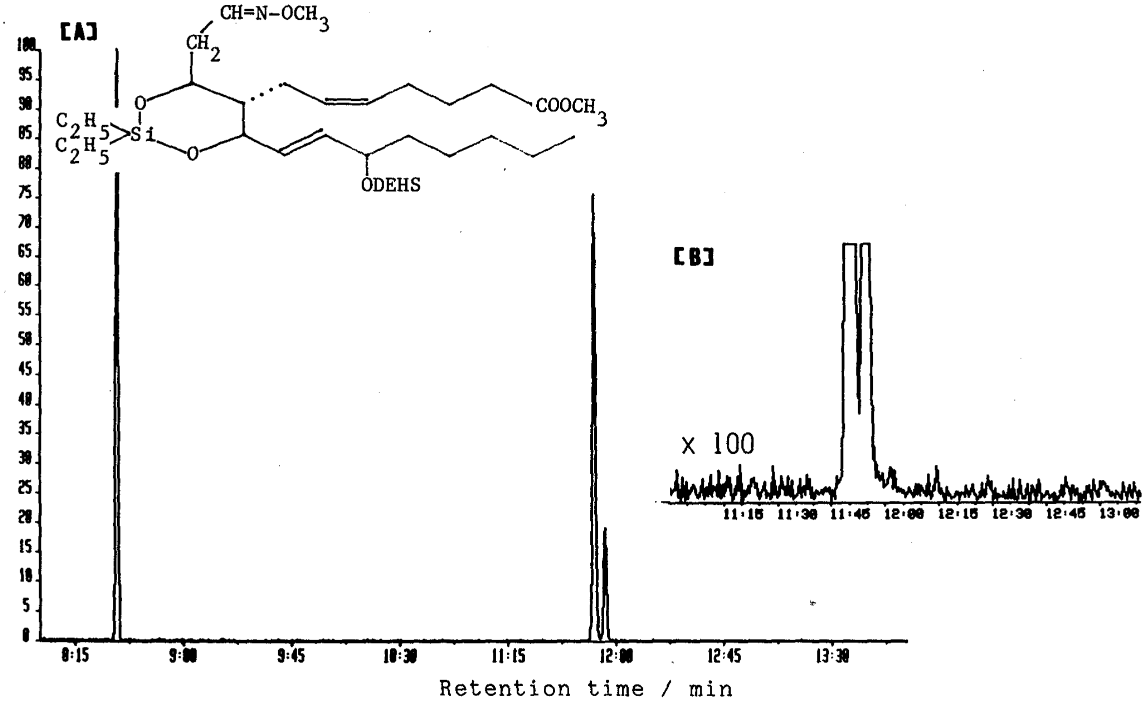

Fig. 2. SIR from TXB_2 25 pg of the DEHS-DES Derivative of Methyl Ester-Methyloxime at a Resolution of 8000 over a GC Retention Time Range of 8-14 min (A)
Insert B shows the same result magnified by 100 times to show the noise level. GC/HR-SIM was performed with the same conditions as in Fig. 1 except that the ionization energy was 35 eV. For GC/HR-SIM, the ion at m/z 269.1573 was focused using the lock mass ion at m/z 268.9824 from perfluorokerosene independently introduced into the ion source.

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