DIMETHYLISOPROPYLSILYL ETHER DERIVATIVE IN GAS CHROMATOGRAPHY/MASS SPECTROMETRY OF 2,3-DINOR-6-KETO-PROSTAGLANDIN $F_{1\alpha}$

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2,3-Dinor-6-keto-prostaglandin $F_{1\alpha}$ (I), extracted from its acidic aqueous solution, was converted to its 1-n-propylamide-6-methyloxime-9,11,15-tris-dimethylisopropylsilyl (DMIPS)-ether derivative by treating it with n-propylamine, O-methylhydroxylamine-HCl and then with DMIPS-imidazole. The chromatogram of the reaction product showed a single broad peak accompanying a shoulder, which was considered to be the unresolved structural syn- and anti-isomer pair. The methylene unit value of this derivative was 34.28, being about 7.5 higher than that of the trimethylsilyl ether derivative of I methyl ester-methyloxime. The reaction product gave a mass spectrum with the ion of $[M-43]^+$ at m/z 669 as a base peak. The molecular ion was observed at m/z 712 with low intensity, and many characteristic ions reflecting the structure with moderate or low intensity. The present I derivative concentrated more than 5% of the total ion current (above m/z 100) into the base peak ion. When Gas chromatography/high resolution selected ion monitoring was carried out at the resolution of 10000 monitoring the ion at m/z 669.4512, specific for the structural integrity of I, the selected ion recording showed a single broad peak with signal-to-noise ratio of more than 10:1 after injection of 2 picograms of the derivative.

KEYWORDS 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$; dimethylisopropylsilyl ether; derivatization; mass spectrum; GC/MS

2,3-Dinor-6-keto-prostaglandin $F_{1\alpha}$ (I), one of the major urinary metabolites of prostaglandin I_2,[1] has been the object of much interest for elucidating the physiological role of its parent compound.[2] Gas chromatography/selected ion monitoring (GC/SIM), in general, has been widely used as a highly specific method in microanalysis of prostanoids in extracts from biological samples. In the case of I, however, there remains a serious problem for its derivatization which plays an important role in microanalysis by GC/SIM. I has the hemiketal hydroxyl group in a relative position favorable for the formation of a gamma-lactone and is prone to gamma-lactonization.[3] In fact, I exists in the spiro-lactone form in an acidic aqueous solution and is extracted as such with organic solvent. Therefore, when this compound was treated sequentially with diazomethane, O-methylhydroxylamine-HCl and silylating agent, the carboxyl group was not initially available to react with diazomethane. To solve this problem, two approaches have been used: The sequence of the derivatization reaction was reversed by first carrying out methyloximation. This enabled I to be obtained in an acyclic form and the formation of an O-methyloxime derivative at C-6 with free terminal carboxyl group without the induction of gamma-lactonization. The revealed carboxyl group would be treated for esterification with N,O-bis(trimethylsilyl)trifluoroacetamide[4] or diazomethane.[3,4]

An alternative solution to the above problem is to convert the unfavourable lactone ring directly into a stable acyclic derivative. Use of the lactone ring-opening reaction with alkylamine for derivatization allows the preparation of specific derivatives reflecting more structural information about the compound of interest. In addition, the reaction product obtained by this lactone ring-opening reaction provides a characteristic spectrum in which significant ions are obtained with prominent intensity in the high mass region. An approach using the lactone ring-opening reaction has been employed to gain more useful information on the microanalysis

of 11-dehydrothromboxane B_2 (11-dehydro-TXB_2).[5] The acyclic derivative of 11-dehydro-TXB_2 was prepared by treating it with n-propylamine and used to investigate its GC and GC/MS properties. As a result, 11-dehydro-TXB_2 methyl ester (ME)-n-propylamide (PA)-dimethylisopropylsilyl (DMIPS) ether derivative gave a mass spectrum in which the $[M-43]^+$ ion was observed as a base peak. This indicates that the choice of this base peak ion may make it a suitable candidate for specific and sensitive microanalysis of 11-dehydro-TXB_2 by GC/SIM. This paper deals with the GC/mass spectrometic property of a novel I 1-PA-6-methyloxime (MO)-9,11,15-tris-DMIPS ether derivative.

The spiro-lactone form of I was extracted from an acidic aqueous solution, and readily converted to its PA-MO-DMIPS ether derivative by treating it with n-propylamine, O-methylhydroxylamine-HCl and then with DMIPS-imidazole. The reaction product was analyzed using a 25 m fused silica capillary column cross-linked with methylsilicone. The resulting derivative showed a single broad peak accompanying a shoulder, which was considered to correspond to the unresolved structural syn- and anti-isomer pair, when eluted with a retention time of 20-30 min. Fortunately, however, the peak shape was observed as a single peak by reducing the retention time by less than a half. The methylene unit value of this derivative was 34.28, being about 4.5 higher than that of the corresponding trimethylsilyl (TMS) ether derivative and about 7.5 higher than that of the TMS ether derivative of I ME-MO. The ethyloxime (EO) and isobutyloxime (IBO) derivatives were also observed as a single broad peak under our GC conditions.

Figure 1 shows the PA-MO-DMIPS ether derivative of I. The molecular ion (m/z 712) and $[M-15]^+$ ion were observed with low intensity. Loss of an isopropyl radical at the DMIPS group from the molecular ion gave rise to the ion of $[M-43]^+$ at m/z 669 as a base peak. The appearance of these ions in the spectrum, typical of DMIPS ether derivatives,[6] confirmed formation of the expected derivative. Many other characteristic fragment ions reflecting the system of I were also observed with moderate or low intensity. The molecular ion lost a methyloxy radical at the methyloxime moiety to give the $[M-31]^+$ ion at m/z 681. Successive losses of the

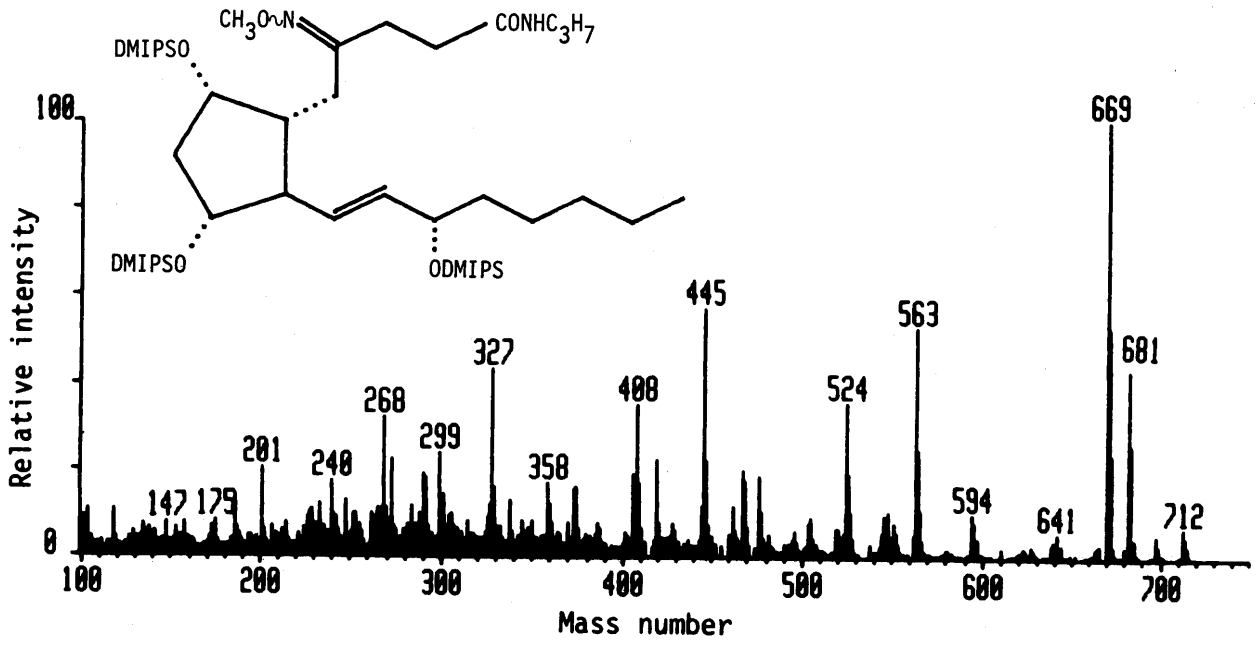

Fig. 1. Mass Spectrum of the 2,3-Dinor-6-keto-prostaglandin F_{1a} n-Propylamide-methyloxime-dimethylisopropylsilyl Ether Derivative

GC/MS was performed using a VG-70SE mass spectrometer (VG Anaritical Ltd., Manchester, UK) interfaced to a Hewlett Packard 5890A gas chromatograph and a VG 11-250J+ Data processing system. GC conditions: column, 10 m x 0.25 mm, a fused silica capillary column cross-linked with methylsilicone (Ultra 1, Hewlett Packard Co, PA, USA); Column temp., 260°C; carrier gas, helium (linear gas velocity 30 cm/min). MS conditions: ion source temp. 200°C; ionization energy, 70 eV; trap current, 100 μA; accelerating voltage, 8 kV.

dimethylisopropylsilanol (118 amu) gave rise to ions at m/z 594, 476 and 405 from the molecular ion and ions at m/z 563, 445 and 358 from $[M-31]^+$ ion respectively. A small part of the ion at m/z 327 shifted to m/z 341 in the mass spectrum of the corresponding EO derivative, but most of this ion remained as it was, it being apparent that this ion at m/z 327 has another ion species of C(1)/C(9) fragment[7] with the same nominal mass as the $[M-3xDMIPSOH]^+$ ion. The ion at m/z 201 originated from the C(15)/C(20) fragment, a characteristic of the prostanoid one and two series. The ion at m/z 186 was considered to correspond to the alpha-chain fragment. The mass fragmentation of PA-EO-DMIPS ether and PA-IBO-DMIPS ether derivatives were closely related to that of the corresponding MO derivative.

The PA-MO-DMIPS ether derivative of I concentrated about 5% of the total ion current (above m/z 100) into the base peak ion at m/z 669. Appearance of a characteristic ion with abundant intensity in a high mass region allows the use of this derivative for the specific and sensitive microanalysis of I by GC/SIM. Figure 2 shows the selected ion monitoring (SIR) of this derivative of I obtained by monitoring the ion of m/z 669.4512 specific for the structural integrity of I with a resolution of 10000. When 2 picograms of I derivative was injected, the SIR showed a single broad peak with signal-to-noise ratio of more than 10:1. This suggests that the present derivative makes it possible to obtain high reliability in the ability to detect picogram levels of I.

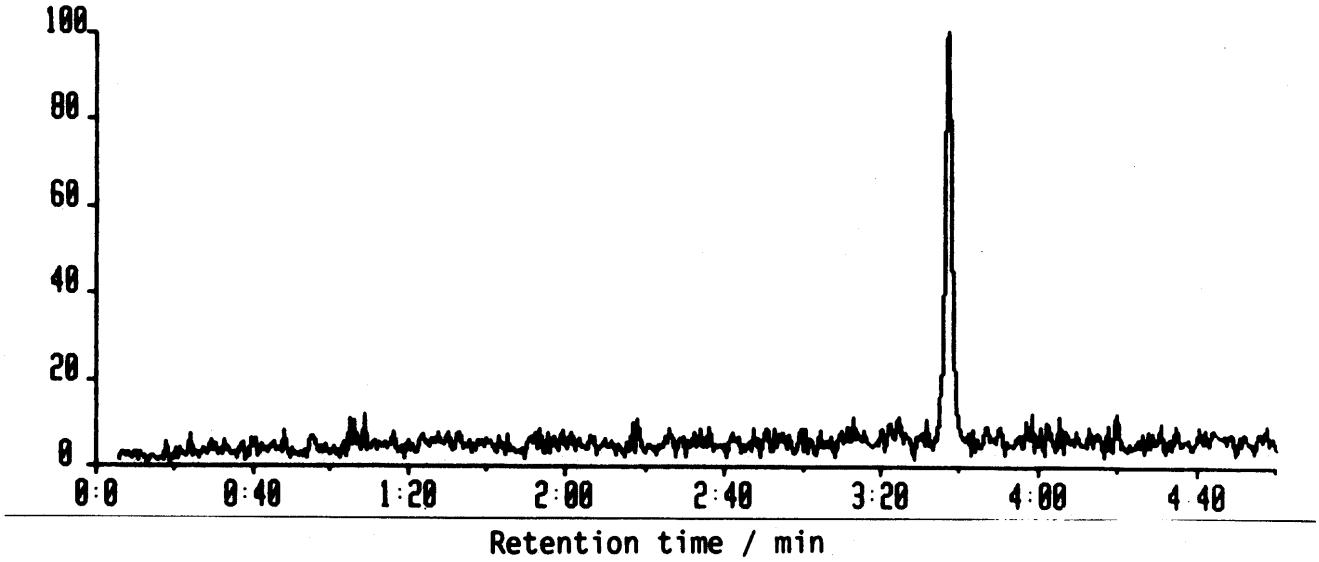

Fig. 2. Selected Ion Recording from 2,3-Dinor-6-keto-prostaglandin $F_{1\alpha}$ 2 Picogram of the n-Propylamide-methyloxime-dimethylisopropylsilyl Ether Derivative at a Resolution of 10000 over a GC Retention Time Range of 0-5 min

GC/high resolution (HR)-SIM was performed with the same conditions as in Fig. 1 with the exception of using an ionization energy of 35 eV. For GC/HR-SIM, the ion at m/z 669.4512 was focused using the lock mass ion at m/z 666.9601 from perfluorokerosene independently introduced into the ion source.

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