Diethylhydrogensilyl-Cyclic Diethylsilylene Derivatives in Gas Chromatography/Mass Spectrometry of Prostanoids. IV. 11-Dehydrothromboxane B_2

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Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) properties of 11-dehydrothromboxane B_2 (11-dehydro-TXB_2) methyl ester-11-*n*-propylamide-15-diethylhydrogensilyl (DEHS)-9,12-cyclic diethylsilylene (DES) derivatives were studied. The methylene unit (MU) value of this derivative was 35.17, being 5.7 higher than that of the 11-dehydro-TXB_2 methyl ester-bis-trimethylsilyl ether derivative. The mass spectrum of this derivative was characterized by the ion at *m/z* 368, which consisted of the fragment of the DES ring and the α-side chain. The major fragmentations were directed by fission of the DES group to give silicon atom-containing ions, indicating that the DES ring takes a leading part in the formation of characteristic ions. Other fragmentations common to the prostanoid derivatives were losses of an ethyl radical from the DEHS or DES group, a C_5H_{11} hydrocarbon fragment and diethylhydrogensilanol. The fragmentation mechanisms are briefly discussed.

Keywords 11-dehydrothromboxane B_2; cyclic diethylsilylene derivative; mass spectrum; mass fragmentation; GC/MS; DEHS-BSTFA

Introduction

11-Dehydrothromboxane B_2 (11-dehydro-TXB_2), one of the major enzymatic metabolite of thromboxane B_2 (TXB_2), has been the object of much interest in connection with the physiological roles of thromboxane A_2 (TXA_2), because it has recently been considered to be more reliable index of thromboxane biosynthesis than TXB_2, which has been monitored as the stable hydrolyzed product of TXA_2.[1,2] In response to the advances in studies of TXA_2 metabolism, microanalytical methods for 11-dehydro-TXB_2 with high sensitivity and high specificity have been developed using gas chromatography/selected ion monitoring (GC/SIM).[3-5]

Lactone-ring opening may be by no means inferior to cyclization as a derivatization reaction to provide structural information on a compound of interest. In general, in order to obtain evidence for lactone-ring structure, the lactone-ring moiety has been treated with sodium methoxide in methanol. This would be expected to convert the lactone-ring to a hydroxycarboxylic acid methyl ester. On the other hand, by treating the lactone-ring moiety with an alkylamine, it is possible to obtain a ring-opened amide compound of different size from the parent compound.

11-Dehydro-TXB_2 having the lactone-ring system was susceptible to alkylamine to give quantitatively an alkylamide group and a hydroxyl group at C-12.[6] The reaction product yields a 1,3-diol group which contains a 1,3-diol group which is suitable for specific extraction of 11-dehydro-TXB_2 from biological samples by the use of a borate column[7] and for cyclization with alkylboronic acid[8] or *N*, *O*-bis(diethylhydrogensilyl)trifluoroacetamide (DEHS-BSTFA).[9,10] Therefore, combined use of the lactone-ring opening reaction followed by re-cyclization for derivatization of 11-dehydro-TXB_2 yields a specific derivative which reflects more structural information and to separate the compound efficiently and easily from a mixture of related homologues.

In our previous papers,[9,10] cyclization was applied to microanalysis of F_α-prostaglandins and TXB_2. Their cyclic diethylsilylene derivatives were prepared by treatment with DEHS-BSTFA and used for the investigation of their GC and gas chromatography/mass spectrometry (GC/MS) properties, being comparable to the corresponding trimethylsilyl (TMS) ether derivatives. Thus, this derivatization reaction was extended to 11-dehydro-TXB_2 which yielded a 1,3-diol group upon lactone-ring opening with *n*-propylamine. This paper deals with the GC and GC/MS properties of 11-dehydro-TXB_2 methyl ester (ME)-11-*n*-propylamide (PA)-15-diethylhydrogensilyl (DEHS)-9,12-cyclic diethylsilylene (DES) derivative [methyl 7-[4-(3-diethylsilyloxy-1-octenyl)-2,2-diethyl-6-(*N*-propylcarbamoylmethyl)-1,3-dioxa-2-silacyclohexan-5-yl]-5-heptenoate].

Experimental

GC A GC-7A gas chromatograph (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a flame ionization detector and a VandenBerg's solventless injector [11] was employed. The column was a 25 m × 0.32 mm fused silica capillary cross-linked with methylsilicone (Ultra 1, Hewlett Packard, 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.

GC/MS A VG Micromass ZAB-HF mass spectrometer (VG Analytical Co. Ltd., Manchester, UK.) interfaced to a Shimadzu GC-9A gas chromatograph with a solventless injector and a DS-2035 data processing system (VG Analytical Co., Ltd.) 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 injection port and transfer line was kept at 290 °C and that of the ion source at 250 °C. The ionization energy and trap current were 70 eV and 200 μA, respectively. The accelerating voltage was 8 kV.

Samples and Reagents 11-Dehydro-TXB_2 was obtained from Cayman Chemicals Ltd. (MI, U.S.A.). Oxygen-18-labelled 11-dehydro-TXB_2 was prepared by exchange of 1- and 11-carbonyl oxygen in 0.1 M LiOH in 98% oxygen-18-enriched water.[12] After one cycle of esterification-hydrolysis, oxygen-18 content was checked by GC-MS following derivatization. The reaction product was a mixture of mainly [$^{18}O_1$]- and [$^{18}O_2$]-labelled analogues (51% and 45%, respectively).

n-Propylamine, *n*-butylamine and tetramethyldisilazane (TMDS) were purchased from Tokyo Kasei Kogyo Ltd. (Tokyo, Japan). DEHS-BSTFA was kindly donated by Dr. M. Hasegawa (Tokyo Kasei Kogyo Ltd.).

Tetradeuterium labelled methanol and oxygen-18 labelled water were obtained from Merck Co., Ltd. (Darmstadt, West Germany) and MSD Isotopes (Quebec, Canada). Diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide.

Derivatization The ME of 11-dehydro-TXB_2 was prepared by treating with diazomethane, and the trideuterium labelled ester was obtained by esterification with tetradeuterium labelled methanol and hydrogen chloride–diethyl ether.

n-Propylamide (PA) and *n*-butylamide (BA) derivatives of 11-dehydro-TXB_2 ME were obtained by treating 11-dehydro-TXB_2 ME with *n*-propylamine or *n*-butylamine at room temperature for 3 h followed by evaporation of the excess of alkylamine under reduced pressure.

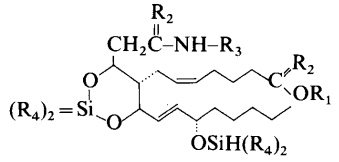
After methylation and amidation, the resulting ME-alkylamide derivatives of 11-dehydro-TXB_2 were treated with TMDS or DEHS-BSTFA in pyridine for 30 min at room temperature. The derivatives were used for GC/MS analysis without removal of excess silylating agent.

Results and Discussion

Gas Chromatographic Properties of the 11-Dehydrothromboxane B_2 Derivative The 11-dehydro-TXB_2 ME-PA-DEHS-DES derivative [methyl 7-[4-(3-diethylsilyloxy-1-octenyl)-2,2-diethyl-6-(*N*-propyl-carbamoylmethyl)-1,3-dioxa-2-silacyclohexan-5-yl]-5-heptenoate] was prepared by treating 11-dehydro-TXB_2 with diazomethane, *n*-propylamine and then with DEHS-BSTFA. The gas chromatogram of the reaction product showed a well-shaped peak when analyzed using a 5%-phenylmethylsilicone cross-linked fused silica capillary column. This suggests that the stepwise derivatization proceeded smoothly and quantitatively and that only the lactone moiety was susceptible to the amide-formation reaction to give an *n*-propylamide group and hydroxyl group when 11-dehydro-TXB_2 was allowed to stand in *n*-propylamine at room temperature for 3 h. The methylene unit (MU) value of the 11-dehydro-TXB_2 derivative was 35.17, being about 4.4 higher than that of the corresponding TMS ether derivative and 5.7 higher than that of the 9,15-bis-TMS ether derivative of 11-dehydro-TXB_2 ME.

Mass Spectrometric Properties of the 11-Dehydrothromboxane B_2 Derivative The mass spectrum of the 11-dehydro-TXB_2 ME-PA-DEHS-DES derivative is shown in Fig. 1. Table I shows the structures of 11-dehydro-TXB_2 ME-PA-DEHS-DES derivative and related derivatives used for elucidation of the fragmentation mechanisms. Table II lists the structures of the characteristic fragment ions found in the mass spectrum of 11-dehydro-TXB_2 ME-PA-DEHS-DES derivative and their elemental compositions. The results from the experiments on the *n*-butylamide homologue derivative and deuterium- and oxygen-18-labelled analogues are also summarized in Table II. Almost all of the characteristic ions were observed with obvious mass unit shifts in the mass spectra of these related derivatives II to IV. In addition, the dimethylhydrogensilyl-cyclic dimethyl-silylene (DMHS-DMS) homologue derivative (V) was prepared and used for estimation of the silicon atom-containing ions by comparison of its mass spectrum with that of the corresponding DEHS-DES de-

TABLE I. Structures of 11-Dehydrothromboxane B_2 Derivatives



Derivative	R_1	R_2	R_3	R_4
I	CH_3	^{16}O	*n*-C_3H_7	C_2H_5
II	CH_3	^{16}O	*n*-C_4H_9	C_2H_5
III	C_2H_5	^{16}O	*n*-C_3H_7	C_2H_5
IV	CH_3	^{18}O	*n*-C_3H_7	C_2H_5
V	CH_3	^{16}O	*n*-C_3H_7	CH_3

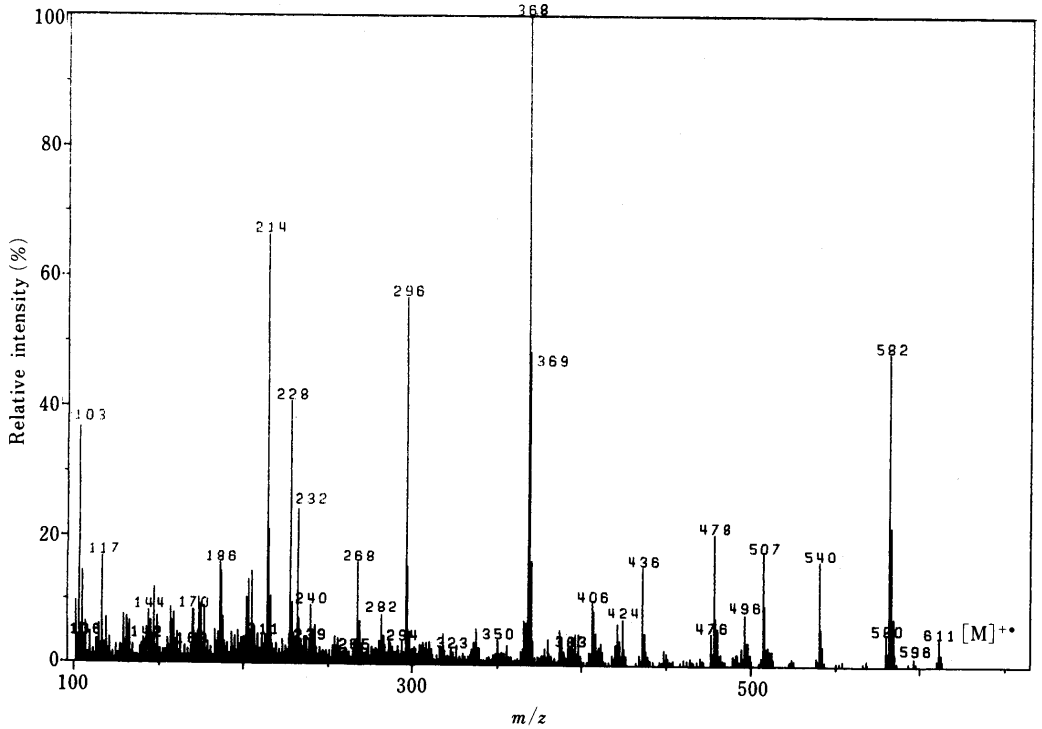


Fig. 1. Mass Spectrum of the 11-Dehydrothromboxane B_2ME-PA-DEHS-DES Derivative

TABLE II. Shift of Ions in Homologue and Analogue Derivatives of the 11-Dehydrothromboxane B_2 ME-PA-DEHS-DES Derivative

m/z	Fragmentation		Elemental composition					Related derivative			
			C	H	N	O	Si	II	III	IV	V
611	$[M]^{+\cdot}$		32	61	1	6	2	625	614	613, 615	555
582	$[M-29]^+$	$M-C_2H_5$	30	56	1	6	2	596	585	584, 586	540 (M−15)
580	$[M-31]^+$	$M-OCH_3$	31	58	1	5	2	594	583	582, 584	524
540	$[M-71]^+$	$M-C_5H_{11}$	27	50	1	6	2	554	543	542, 544	484
507	$[M-104]^{+\cdot}$	M−DEHSOH	28	49	1	5	1	521	510	509, 511	479
478	$[M-29-104]^+$	$M-C_2H_5-$DEHSOH	26	44	1	5	1	492	481	480, 482	464
436	$[M-104-71]^+$	M−DEHSOH$-C_5H_{11}$	23	38	1	5	1	450	439	438, 440	408
424	$[M-187]^+$	M−CH(ODEHS)C_5H_{11}	22	38	1	5	1	438	427	426, 428	396
369		M−C(12)/C(20)−O	19	35	1	4	1	383	372	371, 373	341
368	$[369-1]^+$		19	34	1	4	1	382	371	370, 372	340
296	$[369-73]^+$	369−CH_2COOCH_3	16	30	1	2	1	310	296	296, 298	268
269	$[369-100]^+$	369−$CH_2CONHC_3H_7$	14	24	0	3	1	269	272	271	241
268	$[368-100]^+$	368−$CH_2CONHC_3H_7$	14	23	0	3	1	268	271	270	240
228	$[369-141]^+$	369−C(1)/C(7)	11	22	1	2	1	242	228	228, 230	200
214	$[369-155]^+$	369−C(1)/C(8)−H	10	20	1	2	1	228	214	214, 216	186
187		CH(ODEHS)C_5H_{11}	10	23	0	1	1	187	187	187	159
186		$(C_2H_5)_2=Si=O-C(=CH_2)NHC_3H_7$	9	20	1	1	1	200	186	186, 188	158
101		DEHSO	4	11	0	0	1	103	103	103	—

rivative I. The mass spectrum of V was similar to that of I except for the expected shifts in proportion to the number of silicon atoms. The results are also shown in Table II.

The B/E scanning data confirmed the fragmentation of the molecular ion (*m/z* 611) to give daughter ions at *m/z* 507 and 368, which were structurally diagnostic of the fragmentation. The ion at *m/z* 369 produced ions at 296, 268, 240, 228, 214 and 186. These B/E scanning data suggest that many significant ions in the low mass region may be derived from the ion at *m/z* 369. The intense fragmentation products in the low mass region were silicon atom-containing ions, whose elemental compositions were determined by accurate mass measurement.

A) Fragmentation Initiated by Simple Bond Fission and Related Fragmentations The mass spectrum of the reaction product revealed a series of ions which were determined to be characteristic of the expected 11-dehydro-TXB_2 ME-PA-DEHS-DES derivative. The appearance of the molecular ion at *m/z* 611 was sufficient to confirm the formation of the expected derivative. Losses of ethyl radical, methyloxy radical and diethylhydrogensilanol (DEHSOH: 104 amu) from the molecular ion led to ions at *m/z* 582, 580 and 507, and further loss of DEHSOH from the ion at *m/z* 582 gave rise to $[M-C_2H_5-DEHSOH]^+$ ion at *m/z* 478.

Radical loss of the C(16)/C(20) hydrocarbon fragment from the molecular ion, which is typical of the prostanoid one and two series derivatives, produced the characteristic ion at *m/z* 540. The ion at *m/z* 187 was observed with low intensity, corresponding to the C(15)/C(20) fragment ($[CH(ODEHS)C_5H_{11}]^+$) from the β-side chain and to the ion at *m/z* 173 found in the mass spectra of the TMS ether derivatives of the prostanoid one and two series. Loss of the above C(16)/C(20) fragment from the $[M-DEHSOH]^+$ ion and appearance of the resulting ion have also been utilized for the structural identification of prostanoids. This characteristic and significant ion, $[M-DEHSOH-C_5H_{11}]^+$, at *m/z* 436, which was observed with low intensity, may be considered to be produced by loss of DEHSOH followed by fission of the C(15)–C(16) bond as shown in Chart 1.

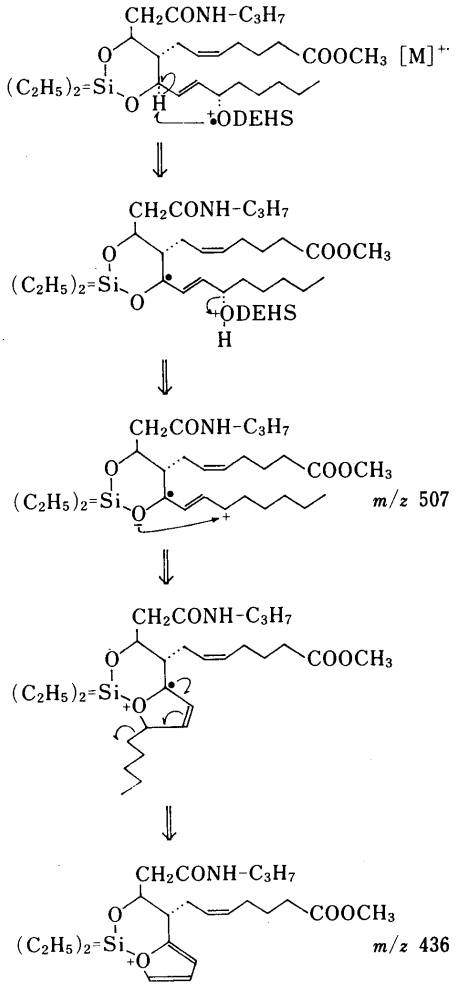


Chart 1

Fragmentation by simple bond fission mechanisms was not an especially favored process compared with the tris-TMS ether derivative and tris-DMIPS ether derivative.[6]

B) Fragmentation Initiated by DES Ring Cleavage and Related Fragmentations Another category of fragmen-

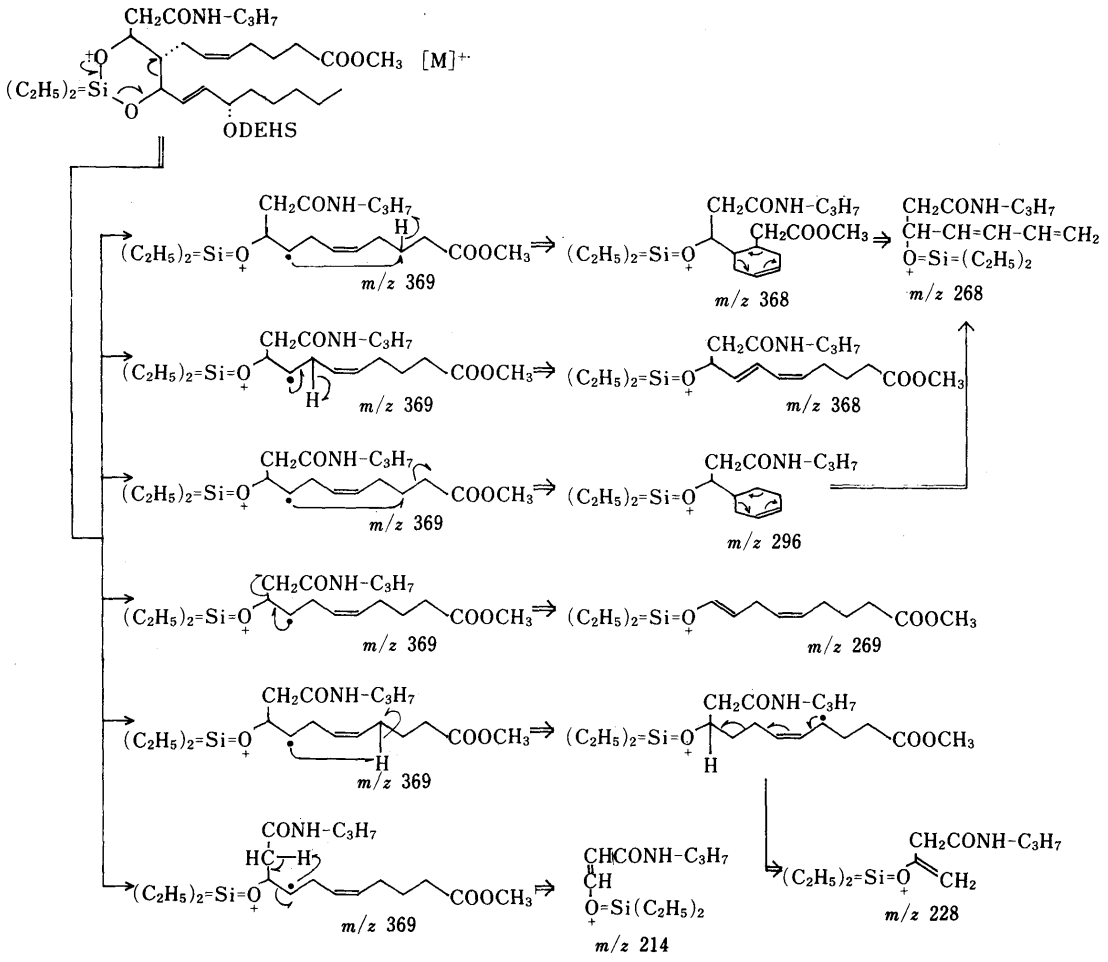


Chart 2

tation was fission of the DES ring accompanied with loss of the β-side chain. This fragmentation mechanism may be rationalized in terms of initial ionization of the oxygen atom at the DES ring. A comparison of the mass spectral data of I with those of related derivatives indicated that the ion at m/z 369 retained one silicon atom, one nitrogen atom and a methyloxycarbonyl group. It is likely that this ion was formed by fission of the DES ring initiated with cleavage of the C(8)–C(12) bond followed by the loss of the β-side chain. A plausible fragmentation is shown in Chart 2, and this fragmentation pathway corresponds to the formation of the ion at m/z 341 in the mass spectrum of TXB_2 methyl ester-methyloxime-DEHS-DES derivative.[9] The elemental composition of this ion at m/z 369, $C_{19}H_{35}N_1O_4Si_1$, is consistent with the observed mass within experimental error.

This ion at m/z 369 further fragments to the ion at m/z 368, the base peak, through two different pathways, by the loss of a hydrogen atom at C-9 and by forming a six-membered ring system with loss of a hydrogen atom at C-3. Expulsion of the C(1)/C(2) fragment (methyloxycarbonyl-methyl radical) instead of a hydrogen atom from the above ion at m/z 369 also resulted in the six-membered-ring-containing ion at m/z 296. This fragmentation process was supported by the mass spectral data in Table II, the accurate mass measurement data and the B/E linked scanning data. Subsequent loss of the protected C-11 carboxylic chain unit (*N*-*n*-propylcarbamoyl group) from the ion of

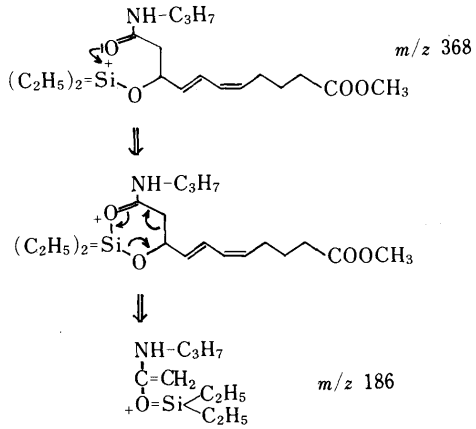


Chart 3

m/z 369 gave the ion at m/z 268. The ion at m/z 228 was produced from the above ion at m/z 369 by a migration of the hydrogen atom at C-4 to C-8 followed by the fission of the C(7)–C(8) bond. In addition, migration of a hydrogen atom from C-10 to C-8 gave rise to the prominent ion at m/z 214. The inferred fragmentation mechanisms for these ions are illustrated in Chart 2.

The labelling data in Table II indicated that the ion at m/z 186 retained one silicon atom and the protected carboxylic chain; the elemental composition of this ion was confirmed to be $C_9H_{20}N_1O_1Si_1$. This ion was considered to

be produced by rearrangement of the ion at *m/z* 368 *via* a six-membered-ring transition state with elimination of the C(1)/C(9) fragment, as shown in Chart 3. This interesting fragment ion may also be formed from the ions at *m/z* 296 and 228 by the same fragmentation process. The B^2/E linked scanning data obtained by focusing of *m/z* 186 revealed that the ions at 296, 268, and 214 were major precursors, with minor contributions from the ions at *m/z* 282, 240 and 228. These silicon atom-containing ions appear to be characteristic of the protected C-11 carboxylic acid moiety which is resulted from a lactone-ring opening reaction with *n*-propylamine.

Comparison with the Corresponding Thromboxane B_2 Derivative The DEHS-DES derivative of TXB_2 ME-methyloxime[9] has the same structure as the corresponding derivative of 11-dehydro-TXB_2 ME-PA, except for the side chain unit C(10)/C(11). The mass spectrum of the 11-dehydro-TXB_2 derivative was considerably simpler than those of the structural isomers of the TXB_2 derivative. Many of the fragmentation products discussed above were also observed with the expected shifts as characteristic common ions of the TXB_2 derivative, but the mass spectrum of the 11-dehydro-TXB_2 derivative lacks the ions formed by elimination of the protected side chain unit and related fragmentations.

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