Dimethylisopropylsilyl Ether Derivative in Gas Chromatography/Mass Spectrometry of 11-Dehydrothromboxane B_2. II. Fragmentation of Methyl Ester-11-*n*-propylamide-dimethylisopropylsilyl Ether Derivative

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The mass spectrum exhibited a series of ions characteristic of the expected 11-dehydrothromboxane B_2 (11-dehydro-TXB_2) derivative. Many of the fragmentation products could be explained in terms of simple bond fission mechanisms. A further series of fragmentations proceeded *via* a six-membered ring transition state or by formation of the cyclic dimethylsilylene ring system. These produced ions in the low mass region containing silicon atoms, characteristic of the protected C-11 carboxylic acid moiety. The appearance of these ions may be used for the identification of 11-dehydro-TXB_2 and its metabolites having a lactone ring. Six homologue and analogue derivatives were prepared for investigation of the fragmentation mechanism. The proposed fragmentation mechanisms have been supported by accurate mass measurement and mass analyzed ion kinetic energy mass spectrometry.

Keywords 11-dehydrothromboxane B_2; dimethylisopropylsilyl ether; GC/MS; mass spectrum; mass fragmentation

11-Dehydrothromboxane B_2 (11-dehydro-TXB_2) is one of the major enzymatic metabolites of thromboxane B_2 (TXB_2). It has recently been considered to be a more reliable index of thromboxane A_2 biosynthesis than TXB_2.[1,2]

11-Dehydro-TXB_2 can occur in a lactone form and a lactone ring-opened (acyclic) form. Gas chromatography (GC) of the methyl ester (ME)-trimethylsilyl (TMS) ether derivative of 11-dehydro-TXB_2, obtained by reaction with diazomethane followed by trimethylchlorosilane–hexamethyldisilazane–pyridine cocktail, revealed the formation of two different derivatives, the expected lactone form and the lactone ring-opened form of methyl 8-[2-methoxycarbonyl-1-(trimethylsilyloxy)ethyl]-9,12-bis-trimethylsilyloxy-5,10-heptadecadienoate.[3] It is essential for microanalysis of 11-dehydro-TXB_2 to keep the derivative in one form during sample preparation. To achieve this, the lactone ring opening reaction by alkylamine was adopted to obtain a chemically stable derivative. The 11-dehydro-TXB_2ME-11-*n*-propylamide (PA)-9,11,15-tris-dimethylisopropylsilyl (DMIPS) ether derivative [methyl 8-(1-dimethylisopropylsilyloxy-2-*N*-*n*-propylcarbamoyl)ethyl-9,12-dimethylisopropylsilyloxy-5,10-heptadecadienoate] was prepared and used for analysis of 11-dehydro-TXB_2 in human urine.[4] The mass spectrum of this 11-dehydro-TXB_2 derivative showed a more complex fragmentation pattern than that of the 11-dehydro-TXB_2ME-9,15-bis-DMIPS ether derivative.[5] Characteristic fragment ions found in the low mass region were produced by migration of the DMIPS group to the carbonyl-oxygen at C-11 *via* a six-membered transition state to give cyclic dimethylsilylene (DMS) ring formation, followed by fission of the DMS ring system. This paper deals with the study of the fragmentation of this 11-dehydro-TXB_2ME-PA-DMIPS ether derivative by isotope labeling experiments in conjunction with mass analyzed ion kinetic energy spectrometry (MIKES) and accurate mass measurement. The mass spectra of homologue derivatives were also examined for comparison.

Experimental

Gas Chromatography/Mass Spectrometry (GC/MS) A VG ZAB-HF mass spectrometer (VG Analytical Ltd., Manchester, U.K.) interfaced to a Shimadzu GC-9A gas chromatograph (Shimadzu Co., Kyoto, Japan) with a solventless injector and a DS-2035 data processing system (VG Analytical Co.) and a VG 70SE mass spectrometer (VG Analytical Ltd.) interfaced to an HP-5890A gas chromatograph (Hewlett Packard Ltd., PA, U.S.A.) with a solventless injector and a VG 11-250J+data processing system (VG Analytical Ltd.) were employed. The column was a 12 m × 0.20 mm fused silica capillary column cross-linked with 5%-phenylmethylsilicone (Ultra 2, Hewlett Packard Ltd.). The temperature of the column oven was maintained at 250—290 °C. The carrier gas was helium with a linear velocity of about 30 cm/s. The temperature of the injection port and the transfer line was kept at 290 °C and that of the ion source at 250 °C. The ionization energy and the trap current were 70 eV and 200 μA, respectively. The accelerating voltage was 8 kV. The mass spectrum of each of the 11-dehydro-TXB_2 derivatives was recorded by repeated scanning (2 s/decade) over the mass range of m/z 900—90 (cycle time: about 3 s) with a dynamic resolution of 2000. High resolution (HR)-MS was carried out under the same GC/MS conditions with a dynamic resolution of 5000. The MIKE spectrum was recorded by data system control of the electric field in the range of $E/E_0=0$—1.10 with a scan cycle time of about 3 s/decade (this condition was modified as necessary for particular purposes). For the collision-induced decomposition (CID)-MIKES helium gas was introduced into the collision cell and the analyzer tube pressure at the 2nd FFR was adjusted to 6×10^{-7} m-bar.

GC/High Resolution-Selected Ion Monitoring (GC/HR-SIM) GC/HR-SIM was performed using a VG 70SE GC/MS system under the same conditions as used in GC/MS with a dynamic resolution of 25000.

Samples and Reagents 11-Dehydro-TXB_2 was obtained from Cayman Chemical Co., Inc. (MI, U.S.A.). Oxygen-18 labeled 11-dehydro-TXB_2 was prepared by exchange of 1- and 11- carbonyl oxygen in 0.1 N LiOH in 98% oxygen-18 enriched water.[6] After one cycle of esterification–hydrolysis, the oxygen-18 content was checked by GC/MS. This revealed the reaction product to be a mixture of $^{18}O_1$- and $^{18}O_2$-labeled analogues (51% and 45% respectively).

n-Propylamine, *n*-butylamine, tetramethyldisilazane (TMDS), trimethylsilyl imidazole (TMSI), dimethylethylsilyl imidazole (DMESI), dimethyl-*n*-propylsilyl imidazole (DMNPSI), and dimethylisopropylsilyl imidazole (DMIPSI) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and tetradeuterium labeled methanol, and oxygen-18 labeled water from Merck Co., Ltd. (Darmstadt, West Germany). Diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide.

Derivatization The ME of 11-dehydro-TXB_2 was prepared by treatment with diazomethane, and the trideuteromethyl ester was obtained by esterification with tetradeuterium labeled methanol and hydrogen chloride-diethyl ether.

n-Propylamide and *n*-butylamide derivatives of 11-dehydro-TXB_2ME were obtained by treating the corresponding 11-dehydro-TXB_2ME with *n*-propylamine or *n*-butylamine at room temperature for 3 h followed by evaporation of the excess of alkylamine under reduced pressure.

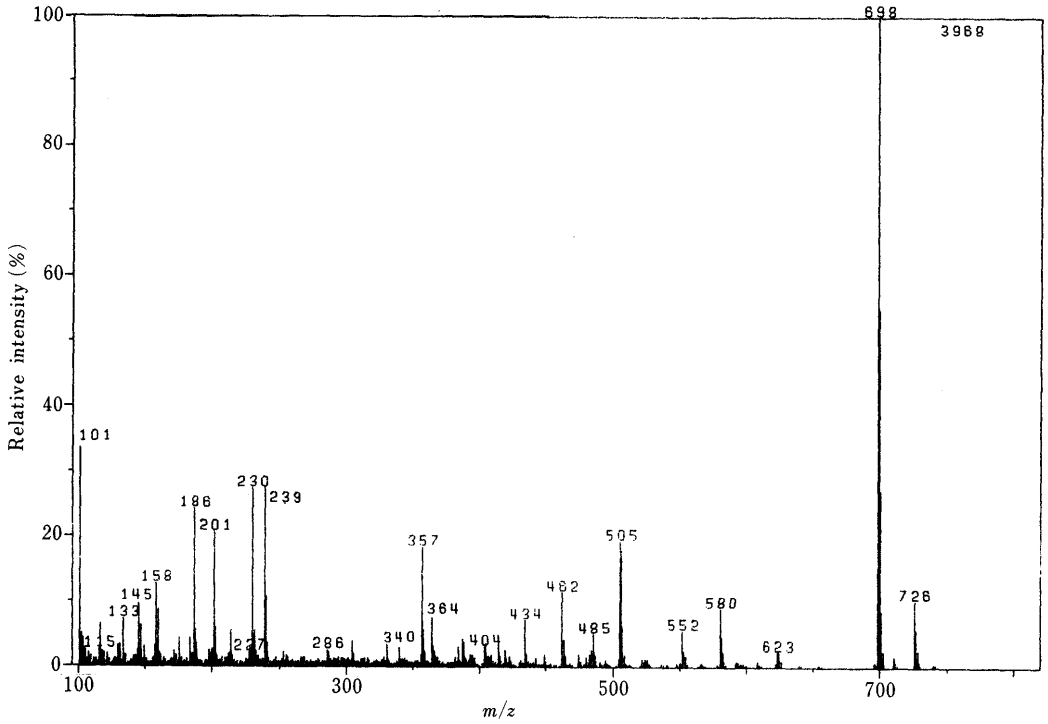
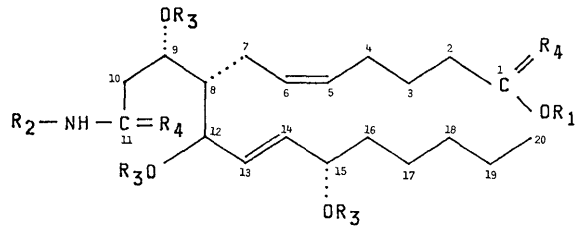


Fig. 1. Mass Spectrum of the 11-Dehydrothromboxane B_2 Methyl Ester-*n*-propylamide-9,11,15-tris-DMIPS Ether Derivative

TABLE I. Structures of 11-Dehydrothromboxane B_2 Derivatives



Derivative	R_1	R_2	R_3	R_4
I	CH_3	*n*-C_3H_7	DMIPS	^{16}O
II	CH_3	*n*-C_3H_7	TMS	^{16}O
III	CH_3	*n*-C_3H_7	DMES	^{16}O
IV	CH_3	*n*-C_3H_7	DMNPS	^{16}O
V	C^2H_3	*n*-C_3H_7	DMIPS	^{16}O
VI	CH_3	*n*-C_4H_9	DMIPS	^{16}O
VII	CH_3	*n*-C_3H_7	DMIPS	^{18}O

TMS, trimethylsilyl; DMES, dimethylethylsilyl; DMNPS, dimethyl-*n*-propylsilyl; DMIPS, dimethylisopropylsilyl.

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

The structures of 11-dehydro-TXB_2 derivatives obtained by the above procedures are shown in Table I, and were used to elucidate the fragmentation mechanisms of the 11-dehydro-TXB_2ME-PA-DMIPS derivative.

Results and Discussion

The mass spectrum of the 11-dehydro-TXB_2ME-PA-DMIPS ether derivative is shown in Fig. 1. The spectrum exhibited a series of ions characteristic of the expected 11-dehydro-TXB_2ME-PA-DMIPS ether derivative. The molecular ion was not observed whereas the ion of $[M-15]^+$

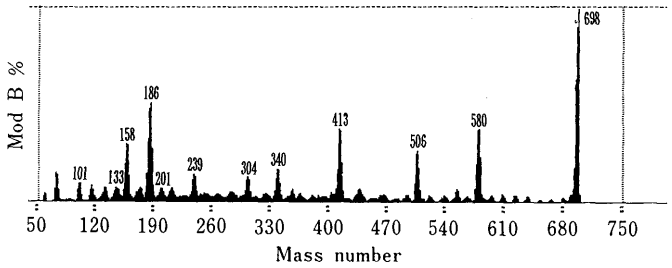


Fig. 2. CID-MIKE Spectrum of the Base Peak Ion at *m/z* 698

at *m/z* 726 was 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* 698 as the base peak. The appearance of these ions in the spectrum, typical of DMIPS ether derivatives, with absence of the molecular ion indicates the incorporation of three DMIPS groups into the 11-dehydro-TXB_2ME-PA derivative. The CID-MIKE spectrum of the base peak ion is shown in Fig. 2. The ions at *m/z* 580, 506, 340, 239, 201, 186, 175, 158, 145, 133, and 101 were identified as fragmentation products of the base peak ion, and these were structurally diagnostic of the fragmentation. The abundant fragmentation products in the low mass region were silicon atom-containing ions. These may have been produced *via* a six-membered ring transition state or by the formation of the DMS ring system, whereas formation of many of the ions could be explained in terms of simple bond fission mechanisms.

The structures of the characteristic fragment ions and their elemental compositions are listed in Table II. The results on the corresponding TMS, DMES and DMNPS ether derivatives, *n*-butylamide derivative, and deuterium- and oxygen-18 labeled derivatives are also summarized in Table II. The mass spectral fragmentation patterns were closely related, and most of the characteristic ions in the

TABLE II. Shift of Ions in Homologue and Analogue Derivatives of the 11-Dehydrothromboxane B_2 Methyl Ester-11-*n*-propylamine-9,12,15-tris-DMIPS Ether Derivative

m/z	Fragmentation		Elemental composition					Related derivatives (*m/z*)					
			C	H	N	O	Si	II	III	IV	V	VI	VII
741	$[M]^{+\cdot}$		39	79	1	6	3	—	—	—	—	—	—
726	$[M-15]^+$	$M-CH_3$	38	76	1	6	3	642	684	726	729	740	728, 730
710	$[M-31]^+$	$M-OCH_3$	38	76	1	5	3	626	668	710	710	724	712, 714
698	$[M-43]^+$	$M-C_3H_7$	36	72	1	6	3	642	670	698	701	712	700, 702
623	$[M-118]^{+\cdot}$	M−DMIPSOH	34	65	1	5	2	567	595	623	626	637	625, 627
580	$[M-43-118]^+$	$M-C_3H_7$−DMIPSOH	31	58	1	5	2	552	566	580	583	594	582, 584
552	$[M-71-118]^+$	$M-C_5H_{11}$−DMIPSOH	29	54	1	5	2	496	524	552	555	566	554, 556
506	$[M-43-118-74]^+$	$M-C_3H_7$−DMIPSOH−$(CH_3)_2Si=O$	29	52	1	4	1	—	492	506	509	520	508, 510
505	$[M-118\times2]^{+\cdot}$	M−DMIPSOH×2	29	51	1	4	1	477	491	505	508	519	507, 509
485		C1/C11+DMIPS	25	51	1	4	2	429	457	485	488	499	487, 489
462	$[M-43-118\times2]^+$	$M-C_3H_7$−DMIPSOH×2	26	44	1	4	1	462	462	462	465	476	464, 466
434	$[M-71-118\times2]^+$	$M-C_5H_{11}$−DMIPSOH×2	19	35	1	4	1	406	420	434	437	448	436, 438
388	$[M-43-118\times2-74]^+$	$M-C_3H_7$−DMIPSOH×2−$(CH_3)_2Si=O$	24	38	1	3	0	—	—	—	391	402	390, 392
387	$[M-118\times3]^{+\cdot}$	M−DMIPSOH×3	24	37	1	3	0	387	387	387	390	401	389, 391
364	$[M-118\times2-141]^+$	M−DMIPSOH×2−α-chain	21	38	1	2	1	336	350	364	364	378	364, 366
357		C12/C20	19	41	0	2	2	301	329	357	357	357	357
340		C1/C11−C_3H_7−H	17	30	1	4	1	—	—	—	343	354	—
239	$[357-118]^+$	357−DMIPSOH	14	27	0	1	1	211	225	239	239	239	239
230		C9/C11: DMIPSO=CH–CH_2–CONH–C_3H_7	11	24	1	2	1	202	216	230	230	244	230, 232
201		C15/C20	11	25	0	1	1	173	187	201	201	201	201
201		DMIPSO–C(=CH_2)–NH–C_3H_7	10	23	1	1	1	173	187	201	201	215	201, 203
186		CH_3–(C_3H_7–)Si=O–C(=CH_2)–NH–C_3H_7	9	20	1	1	1	158	172	186	186	200	186, 188
186		$(CH_3)_2Si$=O–CH=CH–CONH–C_3H_7	8	16	1	2	1	186	186	186	186	200	186, 188
175		$(CH_3)_2Si$=O–DMIPS	7	19	0	1	2	147	161	175	175	175	175
158	$[201-43]^+$	$(CH_3)_2Si$=O–C(=CH_2)–NH–C_3H_7	7	16	1	1	1	158	158	158	158	172	158, 160
145	$[201-56]^+$	DMIPS–OH–CH=CH_2	7	17	0	1	1	117	131	145	145	145	145
133	$[175-42]^+$	$(CH_3)_2Si$=O–SiH(=$(CH_3)_2$)	4	13	0	1	2	—	133	133	133	133	133
101		DMIPS	5	13	0	0	1	—	—	101	101	101	101

spectra had the expected mass shift due to labeling of the related derivatives, II to VII.

Fragmentation by Simple Bond Fission Successive losses of the dimethylisopropylsilanol molecule (DMIPSOH: 118 amu) gave rise to the ions at *m/z* 623, 505 and 387 (nucleic fragment ion) from the molecular ion and ions at *m/z* 580 and 462 from the $[M-43]^+$ ion, respectively. The ions at *m/z* 552 and 434 were formed by elimination of the C(16)/C(20) hydrocarbon fragment (71 amu, typical of the prostanoid one and two series) from the ions at *m/z* 623 and 505. The ion at *m/z* 506, accompanied with that at *m/z* 505 was considered to arise by loss of a dimethylsilicoxide group (74 amu) from the ion at *m/z* 580. Similar fragmentation was observed for the formation of the ion at *m/z* 624 from 698 and that at *m/z* 388 from 462. Cleavage of the C(8)–C(12) bond in the molecule resulted in a characteristic ion at *m/z* 357 and this ion further fragmented to form the ion at *m/z* 239 by loss of a DMIPSOH molecule. The structures of these two ions were identical to those determined in the tris-DMIPS ether derivative of TXB_2ME-methyloxime.[7] A similar series of ions was observed in the mass spectrum of the *n*-butylamide derivative with the exception of a reasonable shift of 14 mass units originating from substitution of a butyl group at the carbamoyl moiety. The observation of the fragmentation products formed by a simple bond fission mechanism is sufficient to confirm formation of the expected derivative.

1. The Ion at *m/z* 364 This ion was observed as one of the daughters of that at *m/z* 505. It remained as it was in the mass spectrum of V with no shift caused by the introduction of the trideuteromethyl ester. These observations suggested that the ion at *m/z* 505 produced this ion by loss of the α-chain fragment of C(1)/C(7) (141 amu).

2. The Ion at *m/z* 230 The C(8)–C(9) bond is weak by virtue of being α to the dimethylisopropylsilyloxy group. This leads to a prediction that the formation of this ion is due to cleavage of the C(8)–C(9) bond, which corresponded to that at *m/z* 202 in the spectrum of the above TXB_2 derivative.[7] This ion structure was supported by the experimental results in Table II and accurate mass measurement.

3. The Ions at *m/z* 145 and 101 The ion at *m/z* 230 further fragmented to give one at *m/z* 145 by loss of an isopropylcyanate molecule initiated with migration of a hydrogen atom from the nitrogen to oxygen at the C-9 dimethylisopropylsilyloxy moiety. The ion at *m/z* 101 was produced from those at *m/z* 230 and 145 by heterolytic Si–O bond cleavage. These fragmentation pathways were supported by the presence of both of the *m/z* 145 and 101 peaks in the CID-MIKE spectrum of *m/z* 230. Additionally, the CID-MIKE spectral studies revealed that the ions at *m/z* 201, 485, 505 and 698 were also precursors of this ion.

Fragmentation Initiated by Migration of DMIPS Group

1. The Ion at *m/z* 485 This ion retained one nitrogen atom, C-11 carbonyl-oxygen atom, two silicon atoms and the methyloxycarbonyl group (Table II), the elemental composition $C_{25}H_{51}NO_4Si_2$ being determined by accurate mass measurement. It is suggested that this ion is produced from the molecular ion by migration of the DMIPS group at C-12 to the carbonyl-oxygen atom in the protected C-11 carboxylic acid moiety followed by loss of the C(12)/C(20)

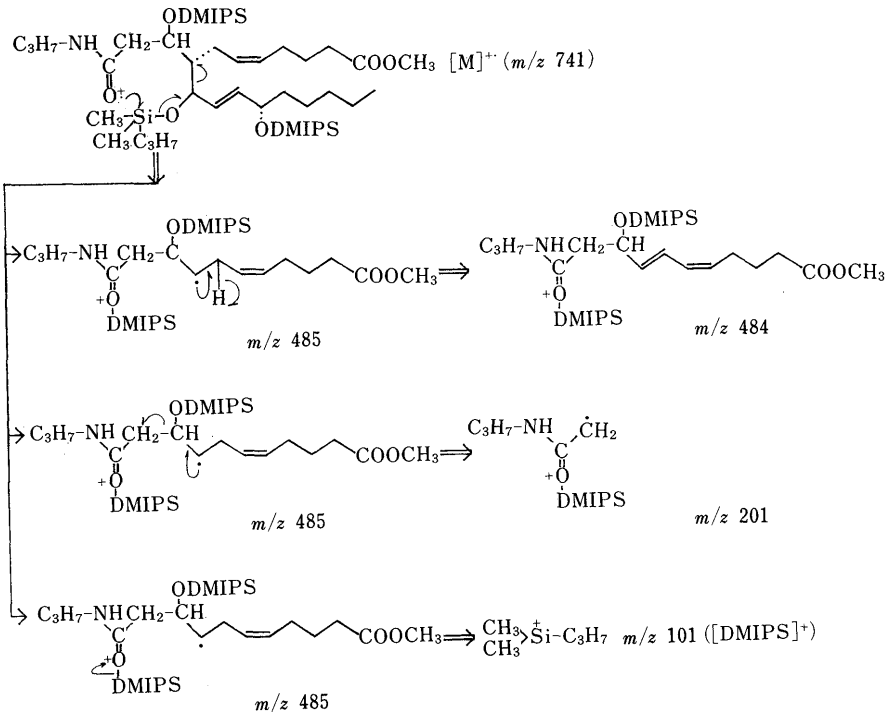


Chart 1

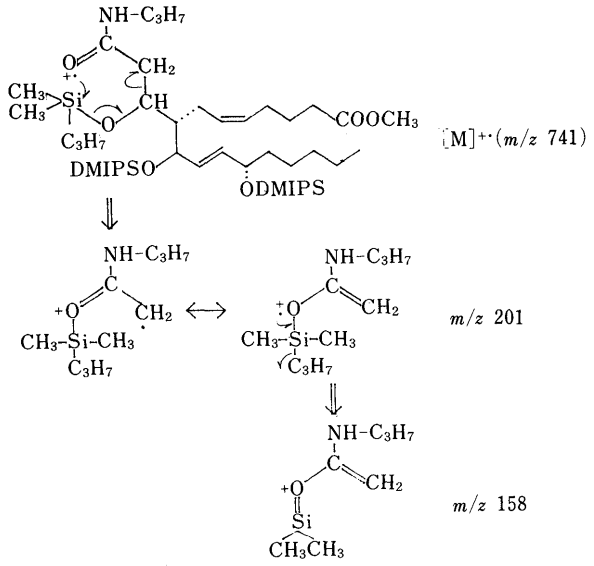


Chart 2

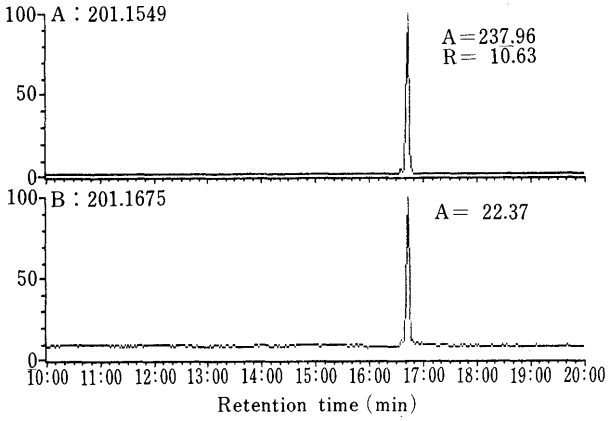


Fig. 3. High Resolution-Selected Ion Recordings of the 11-Dehydrothromboxane B_2 Methyl Ester-11-*n*-propylamide-9,11,15-tris-DMIPS Ether Derivative Obtained by Monitoring the Ions at m/z 201.1549 and 201.1675 at a Resolution of 25000 over GC Retention Times of 10—20 min

fragment. The proposed fragmentation mechanism for formation of this ion is illustrated in Chart 1.

2. The Ion at *m/z* 201 A similar fragmentation with migration of the DMIPS group was found in the formation of the ion at m/z 201. This ion shifted to m/z 215 in the spectrum of the corresponding *n*-butylamide derivative, indicating that this ion has the protected C-11 carboxylic acid moiety. Thus, it was apparent that this ion had not originated from the C(15)/C(20) fragment (a characteristic of the prostanoid one and two series) but had been produced by migration of the DMIPS group at the C-9 to the C-11 carbonyl-oxygen atom followed by fission of the C(9)–C(10) bond, as shown in Chart 2. This fragmentation process was supported by the accurate mass measurement (elemental composition: $C_{10}H_{23}NOSi$, calculated mass: 201.1549, found: 201.1542). This ion may also be derived from the ion at m/z 485 by Chart 1. These were confirmed by the fact that the ion at m/z 485 and the one at m/z 698 were identified as precursors of that at m/z 201. In addition, loss of an isopropyl radical at the DMIPS group from this ion gave rise to the ion at m/z 158, whose elemental composition $C_7H_{16}NOSi$ agreed well with the observed mass within experimental error. Loss of a methyl radical instead of an isopropyl radical could produce the ion at m/z 186. These suggestions are supported by the presence of the m/z 158 and 186 peaks in the CID-MIKE spectrum of m/z 201.

GC/HR-SIM was carried out in order to clarify the contribution of the C(15)/C(20) fragment ion (elemental composition: $C_{11}H_{25}OSi$, calculated mass: 201.1675) to the ion at m/z 201 above. Figure 3 shows the HR-SIM result of

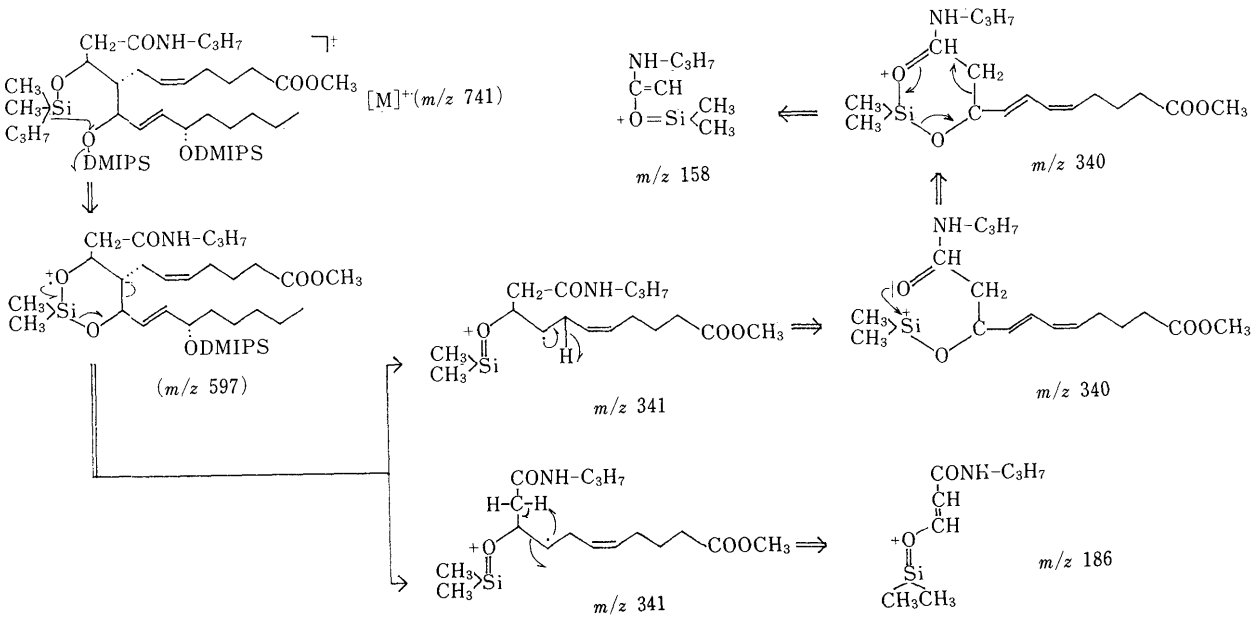


Chart 3

the 11-dehydro-TXB_2ME-PA-DMIPS ether derivative obtained by monitoring these two ions with a resolution of 25000 (10% valley, resolution of more than 16000 was necessary to discriminate these two ions). The peak area ratio of the ions at *m/z* 201.1549 and 201.1675 was approximately 10 : 1.

Fragmentation Initiated by Formation of the Cyclic Dimethylsilylene Ring 1. The Ions at *m/z* 341 and 340 It is suggested that the molecular ion may fragment to produce a hypothetical and unidentified ion having a six-membered 9,12-DMS ring system by loss of an isopropyl radical at the DMIPS group and the other DMIPS group itself, although the corresponding ion is not present in any of the mass spectra of the 11-dehydro-TXB_2 homologue derivatives, II to IV. This hypothetical and unidentified ion could yield an intermediate ion at *m/z* 341 with very low intensity by cleavage of the C(8)–C(12) bond initiated with fission of the DMS ring system, followed by loss of the C(12)/C(20) fragment (β-side chain). A proposed fragmentation is depicted in Chart 3. This intermediate ion is considered to correspond to that at *m/z* 355 in the spectrum of the ME-ethyloxime-15-diethylhydrogensilyl (DEHS)-9,12-cyclic diethylsilylene (DES) derivative of TXB_2[8] and to *m/z* 369 in the mass spectrum of the ME-PA-15-DEHS-9,12-DES derivative of 11-dehydro-TXB_2.[9] Expulsion of a hydrogen atom at C-8 from this ion resulted in the ion at *m/z* 340, which was supported by appearance of the corresponding ion as a minor daughter of the proposed intermediate ion. The ion at *m/z* 268 was identified as a major daughter of this intermediate ion (Fig. 4-A), which, however, could not be observed in the normal spectrum. The ion at *m/z* 340 resulted in an ion at *m/z* 158 by loss of an α-chain fragment initiated with re-formation of the DMS ring system followed by fission of it, as also shown in Chart 3. This was supported by the appearance of a metastable peak, corresponding to the transition ions from *m/z* 340 to 158 (Fig. 4-B).

The 11-dehydro-TXB_2ME-PA-15-dimethylhydrogensilyl(DMHS)-9,12-DMS derivative, the homologue of the above ME-PA-DEHS-DES derivative,[9] was prepared for confirmation of the fragmentation mechanism of the proposed intermediate ion by treating 11-dehydro-TXB_2 ME-PA with TMDS in pyridine, because the structure of this derivative was considered to be identical to that of the hypothetical and unidentified precursor of the intermediate ion. This compound exhibited a series of ions characteristic of the expected derivative (the mass spectrum is shown in Fig. 5). The spectrum was dominated by the base peak ion at *m/z* 340 and gave ions at *m/z* 186, 175, 158, and 133 in the low mass region, which were observed as the characteristic common to the 11-dehydro-TXB_2 ME-PA-DMIPS ether derivative. As can be seen in Fig. 6, the CID-MIKE spectrum of the ion at *m/z* 341 exhibited daughter ions to *m/z* 268, 240, 212, 186, and 158, and in turn, that at *m/z* 340 gave a major ion at *m/z* 158. In a comparison of these CID-MIKE spectra with those in Fig. 4, a major difference in the low mass region is that the

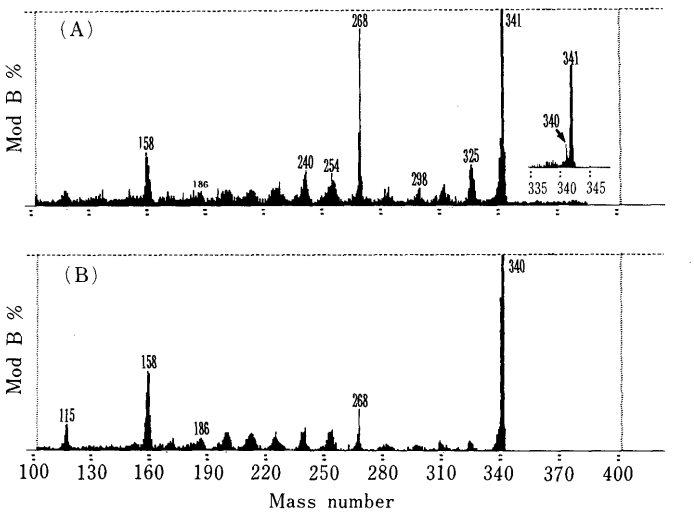


Fig. 4. CID-MIKE Spectra of the Ions at *m/z* 341 (A) and 340 (B)

The partial spectrum inserted in the top is expanded to show the presence of the daughter of *m/z* 340.

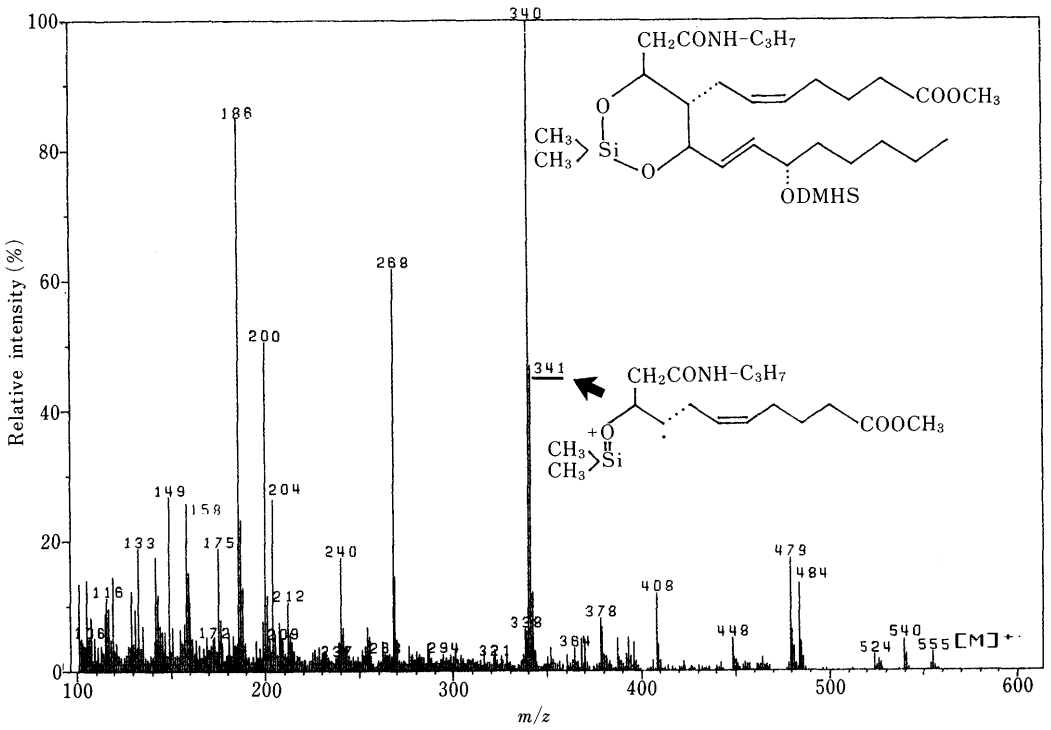


Fig. 5. Mass Spectrum of the 11-Dehydrothromboxane B_2 Methyl Ester-11-*n*-propylamide-15-dimethylhydrogensilyl-9,12-cyclicdimethylsilylene Derivative

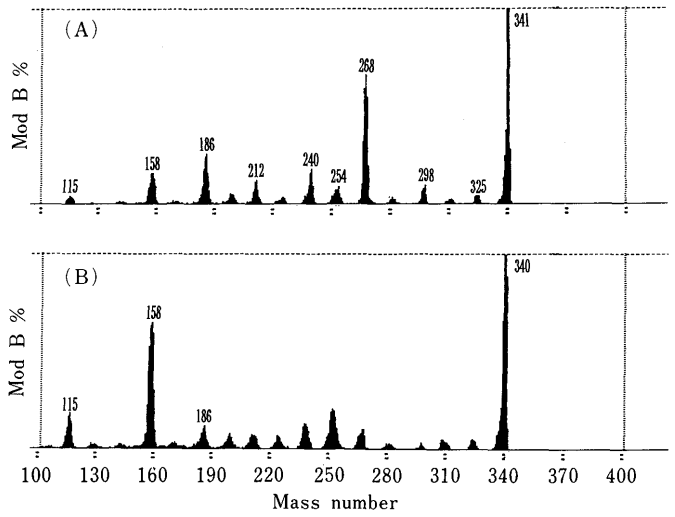


Fig. 6. CID-MIKE Spectra of the Ions at *m/z* 341 (A) and 340 (B) Produced from 11-Dehydrothromboxane B_2 Methyl Ester-11-*n*-propylamide-15-dimethylhydrogensilyl-9,12-cyclicdimethylsilylene Derivative

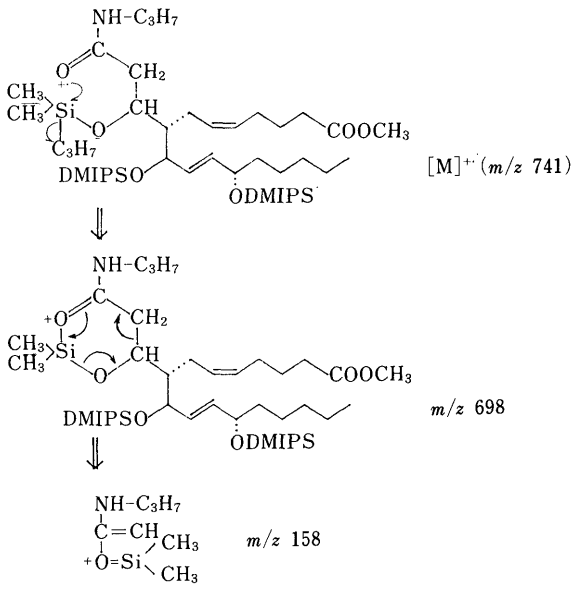


Chart 4

intensity of the *m/z* 186 peak, the transition ions from *m/z* 341 to 186, relative to the *m/z* 158 peak from the derivative of I (Fig. 4-A) is much lower than from the ME-PA-DMHS-DMS derivative (Fig. 6-A). However, it should be emphasized that the CID-MIKE spectra of the respective ions at *m/z* 341 and 340 are in substantial agreement with each other and could be used for our purpose. This implies that the respective ions have identical structure. Accordingly, this does not conflict with our proposal for the intervention of DMS ring formation in the case of the 11-dehydro-TXB_2ME-PA-DMIPS ether derivative.

2. The Ion at *m/z* 186 This ion was observed as a daughter of that at *m/z* 341 in the CID-MIKE spectrum of the above model compound, the 11-dehydro-TXB_2ME-PA-DMHS-DMS derivative, as already mentioned. This ion has one nitrogen atom and the C-11 carbonyl-oxygen atom (Table II). Thus, it was considered that the intermediate ion further fragmented to give the ion at *m/z* 186 by cleavage of the C(8)–C(9) bond with hydrogen abstraction. One of the fragmentation mechanisms is also shown in Chart 3. As mentioned above, the ion at *m/z* 201 could, by loss of a methyl radical, produce the ion at *m/z* 186, which shifted to *m/z* 172 in the corresponding DMES ether derivative. This indicates formation of another ionic species

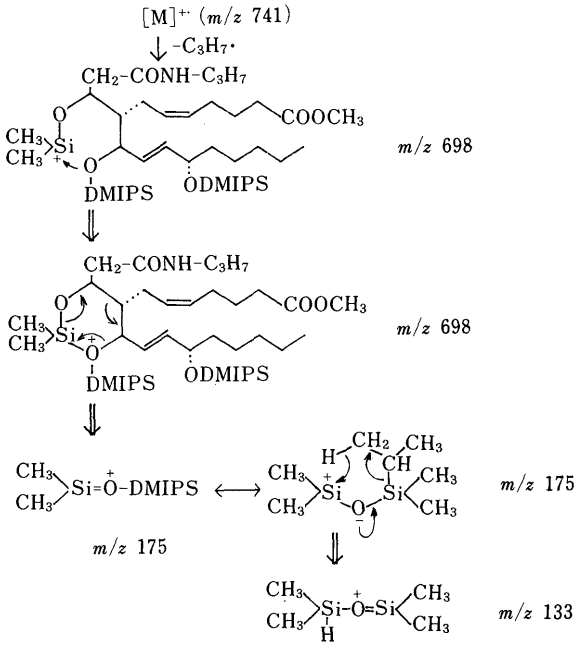


Chart 5

of *m/z* 186 with a different elemental composition from that proposed in Chart 3.

3. The Ion at *m/z* 158 An alternative DMS ring formation with the loss of an isopropyl radical from the molecular ion is also possible. This case includes bond formation between the silicon atom of the C-9 DMIPS group and the C-11 carbonyl-oxygen atom in the protected carboxylic acid moiety. Fission of this DMS ring system followed by expulsion of α- and β-chains (Chart 4), yields the ion at *m/z* 158 having the same elemental composition as the ion proposed for *m/z* 158 in Chart 2 as well as in Chart 3. CID-MIKE spectral studies indicated that the ions at *m/z* 201, 230, 340, 341, 462, 505, 580, and 698 were also precursors of this ion. All of these ions except for the one at *m/z* 201 are considered to produce the ion at *m/z* 158 through a similar six-membered ring transition state.

4. The Ions at *m/z* 175 and 133 A cyclic oxonium ion involving the DMS ring system may also be considered as one of the possible ion structures of the base peak ion. This could fragment to form the ion at *m/z* 175 by fission of the DMS ring followed by loss of the 11-dehydro-TXB_2 moiety by an analogous fragmentation process. The resulting ion further fragments to produce *m/z* 133 by loss of an isopropyl radical with migration of a hydrogen atom in a six-membered transition state. The TMS ether derivative lacks the corresponding ion. This indirectly supports the view that the migration of the hydrogen atom is associated with the formation of the ion at m/z 133 from 175. An inferred fragmentation mechanism is shown in Chart 5.

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