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Positive ion EI mass spectra of 2,3,5-trisubstituted-1,5-benzothiazepines, diltiazem, clentiazem and their fat-soluble metabolites

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Abstract: Diltiazem, clentiazem and their acidic and basic metabolites extractable with *tert*-butyl methyl ether, which are all 2,3,5-trisubstituted-1,5-benzothiazepines, were analysed by positive ion electron ionization (PIEI) mass spectrometry, and the structures of fragment ions and their fragmentation pathways were investigated. In all the mass spectra of these compounds, **b1** ions ($[R^1—CH=CH—OR^2]^{+\cdot}$) or **b2** ions ($[R^1—CH=CH—OH]^{+\cdot}$, **b1** $—CH_2CO$; $R^2 = COCH_3$) were seen as the base peaks or one of the major peaks (R^1 and OR^2 are the substituents at position 2 and 3, respectively). These spectra varied greatly with the nature of the side-chain at position 5: in the spectra of compounds with a 5-(2-dimethylamino)ethyl group, the **a1** ion, $[CH_2=N(CH_3)_2]^+$, was the base peak ion; in those with a 5-(2-monomethylamino)ethyl group (trifluoroacetyl(TFA)-derivatives), no **a1** ion peak was seen, but the **a3** ion, $[CH_2CH_2NCH_3TFA]^+$, was one of the major peaks; in those of the derivatives bearing a 5-(2-amino)ethyl or -carboxymethyl group, no peaks indicating the structures of the side-chain group at position 5 could be seen. GC–mass chromatography using fragment ions such as the **a1**, **a3**, **b1** and **b2** ions enables detection of the unchanged drug and most of its metabolites obtained by extraction with organic solvents under acidic or basic conditions followed by derivatization.

Keywords: *Positive ion electron ionization MS; mass fragmentation; diltiazem; clentiazem; stable isotope; structure elucidation.*

Introduction

Diltiazem (Dil), (+)-(2*S*,3*S*)-2,3-dihydro-3-acetoxy-2-(4-methoxyphenyl)-5-[2-(dimethylamino)ethyl]-1,5-benzothiazepin-4(5*H*)-one, and clentiazem (Clen, 8-Cl derivative of Dil) are both 2,3,5-trisubstituted-1,5-benzothiazepines which have three common substituents, i.e. 2-(4-methoxy)phenyl, 3-*O*-acetyl and 5-(2-dimethylamino)ethyl groups. Dil and Clen, possessing different substituents only at position 8, exert different pharmacological effects [1, 2]. In the study of drug metabolism, gas chromatography–mass spectrometry (GC–MS) can be used to detect and characterize the metabolites rapidly, since comparison can be made with the mass spectral information provided by the spectra of unchanged drugs. The authors investigated the disposition and metabolism of these drugs [3–9], and employed GC–MS as the most useful technique for detection and structure elucidation of the metabolites, and identified them with synthetic reference compounds by comparing their properties [3, 5–7, 9]. This paper describes the characteristic fragmentation pathways of these drugs and metabolites inferred from the mass spectra taken by GC–MS in the positive ion electron ionization (PIEI) mode by using the stable isotope-labelled analogues and various reagents for derivatization.

Experimental

Chemicals and reagents

The authentic compounds of diltiazem (Dil) and its metabolites, M1, M2, M4, M6, M8, MA, MD and A1–A4, clentiazem (Clen) and its metabolites, MB1–MB8 and MA1–MA4 (Figs 1 and 2), and $(CH_3)_2N(^{13}CH_2)_2Cl{\cdot}HCl$ were all synthesized in Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd. The stable isotope analogues of Dil and Clen labelled with deuterium (D) or ^{13}C atoms (Table 1) were synthesized using the corresponding stable isotope analogues of (2-dimethylamino)ethyl chloride·HCl and the respective benzothiazepines according to the

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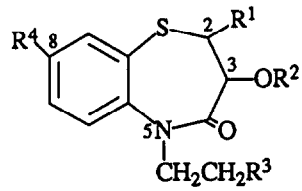


	R1	R2	R3	R4		R1	R2	R3	R4
Dil	X	$COCH_3$	$N(CH_3)_2$	H	Clen	X	$COCH_3$	$N(CH_3)_2$	Cl
M1	X	H	$N(CH_3)_2$	H	MB1	X	H	$N(CH_3)_2$	Cl
M2	X	H	$NHCH_3$	H	MB2	X	$COCH_3$	$NHCH_3$	Cl
M3	Z	H	$N(CH_3)_2$	H	MB3	X	H	$NHCH_3$	Cl
M4	Y	H	$N(CH_3)_2$	H	MB4	Y	H	$N(CH_3)_2$	Cl
M5	Z	H	$NHCH_3$	H	MB5	Y	$COCH_3$	$NHCH_3$	Cl
M6	Y	H	$NHCH_3$	H	MB6	Y	H	$NHCH_3$	Cl
M8	X	H	NH_2	H	MB7	Y	$COCH_3$	$N(CH_3)_2$	Cl
M9	Y	H	NH_2	H	MB8	X	$COCH_3$	NH_2	Cl
MA	X	$COCH_3$	$NHCH_3$	H	MB9	X	H	NH_2	Cl
MB	Y	$COCH_3$	$NHCH_3$	H	MB10	Y	$COCH_3$	NH_2	Cl
MC	Z	$COCH_3$	$NHCH_3$	H	MB11	Y	H	NH_2	Cl
MD	X	$COCH_3$	NH_2	H	MB12	Z	$COCH_3$	$NHCH_3$	Cl
					MB13	Z	H	$N(CH_3)_2$	Cl
					MB14	Z	H	$NHCH_3$	Cl
					MB15	Z	H	NH_2	Cl

X : 4'-methoxyphenyl Y : 4'-hydroxyphenyl

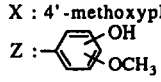
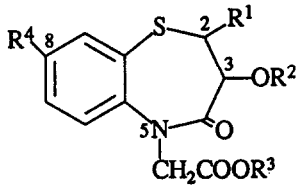


Figure 1
Structures of diltiazem, clentiazem and their basic metabolites. M1–M6, M8, M9, MA–MD: metabolites of diltiazem; MB1–MB15: metabolites of clentiazem.



	R1	R2	R3	R4
A1/MA1	X	$COCH_3$	H	H/Cl
A2/MA2	X	H	H	H/Cl
A3/MA3	Y	$COCH_3$	H	H/Cl
A4/MA4	Y	H	H	H/Cl
A5/MA5	Z	$COCH_3$	H	H/Cl
A6/MA6	Z	H	H	H/Cl

X : 4'-methoxyphenyl Y : 4'-hydroxyphenyl

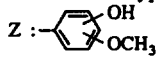


Figure 2
Structures of the acidic metabolites of diltiazem and clentiazem. A1–A6: metabolites of diltiazem; MA1–MA6: metabolites of clentiazem.

synthetic methods of these drugs [2, 10]. The 3-*O*-$COCD_3$ compounds were synthesized using $(CD_3CO)_2O$ (E. Merck, Darmstadt, Germany) and the corresponding 3-*O*-deacetyl compounds. Dimethylethylsilylimidazole (DMESI) was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), *N*-methylbis(trifluoroacetamide) (MBTFA), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG) from Nacalai Tesque, Inc. (Kyoto, Japan). The other chemicals used were of the best grade available.

Sample preparation

The samples for GC–MS were prepared by solvent extraction as follows, and the extracts were concentrated to dryness under reduced pressure.

Urinary metabolites. The urine samples collected after oral administration of diltiazem·HCl or clentiazem maleate to rats (30 mg

Table 1
m/z Values of the molecular ions and the characteristic fragment ions in the MS of diltiazem(Dil), clentiazem(Clen) and their stable isotope analogues

	Dil/Clen-d0	Dil/Clen-E-d4	Dil/Clen-NM-d3	Dil-NM-d6	Dil/Clen-A-d3	Clen-AE-d7	Clen-E-$^{13}C_2$
R1	X	X	X	X	X	X	X
R2	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_3$	$COCD_3$	$COCD_3$	$COCH_3$
R3	$N(CH_3)_2$	$N(CH_3)_2$	NCH_3CD_3	$N(CD_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$
R4	H/Cl	H/Cl	H/Cl	H	H/Cl	Cl	Cl
M^+	414/448	418/452	417/451	420	417/451	455	450
a1	58	60	61	64	58	60	59
a2	71	74	74	77	71	74	73
a3	72	76	75	78	72	76	74
b1	192	192	192	192	195	195	192
b2	150	150	150	150	151	151	150
b3	161	161	161	161	161	161	161
c1	136/170	138/172	136/170	136	136/170	172	171
c2	178/212	182/216	178/212	178	178/212	216	214
d	121	121	121	121	121	121	121

d0, NM-d6, NM-d3, A-d3: 5-$(CH_2)_2R^3$. E-d4, AE-d7: 5-$(CD_2)_2R^3$. E-$^{13}C_2$: 5-$(^{13}CH_2)_2R^3$. X: 4'-methoxyphenyl. As for the mass spectra of Dil-AE-d7, Dil-E$^{13}C_2$ and Clen-NM-d6, see Fig. 3 (bar graphs).

kg^{-1}) and dogs (3 mg kg^{-1}) were adjusted to pH 2 with dil.HCl and extracted with *tert*-butyl methyl ether (tBME). After separation of the tBME layer (the acidic metabolite fraction), the residual aqueous layer was adjusted to pH 9 with dil.ammonia water and re-extracted with tBME to give the basic metabolite fraction.

Synthetic compounds. Since the synthetic compounds were obtained as various salts, they were dissolved in water and extracted with tBME under acidic or basic conditions to obtain them in free form.

Derivatization for GC–MS

The basic compounds dissolved in acetonitrile were made to react with BSTFA or DMESI to obtain *O*-TMS- or *O*-dimethylethylsilyl (DMES)-derivatives, and MBTFA was added for *N*-trifluoroacetyl (TFA) derivatization in the case of compounds having a primary or secondary amino group.

The acidic compounds dissolved in acetonitrile were derivatized with BSTFA or DMESI to trimethylsilylate or dimethylethylsilylate both hydroxyl and carboxyl groups. Otherwise, the acidic compounds, after being dissolved in a small volume of methanol, were made to react with diazomethane or diazoethane (generated from MNNG or ENNG and dissolved in tBME) to obtain their methyl or ethyl esters, and the hydroxyl groups were then silylated.

GC–MS

A Hitachi M-80A Gas chromatograph–mass spectrometer equipped with a Hitachi M-003 data processing system was used to obtain PIEI mass spectra. Durabond-5 Megabore columns (15 or 30 m × 0.53 mm i.d., df = 1.5 μm, J & W Scientific Inc., Folsom, CA, USA) were used for separation of the metabolites and the column oven temperature was programmed at 1°C min^{-1} from 230 to 260°C and then at 4°C min^{-1} from 260 to 290°C for the basic compounds, and at 0.5°C min^{-1} from 245 to 255°C and then at 3°C min^{-1} from 255 to 290°C for the acidic compounds. The temperatures of injection port, interface oven and ion source were 250, 290 and 180°C, respectively. The flow of helium gas was maintained at 15 or 20 ml min^{-1}. The ionization energy, ion accelerating voltage and total emission current were set at 20 eV, 3 kV and 100 μA, respectively.

Results

Dil, Clen and their fat-soluble metabolites bear a 1,5-benzothiazepine ring as a common structural unit and different substituents at position 2, 3, 5 and 8 as shown in Fig. 1 (basic compounds) and Fig. 2 (acidic compounds). The PIEI mass spectra of these drugs and their metabolites were obtained after derivatization such as silylation (TMS or DMES), esterification (CH_3 or C_2H_5) and/or acylation (TFA).

The mass spectra (bar graphs) and *m/z*

values of the characteristic ions together with the substituents of the compounds analysed are shown in Fig. 3 and Table 1 for the unchanged drugs and their stable isotope analogues, in Fig. 4 and Table 2 for some of the basic metabolites, M1, M2, M4, M6, M8, M9, MA, MB, MD, MB1–MB6, MB8, MB9 and MB11, and in Fig. 5 and Table 3 for some of the acidic metabolites, A1–A4 and MA1–MA4.

These mass spectra were analysed and the fragment ions were classified into five groups, i.e., **a** group ions (**a1–a3**) derived from the side-chain at position 5, **b** group ions (**b1–b4**) containing the substituents at position 2 and 3, **c** group ions (**c1–c4**) derived from the benzothiazepine ring carrying the substituent at position 8, tropylium cations (**d** ion) arising from the substituent at position 2 and 2-CH, and **m** group ions (**m1–m3**) formed by elimination of one or two neutral molecules from the molecular ions. The proposed fragmentation patterns and the structures of fragment ions are shown in Figs 6 and 7.

Mass spectra of basic compounds

In the mass spectra of these basic compounds (Figs 3 and 4, Tables 1 and 2) were seen the following chracteristics. The relative

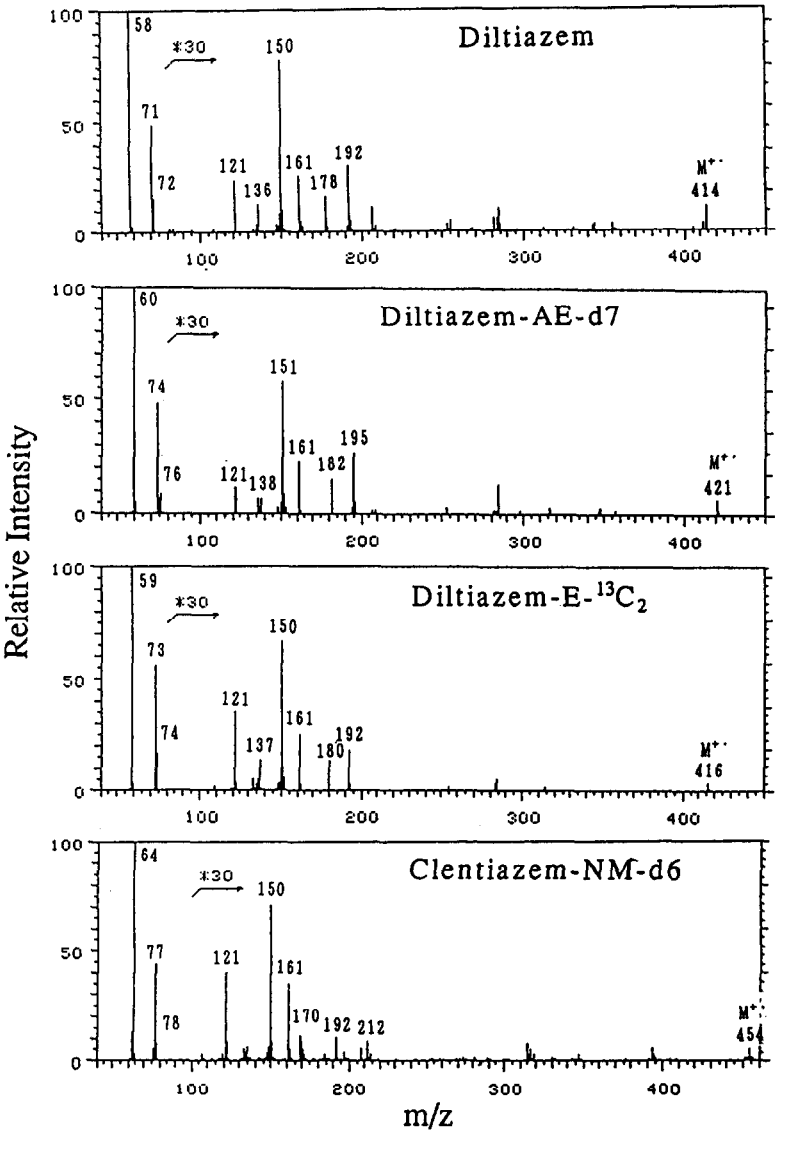


Figure 3
Mass spectra of diltiazem and the stable isotope analogues of diltiazem and clentiazem. AE-d7: 3-$OCOCD_3$, 5-$(CD_2)_2N(CH_3)_2$; E-$^{13}C_2$: 3-$OCOCH_3$, 5-$(^{13}CH_2)_2N(CH_3)_2$; NM-d6: 3-$OCOCH_3$, 5-$(CH_2)_2N(CD_3)_2$.

Table 2
m/z Values of the molecular ions and the characteristic ions in the MS of the derivatives of basic metabolites of diltiazem and clentiazem

	M1(MB1)	MB4(M4)	M2(MB3)	M6/MB6	MB2(MA)	MB(MB5)	MB8(MD)	M8/MB9	M9/MB11
R1	X	Y	X	Y	X	Y	X	X	Y
R2	TMS	TMS	TMS	TMS	$COCH_3$	$COCH_3$	$COCH_3$	TMS	TMS
R3	$N(CH_3)_2$	$N(CH_3)_2$	NCH_3TFA	NCH_3TFA	NCH_3TFA	NCH_3TFA	NHTFA	NHTFA	NHTFA
R4	H(Cl)	Cl(H)	H(Cl)	H/Cl	Cl(H)	H(Cl)	Cl(H)	H/Cl	H/Cl
M^+	444	536	526	584/618	530	554	516	512/546	570/604
a1	58	58	—	—	—	—	—	—	—
a2	71	71	—	—	—	—	—	—	—
a3	72	72	154	154	154	154	—	—	—
b1	222	280	222	280	192	250	192	222	280
b2	—	—	—	—	150	208	150	—	—
c1	136	170	136	136/170	170	136	170	136/170	136/170
c2	178	212	178	178/212	212	178	212	178/212	178/212
d	121	179	121	179	121	179	121	121	179
m2	—	—	436	494/528	470	494	456	422/456	480/514
m3	—	—	408	466/500	442	466	428	394/428	452/486

X: 4′-methoxyphenyl. Y: 4′-TMSO-phenyl.
As for the mass spectra of MB1, M4, MB3, MA, MB5 and MD, see Fig. 4 (bar graphs).

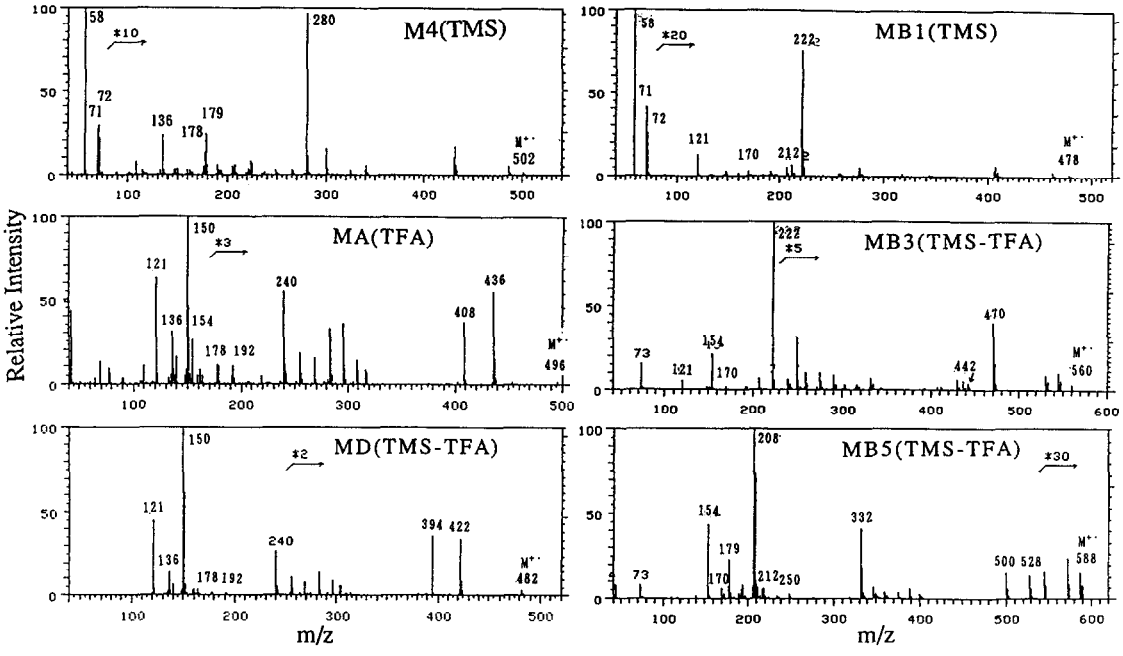


Figure 4
Mass spectra of some basic metabolites of diltiazem and clentiazem. TMS: *O*-trimethylsilyl derivative; TFA: *N*-trifluoroacetyl derivative; TMS-TFA: *O*-trimethylsilyl, *N*-trifluoroacetyl derivative.

Table 3
m/z Values of the molecular ions and the characteristic fragment ions in the MS of the acidic metabolites of diltiazem and clentiazem

	TMS-Derivatives				Me-TMS-Derivatives			
	A1/MA1	A2/MA2	A3(MA3)	A4(MA4)	MA1(A1)	MA2(A2)	A3/MA3	A4/MA3
R1	X	X	Y	Y	X	X	Y	Y
R2	$COCH_3$	TMS	$COCH_3$	TMS	$COCH_3$	TMS	$COCH_3$	TMS
R3	TMS	TMS	TMS	TMS	CH_3	CH_3	CH_3	CH_3
R4	H/Cl	H/Cl	H(Cl)	H(Cl)	Cl(H)	Cl(H)	H/Cl	H/Cl
$M^{+\cdot}$	473/507	503/537	531	561	449	479	473/507	503/537
b1	192	222	250	280	192	222	250	280
b2	150	—	208	—	150	—	208	—
b4	—	249	—	307	—	249	—	307
c1	136/170	136	136	136	170	170	136/170	136/170
c3	266/300	266	266	266	242	242	208/242	208/242
c4	240/274	—	298	—	274	—	298/332	—
d	121	121	179	179	121	121	179	179
m2	413/447	413	471	471	389	389	413/447	413/447
m3	385/419	385	443	443	361	361	385/419	385/419

Me-TMS-Derivative: TMS-derivative of methyl ester. X: 4′-methoxyphenyl. Y: 4′-TMSO-phenyl.
As for the mass spectra of TMS-derivatives of MA3 and MA4, and Me-TMS-derivatives of A1 and A2, see Fig. 5 (bar graphs).

intensities of the $M^{+\cdot}$ peaks were very low, and sometimes could not be observed, especially in the spectra of the compounds bearing the 5-(2-dimethylamino)ethyl group taken with a very small sample volume. However, they could be observed distinctly when a larger sample volume was used. The $M^{+\cdot}$ peaks were suggested by the expected shift of *m/z* values in the spectra of stable isotope analogues and various derivatives of the metabolites. In addition, the $M^{+\cdot}$ peaks were confirmed by observing the peaks of the **m2** ions, as described later, and/or the peaks produced by loss of a methyl or ethyl radical (15 or 29 u) from the TMS or DMES groups of $M^{+\cdot}$ of the respective silylated compounds. The **m2** ions

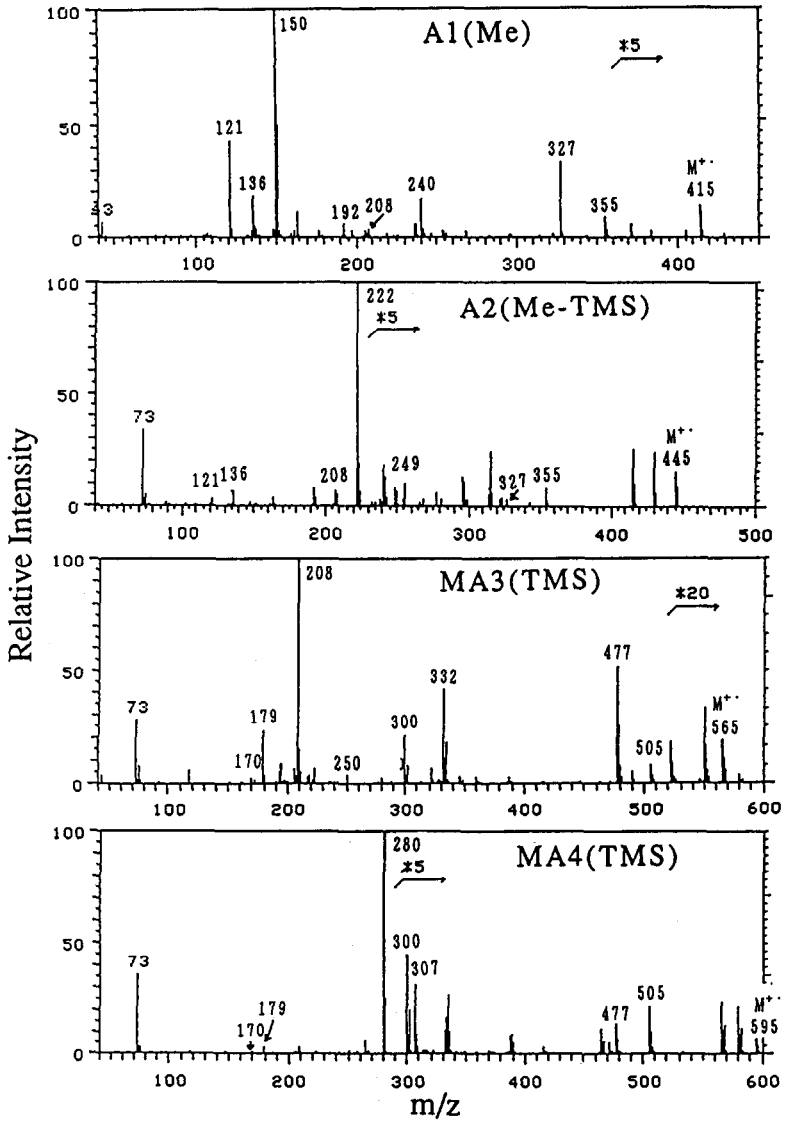


Figure 5
Mass spectra of some acidic metabolites of diltiazem and clentiazem. Me: methyl ester; TMS: *O*-trimethylsilyl derivative; Me-TMS: *O*-trimethylsilyl derivative of methyl ester.

and/or the ions formed by loss of an alkyl radical were observed more clearly in the spectra of the compounds bearing a primary or secondary amino group than in those bearing a tertiary amino group. The base peak ions in the spectra of compounds with a 5-(2-dimethylamino)ethyl group were **a1** ions. On the other hand, with the basic compounds bearing a 5-(2-monomethylamino)ethyl or 5-(2-amino)ethyl group, the base peak ions were **b1** or **b2** ions, which were observed usually as the highest peaks except the **a** group ions with the compounds bearing the 5-(2-dimethylamino)ethyl group.

(1) *Compounds with a 5-(2-dimethylamino)-ethyl group*

Molecular ion and ***m*** *group ions.* The peaks of $M^{+\cdot}$ and the ions in the higher mass region were very small relative to the base peak at *m/z* 58, and the peaks of the **m1**, **m2** and **m3** ions were not clearly observed in most spectra (Figs 3 and 4). The **m1** ions were generated by elimination of the side-chain moiety at position 5 from $M^{+\cdot}$ as a neutral molecule (CH_2=$CHN(CH_3)_2$, 71 u; Scheme 1-2); the **m2** ions by elimination of acetic acid (60 u) or TMS-OH (90 u) (or DMES-OH; 104 u) from $M^{+\cdot}$ of the compounds with 3-*O*-Ac or -*O*-TMS

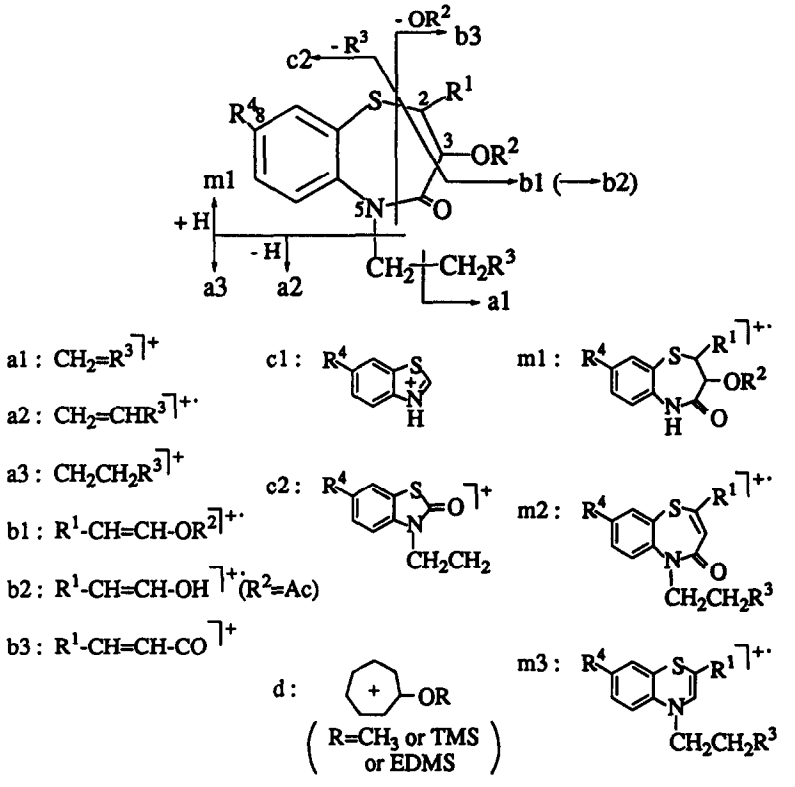


Figure 6
Proposed fragmentations and structures of fragment ions of diltiazem, clentiazem and their basic metabolites in PIEI MS. R^1, R^2, R^3 and R^4 are the same as those in Figs 1 and 2.

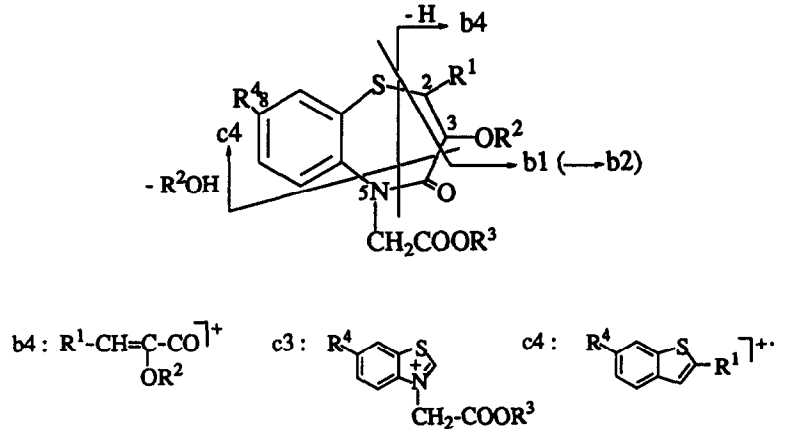
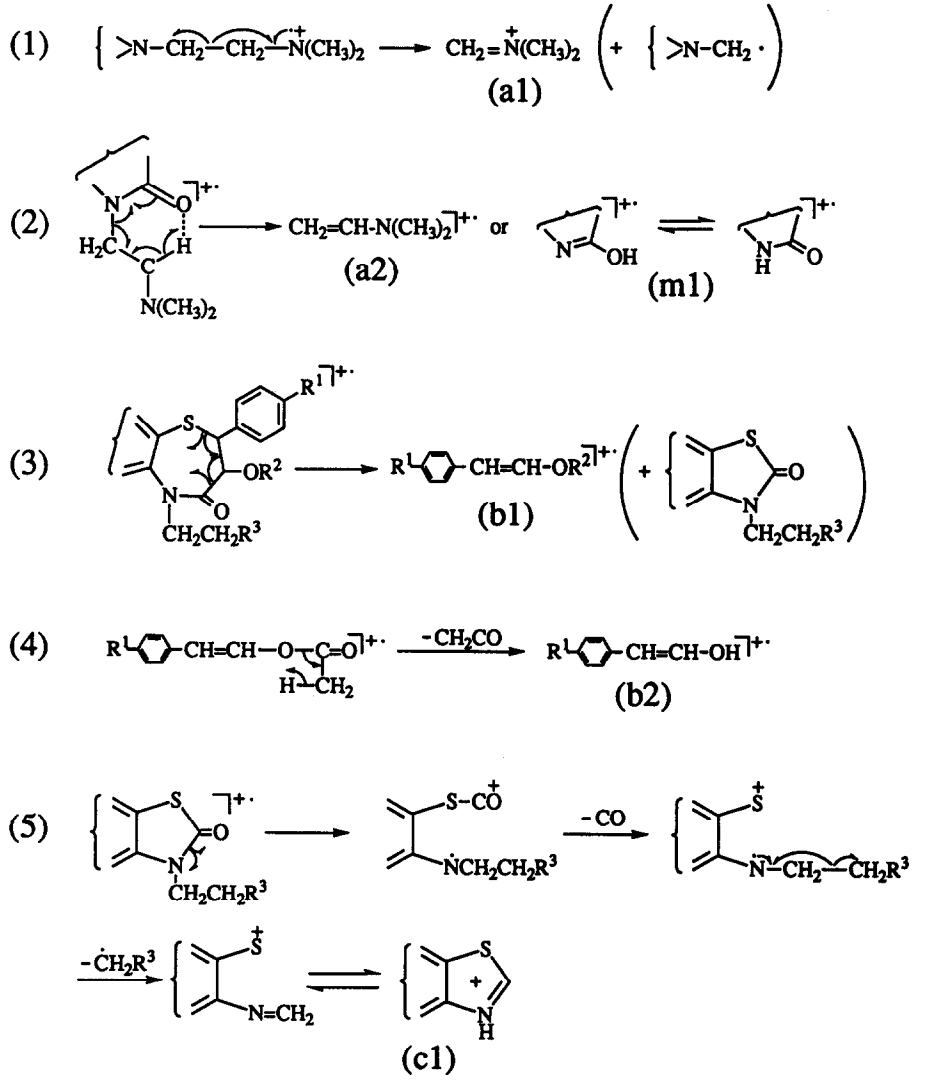


Figure 7
Proposed fragmentations and structures of fragment ions of the acidic metabolites of diltiazem and clentiazem in PIEI MS. R^1, R^2, R^3 and R^4 are the same as those in Figs 1 and 2.

(or -DMES), respectively; and the **m3** ions by loss of CO from the **m2** ions (Fig. 6).

a Group ions. The **a1** ion at *m/z* 58, which was seen as very large base peak, was too abundant to measure their intensities sometimes under the conditions for observing the small peaks of the ions such as $M^{+\cdot}$ and the **m** group ions in the higher mass region. In the spectra of stable isotope analogues, these ions shifted as expected (Table 1 and Fig. 3), which confirmed the formation of **a1** ion by β-cleavage from the 5-side chain as shown in Fig. 6 and Scheme 1-(1). The peaks of **a1** ions tend to be hidden in background peaks because of their small mass numbers, though the GC–



Scheme 1
Possible fragmentation pathways of diltiazem, clentiazem and their fat-soluble metabolites in PIEI MS.

MC peaks of these ions, unexpectedly, were noticed even with samples of a trace quantity. Therefore, the **a1** ion is very useful for detection of the unchanged drugs or metabolites bearing the 5-(2-dimethylamino)ethyl moiety by GC–mass chromatography. The **a2** ions were formed from the side-chain at position 5 through a McLafferty rearrangement followed by the C—N bond cleavage and the charge was left on the fragmented side-chain group as shown in Fig. 6 and Scheme 1-(2) (when the charge was left on the benzothiazepine ring, the **m1** ion was formed). The **a3** ions were formed by heterolytic cleavage of the same C—N bond as above leaving the charge on the side-chain group (Fig. 6). The structures and formation pathways of the **a2** and **a3** ions were elucidated also by analysing the mass spectra of the stable isotopes-labeled compounds (Table 1, Fig. 3). The **a2** and **a3** ions together with the **a1** ions represent the substituents in the side-chain at position 5.

b Group ions. The **b1** (or **b2**) ions were generated from $M^{+\cdot}$ characteristically as 1,2-disubstituted ethylene compound ions, retaining the charge as shown in Fig. 6, Scheme 1-(3) and Scheme 1-(4). The peaks of **b1** (or **b2**) ions were the largest except for those of the **a** group ions, and were the base peaks in the mass spectra of the compounds without the intact dimethylaminoethyl side-chain at position 5. In the case of the compound with 3-*O*-$COCH_3$,

the **b1** ion peaks were small and instead the peaks of **b2** ions, formed by loss of a ketene (CH_2CO, 42 u) from the acetyl group of the **b1** ions, were seen with the intensity similar to that of the **b1** ions from the compounds without 3-*O*-$COCH_3$ (Fig. 6, Scheme 1-4). The expected shift of the **b1** ions in the mass spectra of the compounds with 3-*O*-$COCD_3$(Dil- and Clen-AE-d7, and -A-d3; Table 1, Fig. 3) supported the fragmentation described above. The **b1** and **b2** ions clearly represent the substituents at position 2 and 3 in the structures of Dil, Clen and their metabolites (Tables 1 and 2). The structure of the **b3** ion was assumed to be such as shown in Fig. 6, because (1) the chemical formula ($C_{10}H_9O_2$) was supported by high-resolution mass spectrometry, and (2) no differences were seen in the *m/z* value between the ions from Dil and Clen and among those from the analogues with stable isotope-labelled and non-labelled substituents at position 3 and 5 (Table 1). However, this ion peak was not seen in the spectra of any metabolites. Therefore, the formation of this ion should be investigated further.

c Group ions. The **c** group ions, other than $M^{+\cdot}$, clearly showed the differences between Dil and Clen; i.e. the peaks of **c1** and **c2** ions of Dil-d0 shifted to the *m/z* of these ions of Clen-d0 by 34 u, corresponding to the substitution with Cl at position 8, being observed at *m/z* 170 and 212 with isotope cluster ion peaks (+2 u) of Cl (Table 1, Fig. 3). Therefore, the **c1** and **c2** ions (Fig. 6) reflect the substituent on the benzene moiety of the 1,5-benzothiazepine ring and are useful for structure elucidation of the metabolites. However, these ion peaks were relatively small and sometimes difficult to detect clearly (Figs 3 and 4). In the spectra of the stable isotope analogues, E-d4 or AE-d7, in which the four hydrogens in the ethyl moiety at position 5 are replaced by deuteriums, these ions shifted by +2 u or +4 u to 138 or 182 (Dil-E-d4, -AE-d7) and to 172 or 216 (Clen-E-d4, -AE-d7); and in those of E-$^{13}C_2$, in which the two ^{12}C in the same moiety as above are replaced by ^{13}C, they shifted by +1 or +2 u (Table 1, Fig. 3). These shifts of *m/z* in the spectra of the stable isotope analogues suggested that the structures of the **c1** and **c2** ions (Fig. 6) contain part of the side-chain moiety at position 5 and the speculated formation pathway of the **c1** ion is illustrated in Scheme 1-(5). The chemical formulae of the **c1** and **c2** ions, C_7H_6NS and C_9H_8NOS, respectively, obtained by high-resolution mass spectrometry with Dil-d0, were consistent with the structures postulated above.

d Ion. This ion, a tropylium cation derived from the CH and 4-substituted phenyl at position 2, more directly reflects the structure of the substituent at position 2 than the **b** group ions (Fig. 6). The ion structure was supported by the result of high-resolution mass spectrometry with Dil-d0. This ion is very characteristic, but sometimes could not be observed because of its weak relative intensity.

(2) *Compounds with a 5-(2-monomethylamino)ethyl group*

Secondary amino groups such as 5-$CH_2CH_2NHCH_3$ may not be derivatized under the usual silylation conditions described above or the silyl group of *N*-silyl derivatives may be readily eliminated. Therefore, the peaks of the **a1** ion in these compounds shifted by −14 u to *m/z* 44 (data not shown) from *m/z* 58 of the compounds with a 5-(2-dimethylamino)ethyl group. In GC–MS, it was difficult to obtain sharp peaks in the chromatograms of the metabolites bearing underivatized primary and/or secondary amino groups, especially when the sample volume was very small, because these compounds tend to be adsorbed on solid material such as GC columns or unstable even if the other functional groups are silylated. Therefore, the authors measured their GC–MS after derivatization to *N*-trifluoroacetyl (*N*-TFA) compounds by adding MBTFA directly to the silylation mixtures.

In the spectra of *N*-TFA or *O*-TMS (or -DMES)-*N*-TFA derivatives, the peaks of **a1** and **a2** ions (*m/z* 140, 153) were not usually observed and instead the peak of **a3** ion (*m/z* 154) was seen as one of the major peaks (Table 2, Figs 4 and 6). This ion structure was supported by the shift of *m/z* by +4 u in the spectra of deuterated metabolites of Dil-AE-d7 and Dil-E-d4; i.e. MA-AE-d7 and MA-E-d4, respectively (data not shown). The base peaks were derived from the **b1** (or **b2**) ions. The peaks of **c1**, **c2** and **d** ions were observed similarly to the spectra of compounds bearing the 5-(2-dimethylamino)ethyl group as described above. In the mass spectra of the metabolites bearing a 3-*O*-$COCH_3$ and a 5-(2-monomethylamino)ethyl group (MA and MB, metabolites of Dil; MB2 and MB5, metabolites

of Clen), the **m2** and **m3** ions were observed as major peak ions. Another characteristic ion peak was seen in each spectrum at *m/z* 240 (MA, 4′-*O*-CH_3) (Fig. 4), 298 (MB, 240 + 58 u, 4′-*O*-CH_3 → 4′-*O*-TMS) (data not shown), 274 (MB2, 240 + 34 u, 8-H → 8-Cl) (data not shown) and 332 (MB5, 274 + 58 u, 4′-*O*-CH_3 → 4′-*O*-TMS) (Fig. 4). These peak ions possibly contain part of the substituents at positions 2 and 8, though their structures and formation pathways are unknown.

(3) *Compounds with a 5-(2-amino)ethyl group*

GC–MS of the compounds with 5-(2-amino)ethyl group were measured after silylation and trifluoroacetylation. The substituents at position 5 of these derivatives were considered to be CH_2CH_2NHTFA from the mass numbers of the molecular ions, though the peaks corresponding to the **a3** ion (*m/z* 140) were not observed distinctly (Fig. 4). The base peak ions were the **b1** (or **b2**) ions as in the case of the compounds bearing a 5-(2-monomethylamino)ethyl group and peaks of **c1**, **c2** and **d** ions were in common with those in the spectra of the other basic compounds described in (1) and (2) (Table 2, Fig. 4).

Mass spectra of acidic metabolites

Fat-soluble acidic metabolites, which contain a carboxymethyl group at position 5 (Fig. 2), are the major metabolites of Dil and Clen. The mass spectra of Me-, TMS- and Me-TMS-derivatives of some acidic metabolites are shown in Fig. 5 and the proposed fragmentation patterns and structures of the fragment ions in Fig. 7. The substituents of the derivatives and *m/z* values of the characteristic fragment ions are listed in Table 3.

The mass numbers of molecular ions are odd since the number of nitrogen atoms in the molecule is reduced to one by oxidative deamination of the 5-side-chain followed by oxidation of the resulting aldehyde group to form a carboxymethyl group. The $M^{+\cdot}$ peaks, judged by a similar manner as with the MS of the basic compounds, were observed clearly, although not as large as those in the case of the unchanged drugs and basic metabolites (Table 3, Fig. 5). The peaks of the ions derived from the substituent at position 5 such as $[CH_2COOR]^+$ were not observed (Table 3, Fig. 5).

The base peak ions were the **b1** or **b2** ions, indicating the substituents at position 2 and 3 like those of the basic metabolites with a primary or secondary amino group. In the spectra of the compounds bearing 3-*O*-TMS group, the peaks of **b4** ions were observed (Table 3, Fig. 5). The probable structures of these ions are shown in Fig. 7, and were not seen in the MS of basic metabolites.

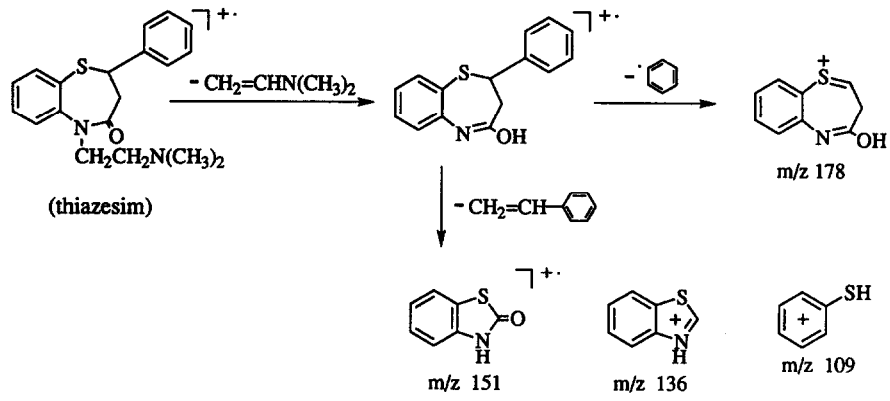
The characteristic **c** group ions included two major peaks of **c3** and **c4** ions, in addition to the **c1** ion peak, and needless to say, there was no peak corresponding to the **c2** ion (Table 3, Fig. 5). The possible structures of the **c3** and **c4** ions inferred from their mass numbers in various derivatives are illustrated in Fig. 7. The peaks of **d**, **m2** and **m3** ions were seen like those of the basic metabolites (Table 3, Fig. 5).

Discussion

Concerning the MS of 1,5-benzothiazepines, PIEI MS of dihydro-and tetrahydro-1,5-benzothiazepines [11, 12], and thiazesim and its metabolites [13] have been reported. The mass spectra of Dil and its fat-soluble basic metabolites have been obtained by direct introduction MS without derivatization [14] and by GC–MS after silylation [15, 16]. The authors have investigated the GC–MS of Dil, Clen and their fat-soluble acidic and basic metabolites [3, 5–7, 9].

The above dihydro- and tetrahydro-1,5-benzothiazepines were 2,4-substituted compounds with no carbonyl group at position 4 and the major ions in their mass spectra were the molecular ions and fragment ions formed by fragmentation accompanied by ring fission [11, 12]. Consequently, their fragmentation patterns are different from those in Dil, Clen, thiazesim and their metabolites which are 2,3,5-trisubstituted or 2,5-disubstituted 2,3-dihydro-1,5-benzothiazepines with a carbonyl group at position 4.

Thiazesim analogues including the unchanged drug and its two metabolites (Metabolite 1 and 2) are 2,5-disubstituted-2,3-dihydro-1,5-benzothiazepines which have no substituent at position 3 (Scheme 2) [13]. Since the mass spectra of thiazesim analogues were obtained by direct introduction of the underivatized samples [13], they cannot be compared directly with the spectra of Dil, Clen and their metabolites (Dil analogues) of the authors' study by GC–MS after derivatization. In the mass spectra of thiazesim analogues, the peak corresponding to the **a1** ion (*m/z* 58) was



Scheme 2
Structures and selected fragment ions of thiazesim in PIEI MS modified after Dreyfuss *et al.* [13].

expressed as the base peak only in one spectrum and in the other spectra the relative intensities of the peaks at *m/z* 58 were expressed to be higher than 100%. Thus, it is difficult to compare the relative intensities of other peaks [13].

In the spectra of Dil analogues, the peaks of $M^{+\cdot}$ and the **m1** ions were very small and the peaks of **b1** (or **b2**) ions were the next highest to those of the **a** group ions. In the case of thiazesim analogues, the peaks of the ions corresponding to **b1** at *m/z* 104, 120 and 136 were only 3–5 times higher than those of their respective $M^{+\cdot}$ and the peaks corresponding to **m1** ions were observed clearly with peak heights more than 1/2 of those of $M^{+\cdot}$ [13]. These differences in the intensity of these ions between thiazesim analogues and Dil ones propbably come from the stability differences between the respective ions with and without the substituent, *O*-acetyl or *O*-silyl group at 3-position.

As the results of high-resolution mass spectral analyses of thiazesim analogues, the structures of two major fragment ions at *m/z* 136 and 178 were proposed to be formed from the ion corresponding to the **m1** ion as shown in Scheme 2 [13]. The MS of Dil-d0, the peaks of the **c1** and **c2** ions were seen at the same *m/z* (136, 178) as those in thiazesim (Table 1, Fig. 3), and these ions in Dil analogues are also considered to indicate substituents in the benzene moiety of the benzothiazepine ring (Fig. 6). However, from the findings concerning the *m/z* shift of these ions in the stable isotope analogues of Dil and Clen (Table 1, Fig. 3) together with the chemical formulae by high-resolution mass spectrometry, the possible structures of the fragment **c1** and **c2** ions were considered to contain (part of) the ethyl moiety of the side-chain at position 5 (Fig. 6), and differ from the proposed fragmentation pattern of thiazesim analogues, and the possible formation pathways of the **c1** was thus speculated as shown in Scheme-1(5).

When the authors investigated the metabolites of Dil and Clen in rat, dog and man, the **b1** and **b2** ions in PIEI MS were very useful for detection and structure elucidation of the fat-soluble metabolites of these drugs [3, 5–7, 9]. That is to say, from the *m/z* values of these ions, the substituents at position 2 and 3 of these drugs (2,3,5-trisubstituted-1,5-benzothiazepines) were easily determined. The fat-soluble basic metabolites of Dil in rat, M3 and M5, have been reported to possess both -OH and $-OCH_3$, attached to the 2-phenyl group [3, 14]. By GC–mass chromatography using their **b1** and **b2** ion, *m/z* 310 (3-*O*-TMS) and *m/z* 238 (3-*O*-Ac-CH_2CO), respectively, the fat-soluble acidic metabolites of Dil, A5 and A6 [5, 6], and the fat-soluble metabolites of Clen MB12–MB15, MA5 and MA6 [9] were detected in rat. On the other hand, the **a** group ions indicate the structure and presence of 2-(tertiary or secondary amino)ethyl moiety at position 5. GC-mass chromatography using the **a** group (mainly **a1** and **a3**) ions and **b1** (3-*O*-Silyl) and **b2** (3-*O*-H from 3-*O*-Ac) ions is capable of analysing most fat-soluble metabolites in the samples obtained with organic solvent extraction.

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