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Liquid Secondary Ionization Mass Spectrometry and Collision-induced Dissociation Study of 2-Chloro-N^{10}-substituted Phenoxazines

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Positive-ion liquid secondary ionization mass spectrometry in combination with 3-nitrobenzyl alcohol as the liquid matrix was used to investigate the mass spectral features of a set of 21 N^{10}-substituted derivatives of 2-chlorophenoxazine. The N-10 substitution included propyl, butyl and acetyl groups containing various secondary amines (*N*,*N*-diethylamine, *N*,*N*-diethanolamine, morpholine, piperidine, pyrrolidone or β-hydroxyethylpiperazine) or a chloro group. These compounds are potent multi-drug resistance modulators. The molecular ions are observed as $M^{+\cdot}$ and $[M + H]^+$ ions. In general, the fragmentation pathways of these molecules are similar and very straightforward. The phenoxazine ring system remains stable under the Cs^+ ion beam bombardment conditions, while fragmentations are observed along the length of the alkyl and acetyl side-chains. The fragmentation reactions were corroborated by acquiring product ion and constant neutral loss tandem mass spectrometric scans of the pertinent ions. © **1997 John Wiley & Sons, Ltd.**

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INTRODUCTION

Phenoxazine (**1**), a tricyclic compound, is known to potentiate the uptake of anticancer agents vincristine and vinblastine in MDR cells to a greater extent than verapamil.[1] However, **1** is less stable in a serum-containing medium. Therefore, a search for the development of highly potent, stable and less toxic therapeutic agents possessing multi-drug resistance (MDR) modulating properties has assumed a great importance.[2] In this quest, we have synthesized a number of phenoxazine derivatives, in which the C-2 hydrogen is replaced by chlorine and the N-10 hydrogen by propyl, butyl and acetyl groups containing various secondary amines (*N*,*N*-diethylamine, *N*,*N*-diethanolamine, morpholine, piperidine, pyrrolidone and β-hydroxyethylpiperazine) or chlorine.[3] Many of these compounds have been shown to possess antiproliferative and anti-MDR activities. The structures of these compounds are shown in Fig. 1.

Mass spectrometry plays a vital role in the development of therapeutic drugs by providing methods for their characterization and quantification. High levels of structure specificity and sensitivity are the hallmarks of mass spectrometric procedures. High molecular specificity is achieved because of the incontrovertible genealogical relationship between precursor and product ions. A mass spectrum is usually a signature of the analyte because it contains both molecular mass (M_r) and compound-specific fragment ions.

Recently, a set of eight 2-trifluoromethyl-N^{10}-substituted phenoxazines were characterized by using electron ionization (EI) and liquid secondary ionization mass spectrometric (LSIMS) techniques.[4] The present study is a sequel to this previously reported work. In this comprehensive study, we included 21 derivatives of 2-chlorophenoxazine mentioned above. LSIMS was used to obtain mass spectral data. LSIMS has emerged as a powerful analytical tool for the characterization of a variety of biomolecules. A correlation between the structure of these chemosensitizers and the fragmentation patterns observed when they are subjected to a beam of high-energy Cs^+ ions is discussed. Fragmentation pathways were corroborated by use of tandem mass spectrometric (MS/MS) techniques. MS/MS is a

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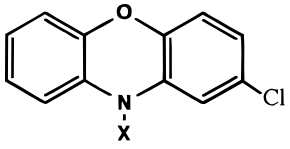


compound	X	MW
2	H	217
3	$(CH_2)_3N(C_2H_5)_2$	330
4	$(CH_2)_4N(C_2H_5)_2$	344
5	$(CH_2)_3$-N O	344
6	$(CH_2)_4$-N O	358
7	$(CH_2)_3$-N	342
8	$(CH_2)_4$-N	356
9	$(CH_2)_3$-N	328
10	$(CH_2)_4$-N	342
11	$(CH_2)_3$-N N-CH_2CH_2OH	387
12	$(CH_2)_4$-N N-CH_2CH_2OH	401
13	$(CH_2)_3N[(CH_2)_2OH]_2$	362
14	$(CH_2)_4N[(CH_2)_2OH]_2$	376
15	$(CH_2)_3Cl$	293
16	$(CH_2)_4Cl$	307
17	$COCH_2Cl$	293
18	$COCH_2N(C_2H_5)_2$	330
19	$COCH_2$-N O	344
20	$COCH_2$-N	342
21	$COCH_2$-N	328
22	$COCH_2$-N N-CH_2CH_2OH	387

Figure 1. Structures of phenoxazines.

powerful tool that provides an incontrovertible connectivity relationship between precursor and product ions.[5] An additional advantage of MS/MS is that the contribution of the matrix ions, often a source of ambiguity in the conventional LSI mass spectrum, is eliminated. Both product ion scan and constant neutral loss scan were used in this MS/MS experiment.[6] Previous work on phenoxazines includes an EI study of ring substituted phenoxazines.[7]

EXPERIMENTAL

All mass spectral data were acquired in the positive-ion mode by using an AutoSpec Q hybrid tandem mass spectrometer of E_1BE_2–qQ geometry (where E is an electric sector, B a magnetic sector, q an r.f.-only quadrupole and Q a quadrupole mass analyzer) (Micromass, Manchester, UK). Ionization used a beam of Cs^+ ions produced by typically operating the Cs^+ ion gun at voltages between 30 and 40 keV and an emission of ~1.1 μA. These parameters were optimized to provide maximum ion signals. The ions were accelerated out of the source at a potential of 8 kV. The conventional mass spectra were obtained by scanning the magnet in the mass range 50–500 mass units at a scan speed of 7 s per decade and at a mass resolution of 1000. The data accumulation and manipulation were under the control of Digital Vax Station 3100-based Opus software. To obtain the mass spectra, a few micrograms of each compound and 1 μl of 3-nitrobenzyl alcohol (NBA) were mixed thoroughly on the stainless-steel target and inserted into the ion source.

The product ion spectra were acquired using the constant *B/E* linked-field scan technique.[6] The instrument was calibrated in the mass range 10–470 mass units using glycerol as the mass calibrant. The pertinent precursor ions generated by bombarding the analyte–NBA mixture with the Cs^+ ions beam were mass selected and subjected to high-energy collision-induced dissociation (CID) in the first field-free region of the hybrid tandem mass spectrometer mentioned above. Helium was used as the collision gas. Constant neutral loss data were also acquired by mass selecting the ions in the first field-free region.

All compounds were synthesized in our laboratory and separated to the desired purity by liquid chromatography. Their purity was checked by UV–visible, infrared and NMR spectrometric methods. Details of their synthesis along with evaluation of their bioactivity have been reported elsewhere.[3]

RESULTS AND DISCUSSION

Fast atom bombardment (FAB)[8] and its variation LSIMS[9] have become standard mass spectrometric techniques for the characterization of biomolecules. One of the advantages of FAB or LSIMS is that a compound-specific mass spectrum can be obtained for a variety of non-volatile compounds. On the one hand, these techniques yield abundant molecular ion signals in the form of protonated or deprotonated ions, from which the molecular mass of a compound can be determined. On the other hand, the energy deposited during the desorption/ionization process is sufficient, especially for small molecules, to produce a reasonably large number of structure-specific fragment ions. A liquid matrix, however, is a critical component in the use of these techniques. A useful matrix should be able to dissolve the analyte and provide a medium that can facilitate the generation of molecular ions of the analyte. Although glycerol is by far the most commonly used liquid matrix,[10] certain compounds such as quinones[11] and anthracyclines[12] do not produce meaningful data with this matrix. NBA has been shown to be an effective substitute for certain classes of compounds.[13,14]

Recently, we used both electron ionization (EI) and LSIMS to obtain the mass spectra of eight 2-trifluoromethyl derivatives of phenoxazine.[4] Glycerol, sulfolane and NBA were used as liquid matrices in the LSIMS analysis. Although EI produced good spectral data, the $M^{+\cdot}$ ion abundance was poor for some com-

pounds. On the other hand, LSI mass spectra obtained using NBA were well endowed with both the molecular ions signal and characteristic fragment ions. Except for one compound, the molecular ion signal (as either $M^{+\cdot}$ or $[M + H]^+$) formed the base peak for all compounds. The use of sulfolane resulted in less abundant molecular ions. An additional drawback of using sulfolane as a matrix is that it does not last very long under the ion source temperature and vacuum conditions. Glycerol, on the other hand, was not a compatible liquid matrix for these compounds; the matrix ions overwhelmed the analyte signal in the LSI mass spectra.

With these observations in mind, mass spectra of the above-mentioned 2-chlorophenoxazine derivatives were obtained using LSIMS as the ionization method and NBA as the liquid matrix. To obtain detailed and comprehensive data on fragmentation pathways of phenoxazine derivatives, we included 21 compounds in this study. Although the basic structural unit in these compounds is the same, the differences in their structures arise because the substituents attached to the N-10 position are of diverse functionality. They can be broadly classified into two groups, one containing alkyl (propyl and butyl) derivatives and other acetyl derivatives. These derivatives further differ from each other because the terminal hydrogen of an alkyl or acetyl group is replaced by either chlorine, *N*,*N*-diethylamine, *N*,*N*-diethanolamine, morpholine, piperidine, pyrrolidone or β-hydroxyethylpiperazine.

The LSI mass spectra of all of the N^{10}-substituted 2-chlorophenoxazines studied are given in Table 1. To provide a visual picture, the mass spectrum of 10-(3′-dimethylaminopropyl)-2-chlorophenoxazines (**3**) is illustrated in Fig. 2. For clarity, the mass spectral data in Table 1 are presented in three different columns. In one column, the molecular ion region is shown. The ions formed due to losses of the N-10 side-chain components (i.e. ions containing the phenoxazine moiety) are included in the middle column. The ions formed due to retention of charge by the side-chain fragments are shown separately in the last column.

The data in Table 1 reveal that all 2-chlorophenoxazine derivatives yield abundant molecular ions when bombarded with a beam of Cs^+ ions. The molecular ion is the base peak in the mass spectra of these compounds, except in the spectra of 10-(*N*-morpholinoacetyl- (**19**) and 10-(*N*-pyrolidinoacetyl)-2-chlorophenoxazines (**21**); the relative abundances of the molecular ions of these compounds are 42 and 72%, respectively. The molecular ions were observed either in the form of radical cations ($M^{+\cdot}$) or as protonated molecules ($[M + H]^+$. The 2-chlorophenoxazines that contain secondary amines in the N-10 side-chain produce more abundant $[M + H]^+$ ions compared with $M^{+\cdot}$ ions. The only exception is the 10-[3′-(*N*-bishydroxyethyl)amino]propyl derivative (**13**). The abundance of $M^{+\cdot}$ further decreases in the acetyl derivatives. In contrast, $M^{+\cdot}$ is the most abundant of the molecular ion cluster for the unsubstituted compound (**2**) and for the chloropropyl- (**15**), chlorobutyl- (**16**) and chloroacetyl (**17**) derivatives. Several other examples of the formation of radical cations of certain analyte when

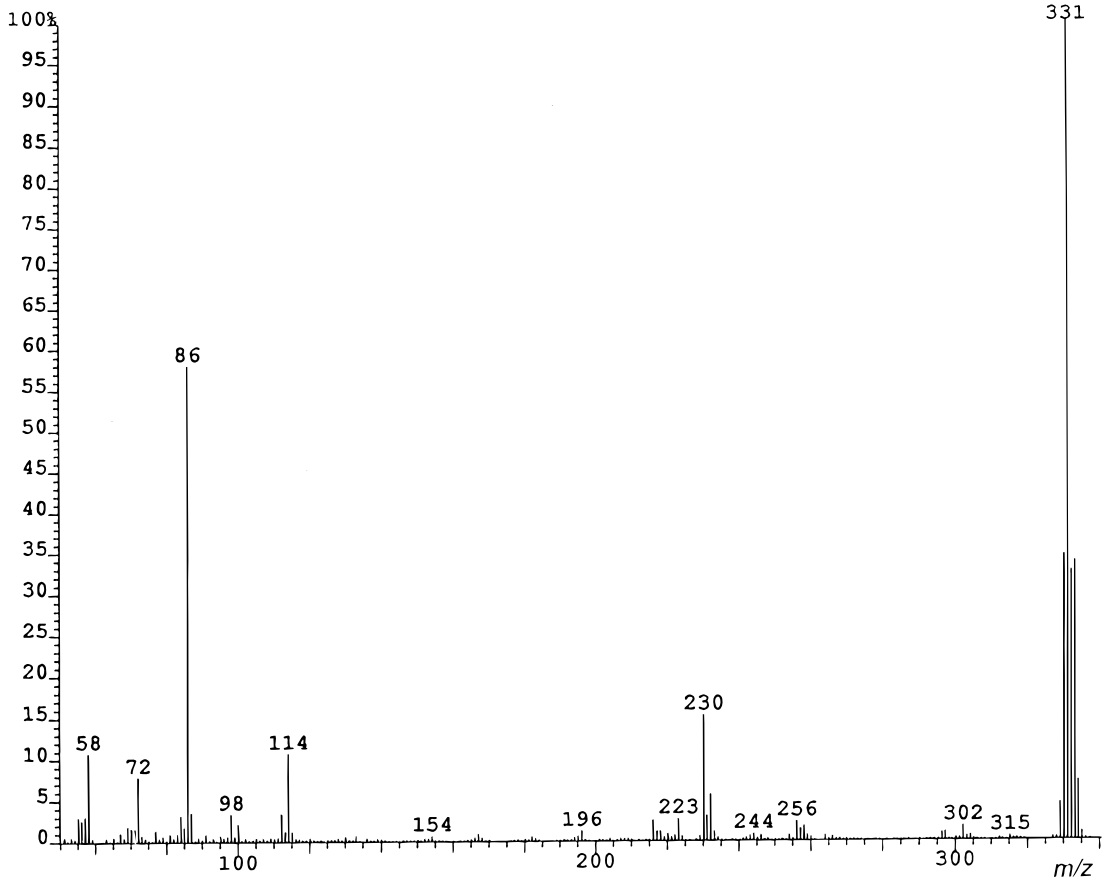


Figure 2. Conventional mass spectrum of 10-(3′-*N*-dimethylaminopropyl)-2-chlorophenoxazine (**3**). NBA was used as the liquid matrix.

Table 1. Mass spectra of 2-chlorophenoxazine derivatives (*m*/*z* with relative intensities (%) in parentheses)

Compound	Molecular ion cluster region	Loss of side-chain moieties	Side-chain fragments
2	219 (35), 218 (29), 217 (100), 216 (12)	183 (5), 182 (2).	
3	333 (34), 332 (33), 331 (100), 330 (35)	230 (15), 223 (3), 216 (3)	114 (11), 100 (2), 98 (3), 86 (58), 72 (8), 58 (11)
4	347 (34), 346 (32), 345 (100), 344 (30)	272 (9), 237 (7), 230 (15), 217 (6), 216 (9)	128 (57), 114 (4), 86 (77), 72 (10), 58 (22)
5	347 (34), 346 (42), 345 (100), 344 (67)	258 (2), 230 (15), 223 (2), 217 (2), 216 (4)	128 (14), 114 (1), 100 (39), 86 (2), 70 (3)
6	361 (34), 360 (42), 359 (100), 358 (66)	272 (5), 237 (3), 230 (13), 217 (5), 219 (9)	142 (83), 126 (3), 100 (33), 98 (5), 86 (3), 84 (5), 70 (3)
7	345 (34), 344 (37), 343 (100), 342 (47)	258 (1), 230 (9), 223 (1), 217 (1), 216 (2)	126 (11), 112 (2), 98 (36), 84 (6), 83 (2), 70 (2), 69 (4), 56 (2)
8	359 (33), 358 (37), 357 (100), 356 (50)	272 (5), 237 (3), 230 (7), 217 (2), 216 (5)	140 (75), 138 (10), 124 (3), 98 (40), 84 (10), 70 (2), 56 (2)
9	331 (34), 330 (37), 329 (100), 328 (47)	258 (1), 230 (12), 223 (2), 217 (1), 216 (3)	112 (13), 110 (4), 98 (2), 84 (47), 83 (6), 70 (6)
10	345 (33), 344 (37), 343 (100), 342 (48)	272 (5), 237 (3), 230 (6), 217 (2), 216 (5)	126 (76), 124 (10), 112 (1), 110 (3), 98 (1), 84 (36), 70 (7)
11	390 (34), 389 (37), 388 (100), 387 (46), 386 (12)	356 (3), 256 (2), 230 (17), 217 (2), 216 (3)	171 (6), 157 (2), 143 (10), 141 (4), 128 (2), 113 (4), 100 (4), 98 (4), 97 (4)
12	404 (28), 403 (38), 402 (100), 401 (68), 400 (71)	384 (3), 370 (13), 355 (4), 272 (11), 237 (6), 230 (17), 217 (6), 216 (11)	185 (92), 183 (24), 169 (6), 167 (5), 155 (8), 153 (10), 143 (22), 141 (11), 129 (6), 128 (11), 125 (9), 111 (5), 110 (8), 100 (17), 98 (18), 97 (18), 84 (19), 83 (9), 70 (16)
13	365 (24), 364 (45), 363 (76), 362 (100), 361 (21)	345 (3), 331 (14), 258 (3), 256 (3), 230 (44), 232 (16), 223 (6), 217 (3), 216 (6))	146 (8), 144 (7), 118 (52), 100 (4), 88 (46), 74 (7
14	379 (34), 378 (36), 377 (100), 376 (42)	345 (14), 272 (8), 237 (5), 230 (11), 216 (7)	160 (15), 130 (4), 128 (3), 118 (10), 116 (4), 114 (3), 104 (1), 88 (2), 74 (8)
15	297 (12), 296 (18), 295 (67), 294 (30), 293 (100)	259 (4), 230 (16), 217 (2), 216 (8)	77 (1)
16	311 (12), 310 (17), 309 (65), 308 (29), 307 (100)	273 (3), 230 (14), 217 (3), 216 (8)	91 (2)
17	297 (18), 296 (50), 295 (75), 294 (78), 293 (100)	259 (9), 217 (38), 216 (61)	77 (9)
18	333 (34), 332 (21), 331 (100), 330 (1)	217 (3), 216 (3)	114 (3), 86 (45), 72 (2), 58 (8)
19	347 (12), 346 (9), 345 (42), 344 (6), 343 (19)	217 (5), 216 (8)	100 (100), 98 (18)
20	345 (34), 344 (23), 343 (100), 342 (2)	217 (3), 216 (3)	123 (3), 98 (55), 84 (2), 70 (2), 69 (2)
21	331 (21), 330 (15), 329 (72), 328 (8), 327 (33)	217 (6), 216 (7)	112 (20), 110 (4), 84 (100), 70 (2)
22	390 (33), 389 (24), 388 (100), 387 (10), 386 (28)	370 (6), 356 (5), 217 (38), 216 (14)	171 (6), 143 (73), 141 (21), 129 (6), 128 (11), 113 (6), 111 (8), 100 (11), 97 (18), 84 (7), 83 (10), 70 (14), 69 (14)

impacted by an atom or ion beam can be found in the literature.[12,15] Some of the 2-trifluoromethylphenoxazines studied earlier also produced $M^{+\cdot}$ ions.[4] NBA acts as an electron scavenger and thus facilitates one-electron oxidation of certain analytes. With the presence of a basic functionality (secondary amines and carbonyl group) in the side-chain of phenoxazines, protonation at that site competes more favorably with the one-electron oxidation, resulting in more abundant $[M + H]^+$ ions.

In general, the mass spectral features of 2-chlorophenoxazine derivatives are similar. The phenoxazine ring system remains stable, whereas fragmentation reactions are observed due to cleavages of bonds in the N-10 side-chain portion of these compounds. The mass spectral characteristics of these compounds are discussed in terms of the following representative examples.

2-Chlorophenoxazine (2)

LSIMS ionization of **2** produces both a radical cation (as a base peak) and a protonated ion (of about 16% abundance relative to $M^{+\cdot}$ after correcting for the ^{13}C contribution from $M^{+\cdot}$ ion). Apart from the cluster of ions in the molecular ion region, the only other analyte-related ions observed are at *m/z* 183 and 182. Loss of a chlorine atom from $[M + H]^+$ and $M^{+\cdot}$ ions, respectively, yield these two ions. This spectrum is a testimony to the stability of the phenoxazine ring system.

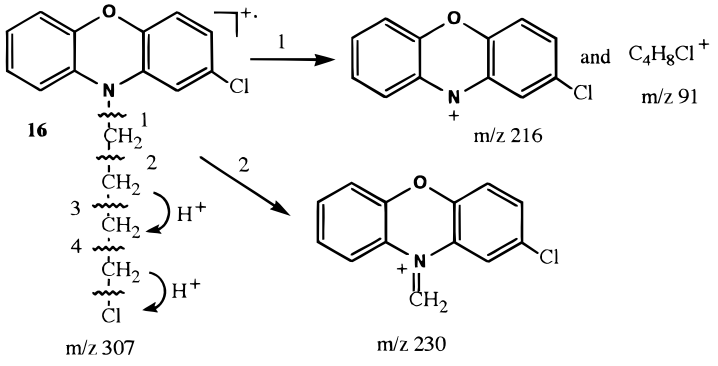
Chloroalkyl- and chloroacetyl derivatives

Three compounds (**15–17**) containing these structural features were studied. A characteristic feature of the spectra of these compounds is the presence of dominant molecular ions and a few fragment ions. As noted above, the molecular ions are present mainly as radical cations, with the $[M + H]^+$ ion being relatively more pronounced only in **17**. As a typical example, the fragmentation pathways of **16** are shown in Scheme 1. In both 10-(*N*-chloropropyl)- (**15**) and 10-(*N*-chlorobutyl)-2-chlorophenoxazines (**16**), the prominent fragmentation is cleavage of the bond α to the ring nitrogen (reaction (2)); the charge bearing fragment is mainly the phenoxazine moiety (*m/z* 230). Fission of the bond linking the side-chain to the phenoxazine ring also occurs (reaction (1)), producing *m/z* 216 and 77 in **15** and *m/z* 216 and 91 in **16**; the side-chain fragments have less propensity for the charge. However, cleavage of this bond is highly favored in the chloroacetyl derivative (**17**), probably because of the close proximity of an additional charge center (the carbonyl group). Protonation of the ring is also observed, yielding *m/z* 217. The product ion spectrum of the $M^{+\cdot}$ ion (*m/z* 307) of **16** (Fig. 3) is in agreement with the proposed fragmentations. The two major product ions in that spectrum are at *m/z* 230 and 216. In addition, CID of *m/z* 307 also produces *m/z* 271 (loss of HCl) and 243 (loss of C_2H_5Cl; see reactions (3) and (4) in Scheme 1).

Alkyl derivatives containing secondary amines

Eight compounds (**3–10**) are included in this discussion. In these compounds also, the phenoxazine ring remains intact, whereas fragmentations occur with greater facility along the length of the alkyl side-chain. The general patterns of fragmentations are similar to those discussed above for the chloro derivatives. However, with the introduction of an additional charge-bearing functionality, the side-chain fragments are more abundant in the spectra. The mass spectral data of 10-(3′-dimethylaminopropyl)-2-chlorophenoxazines (**3**) and 10-(3′-*N*-piperidinopropyl)-2-chlorophenoxazines (**7**) are typical of this group of compounds and are illustrated in Schemes 2 and 3, respectively.

In **3**, the origin of all of the fragment ions can be attributed to fragmentation of bonds in the alkyl chain. For example, the ions at *m/z* 216 (3%) and 230 (15%) are formed due to cleavage of the bond that connects the alkyl chain to the phenoxazine ring (reaction (1)) and the bond α to the phenoxazine nitrogen (reaction (2)), respectively. The fragment ion (*m/z* 114) complementary to *m/z* 216 is of reasonable abundance (11%), whereas the fragment ion complementary to *m/z* 230 (*m/z* 100) is very weak. The major fragmentation pathway, however, is homolytic cleavage of the bond α to the dimethylamino nitrogen (reaction (3)). This reaction produces a strong signal at *m/z* 86 (58%). The corresponding phenoxazine ring-containing fragment is not observed. The *m/z* 86 ion is also formed by the loss of C_2H_4 from *m/z* 114. The evidence for this proposal is the product ion spectrum of *m/z* 114 (Table 2) and the constant neutral scan loss of 28 Da (not shown), both of which establish unambiguously the connectivity relation between *m/z* 114 and 86. The ion of *m/z* 72 (8%) is formed by the cleavage of the C—N bond that connects the dimethylamino group with the propyl group. This



Scheme 1

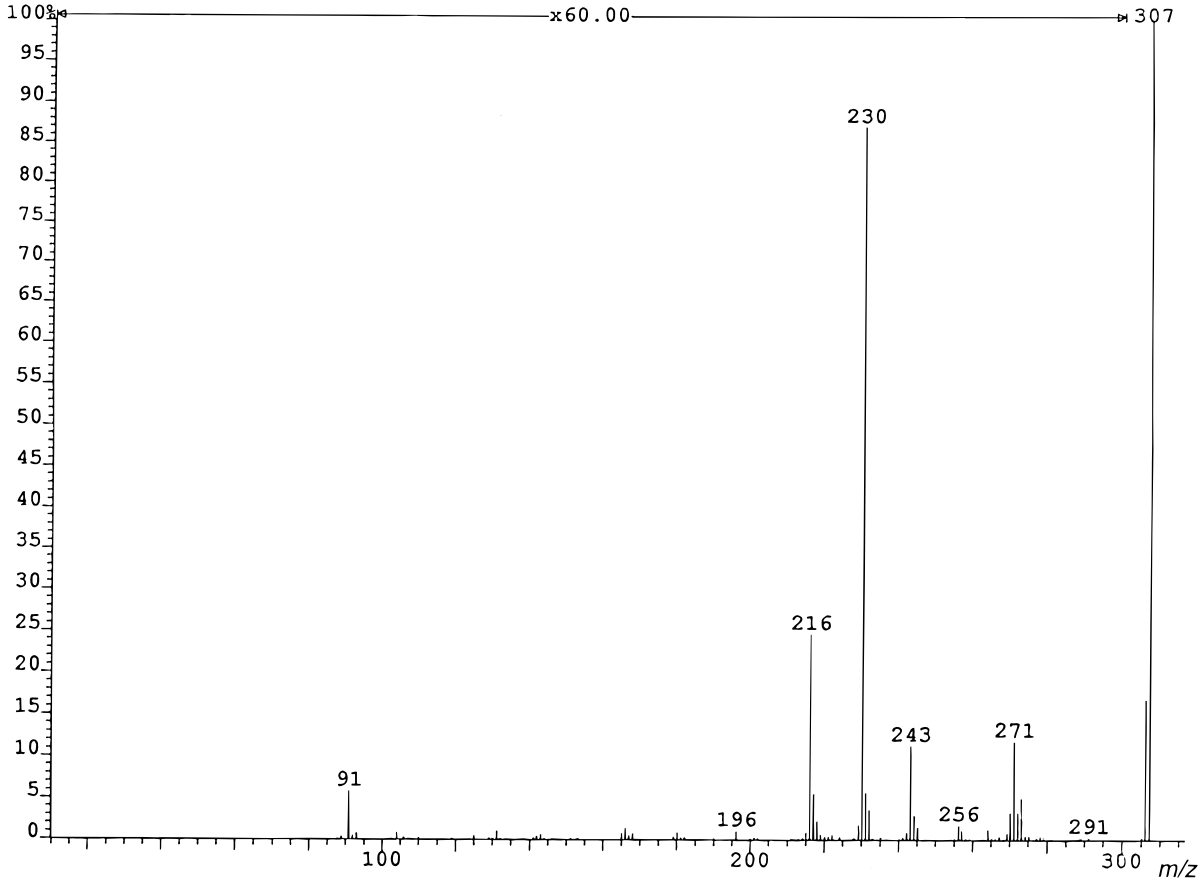
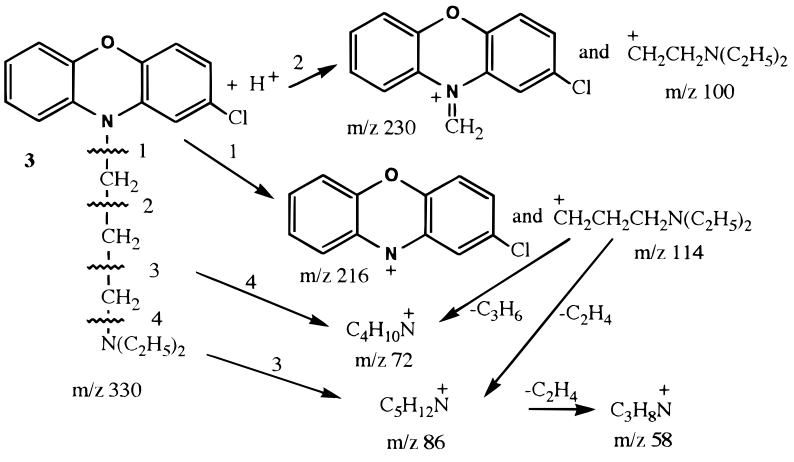
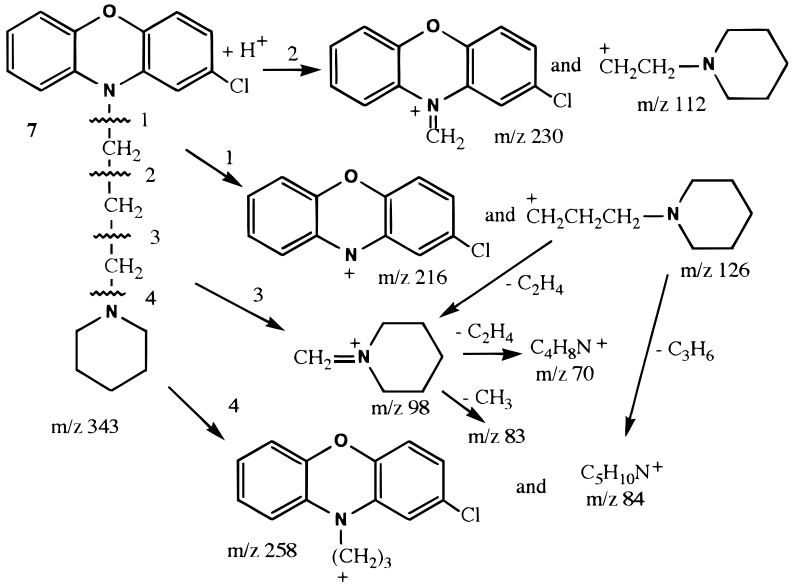


Figure 3. Product ion spectrum of the $M^{+\cdot}$ ion of 10-(*N*-chlorobutyl)-2-chlorophenoxazine (**16**).

ion is one of the prominent ions in the product ion spectrum of *m/z* 114, suggesting that alternative route for its formation is loss of C_3H_6 from *m/z* 114 (see Scheme 2). No substantial evidence of the product–precursor relationship between *m/z* 86 and 72 was obtained from the product ion spectrum of *m/z* 86 (Table 2) and the constant neutral loss of 14 Da. Another significant ion in the spectrum is at *m/z* 58. The product ion spectrum of *m/z* 86 suggests that *m/z* 58 is derived from *m/z* 86 by the loss of C_2H_4. Further supportive evidence of this reaction is the neutral loss scan of 28 Da. The *m/z* 58 probably has the structure $CH_3CH_2\overset{+}{N}H{=}CH_2$. The fragmentation pathways of **3** were confirmed by acquiring product ions spectra of both $M^{+\cdot}$ and $[M + H]^+$ ions. The prominent product ions derived from the $M^{+\cdot}$ ion (*m/z* 330) of **3** are at *m/z* 86, 114, 223, 230, 231, 257 and 258 with minor contributions of *m/z* 72, 100, 196, 217, 295, 301, 302 and 305 (Fig. 4). The product ion spectrum of the $[M + H]^+$ ion is also similar to the spectrum of $M^{+\cdot}$ except that *m/z* 258 is more abundant than *m/z* 257 (see inset in Fig. 4). The formation of ions of *m/z* 196, 223 and 295 is rationalized to involve the loss of C-2 chlorine atom from ions of *m/z* 231, 258 and 331.



Scheme 2



Scheme 3

In **7**, cleavage of bonds takes place all along the alkyl chain (Scheme 3). Of these reactions, homolytic cleavage of the bonds α to the phenoxazine (reaction (2)) and piperidine ring nitrogens (reaction (3)) is more significant; the charge is retained by the nitrogen-containing fragments, producing ions of *m/z* 230 (9%) and 98 (36%), respectively. The respective complementary fragments are either of very low abundance (e.g. *m/z* 112, 2%) or absent (e.g. *m/z* 244) in the conventional mass spectrum. Fission of the bond that connects the side-chain to the phenoxazine ring also occurs (reaction (1)), resulting in the formation of ions of *m/z* 216 (2%) and 126 (11%). The bond that connects the piperidine ring to the propyl group is also labile under the LSIMS conditions. Rupture of that bond (reaction (4)) produces ions of *m/z* 258 (1%) and 84 (6%). An alternative route to the formation of *m/z* 98 is the loss of C_2H_4 from *m/z* 126. This evidence was gathered from the product ion spectrum of *m/z* 126 (Table 2). The neutral loss scan of 28 Da (not shown) provides additional evidence for this proposal. The product ion spectrum of *m/z* 126 shows that *m/z* 84 is also derived from *m/z* 126 (loss of C_3H_6). Although the phenoxazine ring is stable when **7** is bombarded with a keV energy beam of Cs^+ ions, the piperidine ring is very fragile. The ions of *m/z* 70 (2%), 69 (4%) and 56 (2%) are thought to originate from the piperidine ring. The product ion spectrum of *m/z* 98 (Table 2) suggests a genealogical relation between these ions and *m/z* 98. The ions of *m/z* 83, 70, 69, 55, 44 and 42 are present in that spectrum. Additional evidence in support of the pathways shown in Scheme 3 also comes from the product ion spectra of the $M^{+\cdot}$ and $[M + H]^+$ ions of **7** (Fig. 5). These spectra are identical except for the relative abundances of *m/z* 258 and 257, the former being more abundant in the spectrum of $[M + H]^+$ ion (see inset in Fig. 5). Similarly to the data in Fig. 4, the formation of ions of *m/z* 196, 223 and 307 involves the loss of the C-2 chlorine atom.

Acetyl derivatives containing secondary amines

Compounds **18–21** are discussed in this section. Structurally, they differ from the corresponding alkyl derivatives discussed above in that they contain a carbonyl group in the side-chain in place of the CH_2CH_2 moiety. Fragmentation reactions of this group of compounds are fewer compared with the corresponding propyl and butyl derivatives. 10-(3′-*N*-Piperidinoacetyl)-2-chlorophenoxazine (**20**) is chosen as a typical example to illustrate the reactions of these four compounds. The dominant fragmentation pathway in **20** is homolytic cleavage of the bond between the carbonyl carbon and the CH_2 group, with charge retention by the side-chain

Table 2. CID mass spectra of certain important fragment ions

Precursor compound	Precursor ion (*m/z*)	*m/z* of the product ions[a]
3	114	**98**, **86**, 85, 84, **72**, 71, 58, 57
3	86	70, **58**, 44, 330
7	126	**110**, **98**, 96, **84**, 83, 69
7	98	**83**, 81, **70**, **69**, **55**, **44**, 42
20	126	108, 107, 100, **98**, **97**, 84, **83**, **70**, **69**, 55
20	98	**83**, 81, **70**, **69**, **55**, **44**, 42
12	185	167, 153, 143, 139, 128, 116, 112, 110, 100, **98**, **84**, 70, 56
12	143	128, 116, 113, 112, **100**, **98**, 86, **70**, 62, 42
12	128	99, 98, **97**, 86, 85, 84, 71, 70, 64

[a] The ions shown in bold are the prominent product ions in the spectra.

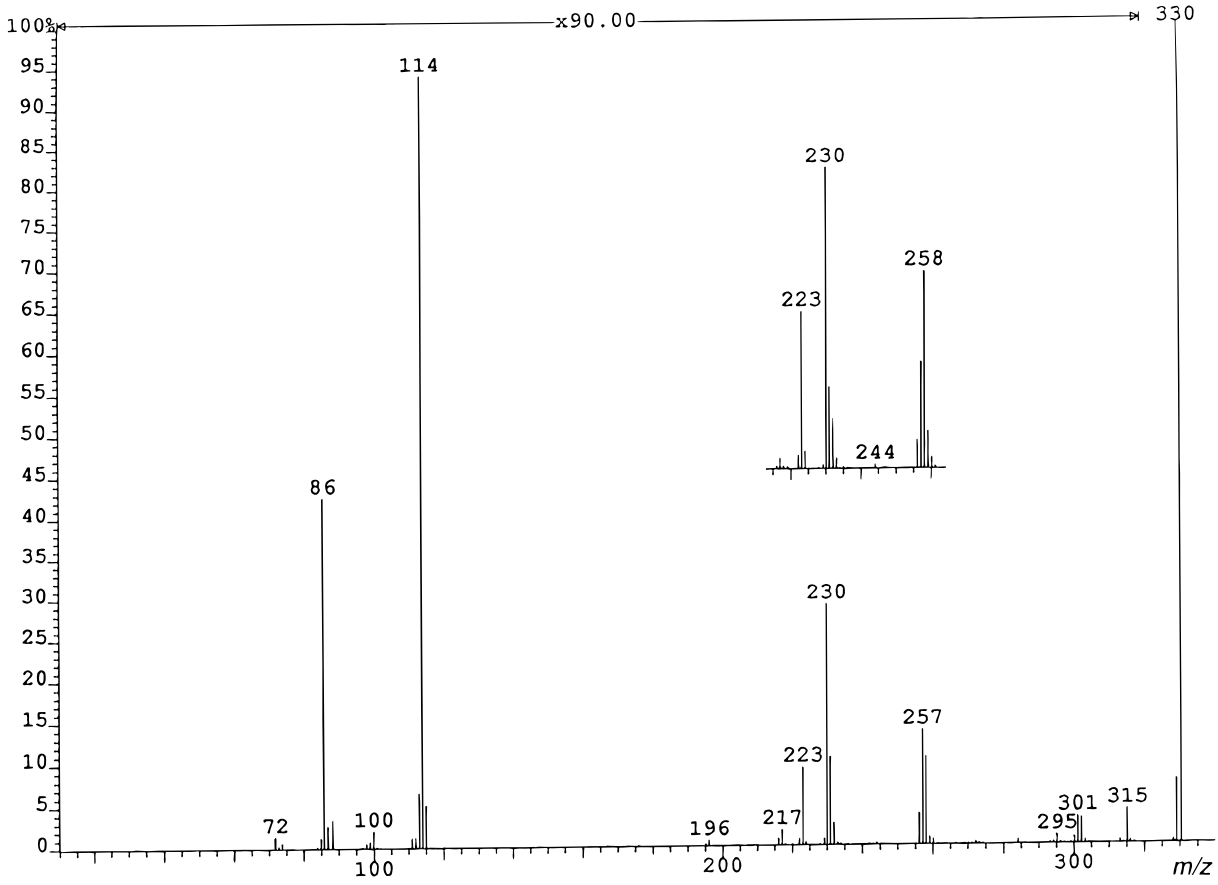


Figure 4. Product ion spectrum of the $M^{+\cdot}$ ion of 10-(3′-*N*-dimethylaminopropyl)-2-chlorophenoxazine (**3**).

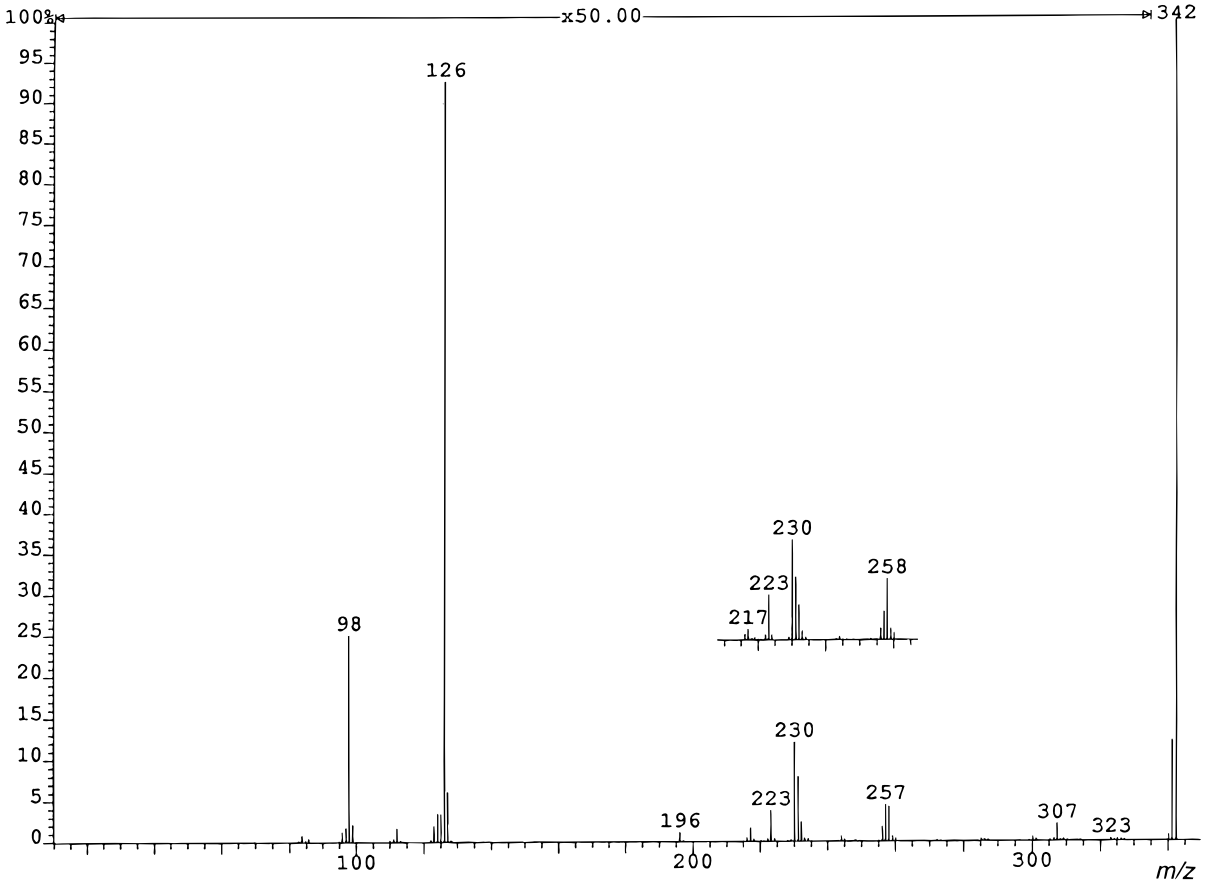
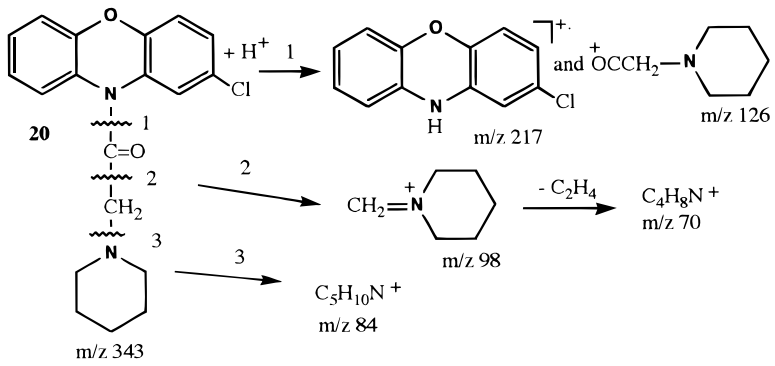


Figure 5. Product ion spectrum of the $M^{+\cdot}$ ion of 10-(3′-*N*-piperidinopropyl)-2-chlorophenoxazine (**7**).



Scheme 4

nitrogen-containing fragments (reaction (2) in Scheme 4). The adjacent bonds are also broken but to a limited extent (reactions (1) and (3)). In the mass spectrum of **20**, other compound-specific fragment ions present are at *m/z* 216, 70 and 69. The molecular ion of **20** is even more stable under CID conditions. The ion of *m/z* 98 is the only major ion present in the product ion spectrum of $[M + H]^+$ (Fig. 6). The ions of *m/z* 125, 126, 216, 217, 230, 231, 259, 286 and 314 are of minor importance. The product ion spectrum of *m/z* 126 (Table 2) shows that a small population of *m/z* 98 is also derived from this ion (not shown in Scheme 4). The connectivity relation between *m/z* 98 and *m/z* 83, 70 and 69 was also established from the product ion spectrum of *m/z* 98 (Table 2). The formation of *m/z* 70 via loss of C_2H_4 from *m/z* 98 is supported by the neutral loss scan of *m/z* 28. The product ion spectra of *m/z* 98 derived from **7** and **20** are almost identical; obviously both have an identical structure. On the other hand, the product ion spectra of *m/z* 126 derived from these two precursors are not similar, consistent with the fact that the two are isobaric compounds and thus have different structures.

β-Hydroxyethylpiperazino and bishydroxyethylamino derivatives

Five compounds are discussed in this section, of which three contain a hydroxyethylpiperazino functionality, 10-{[(β-hydroxyethyl)piperazino]propyl}- (**11**), -butyl}- (**12**), and -acetyl}-2-chlorophenoxazines (**22**) and two (**13**

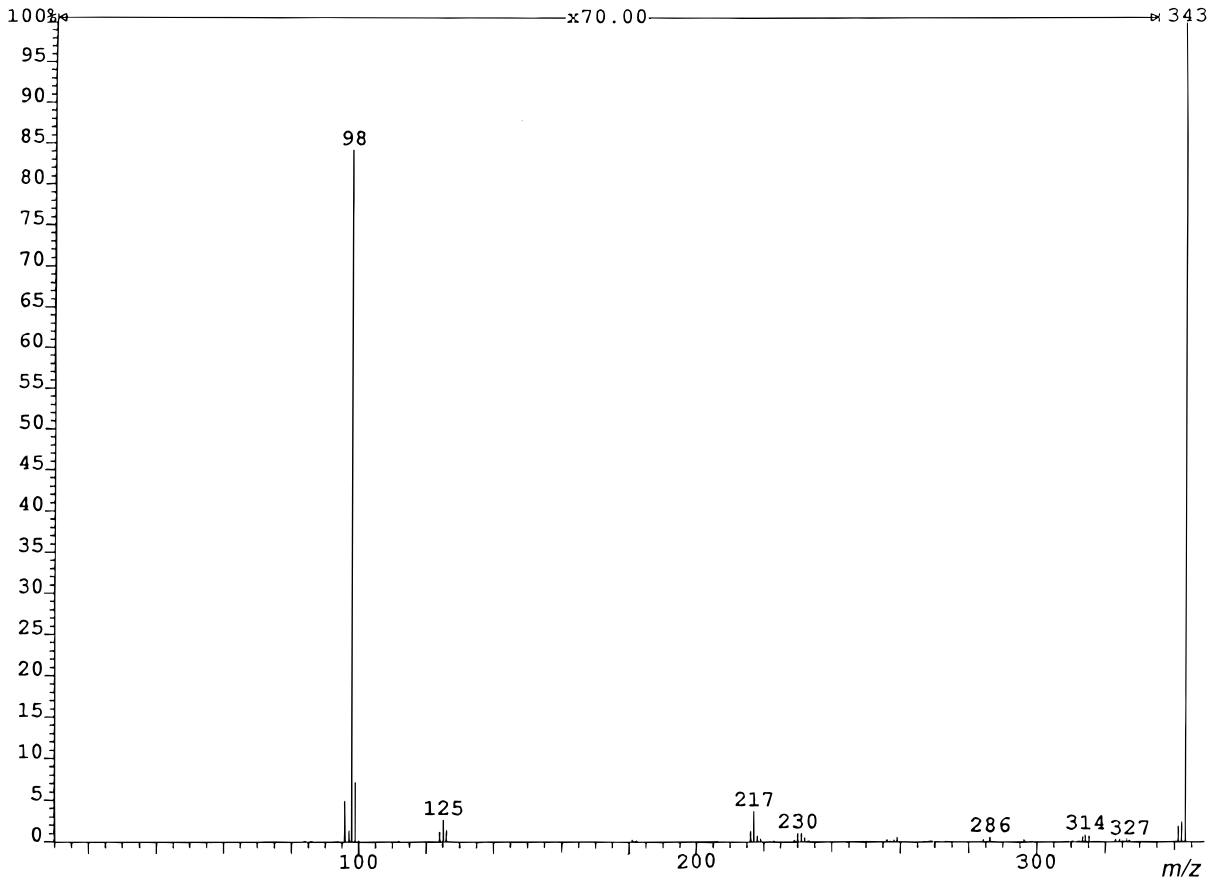
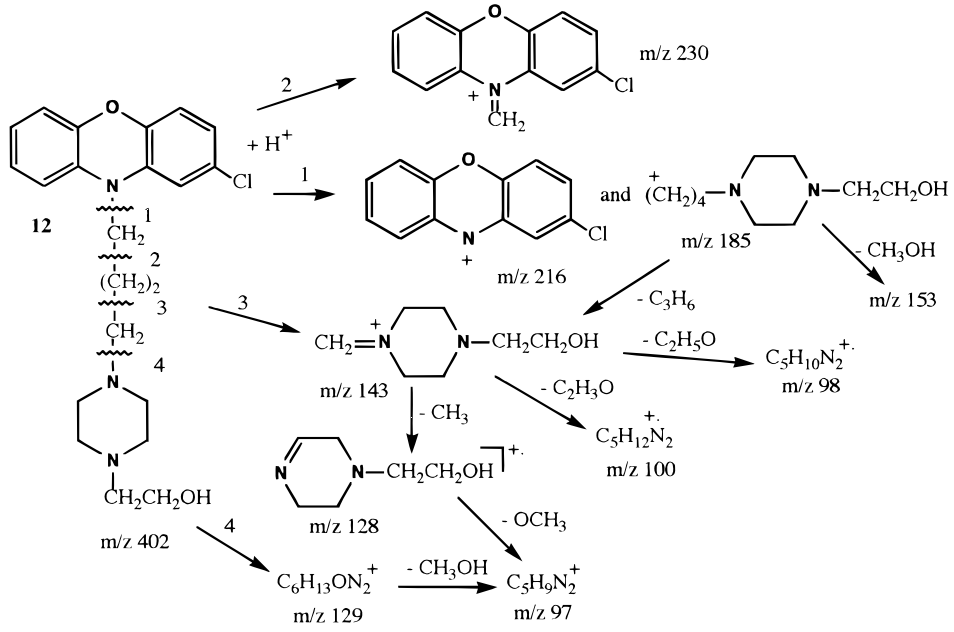


Figure 6. Product ion spectrum of 10-(3′-*N*-piperidinoacetyl)-2-chlorophenoxazine (**20**).



Scheme 5

and **14**) a bishydroxyethylamino group. All of these compounds show extensive and complicated fragmentation patterns. The reason for this feature is the presence of the hydroxyethyl moiety, which introduces the possibility of loss of several neutral fragments, such as H_2O, CH_2O, CH_2OH and CH_3OH. The fragmentation reactions of the trifluoromethyl analog of **13** and the corresponding side-chain moiety $(CH_2)_3N(CH_2CH_2OH)_2$ have been discussed in detail in a previous publication and will not be elaborated here. Fragmentation pathways of **12** are depicted in Scheme 5. The conventional mass spectrum of **12** contains a large number of ions (Table 1). As is the case with other phenoxazine compounds studied here, these ions are formed as a result of

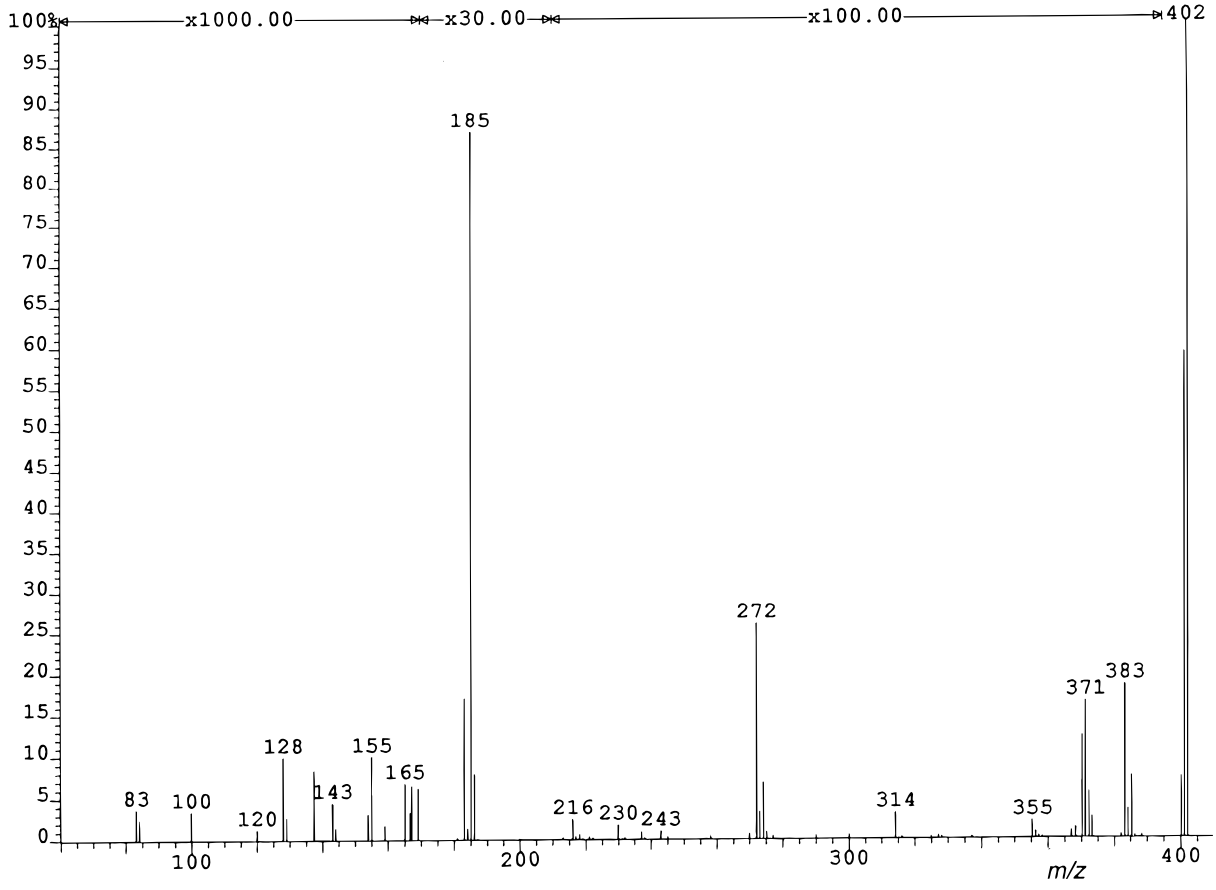


Figure 7. Product ion spectrum of the $[M + H]^+$ ion of 10-{[(β-hydroxyethyl)piperazino]butyl}-2-chlorophenoxazine (**12**).

cleavage of bonds in the (β-hydroxyethyl)piperazinobutyl moiety, whereas the chlorophenoxazine ring remains intact. The most dominant fragmentation reaction is cleavage of the bond connecting the (β-hydroxyethyl)piperazinobutyl moiety with the phenoxazine nitrogen (reaction (1)), producing *m/z* 216 (11%) and 185 (92%). Cleavage of the bond linking the butyl group to the piperazino nitrogen produces *m/z* 272 and 129 (reaction (4)). The C—C bonds α to the phenoxazine and piperazino nitrogens are also labile (reactions (2) and (3), respectively). These reactions produce ions of *m/z* 230 and 143, respectively. Several other fragment ions are formed by the expulsion of H_2O, CH_3, CO, CH_2O, CH_2OH or CH_3OH either from $[M + H]^+$ or from these primary fragment ions. For example, $[M + H]^+$ expels H_2O and CH_3OH to produce *m/z* 384 and 370, *m/z* 185 expels H_2O, CH_3OH and C_3H_6 to produce *m/z* 167, 153 and 143 and *m/z* 143 expels CH_3, H_2O, C_2H_3O and C_2H_5O to produce *m/z* 128, 125, 100 and 98, respectively. The supporting evidence for these reactions is the product ion spectra of the $[M + H]^+$ ion of **12** (Fig. 7), *m/z* 181, 143 and 128 (see Table 2 for the last three spectra). Additional support for these proposals comes from the constant neutral loss of 32 and 31 Da. The constant neutral loss scans of 32 and 31 also suggest that *m/z* 97 is formed from both *m/z* 129 and 128.

CONCLUSIONS

This study has clearly shown that liquid-SIMS in combination with NBA as the matrix is a useful technique for characterization of a variety of 2-chlorophenoxazine derivatives. In general, mass spectral features of these compounds are similar. All compounds yield abundant molecular ions in the form of $M^{+\cdot}$ or $[M + H]^+$ ions. The mass spectra are testimony to the stability of the phenoxazine ring system. No fragmentation is observed in the phenoxazine ring, whereas all bonds in the N-10 side chain portion are prone to cleavage under LSIMS conditions. Common diagnostic fragmentations are cleavage of the C—C bond α to the phenoxazine nitrogen and the bond that connects a side chain to this nitrogen. The acetyl derivatives produce fewer fragments than the corresponding propyl and butyl derivatives.

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