bonate, filtered, and concentrated to dryness on a rotary evaporator. Further drying in vacuum oven gave 6.12 g (90%) of a light-yellow crystalline solid. The solid was washed with hot hexane, and dried in a vacuum oven, giving 5.46 g (85%) of *p*-nitrobenzyl alcohol, mp 92-93.5 °C (lit.[13] mp 93 °C), with IR and NMR spectrum identical with the authentic material.[14]

Acknowledgement. We are grateful to the Ministry of Education for the financial support of this work.

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Tandem Mass Spectrometry of some s-Triazine Herbicides

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Mass spectral fragmentations of some s-triazine herbicides have been investigated using tandem mass spectrometry. Major pathways driven by the side chains have been confirmed. However, most of the previously proposed pathways which were thought to be characteristic of the ring have been found unlikely. A class of ring cleavage reactions characteristic of s-triazine rings with alkylamino side chains has been found and the mechanism has been proposed. In addition, tandem mass spectrometry has been utilized to differentiate tautomeric structures and to analyze the fragmentation reactions occurring from the mixture of isobaric ions.

Introduction

Mass spectrometry is a powerful method for the structural determination and quantitation of organic, inorganic, and biological molecules.[1,2] Various ionization methods such as electron ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), etc. are used to produce molecular ions or quasi-molecular ions from samples. These ions undergo further fragmentation in the ion source depending on their internal energies, producing fragment ions. The resulting ions are analyzed according to their mass-to-charge (m/z) ratios.

For the interpretation[3] of mass spectra and hence for the structural determination various empirical correlations such as the mechanistic pathways are employed to explain the various ions appearing in the spectra. Also, determination of the accurate masses through high resolution mass spectrometry aids the spectral interpretation by providing unequivocal elemental compositions of ions. However, lack of detailed knowledge about the fragmentation pathways for a compound class has been a major weakness in the interpretation procedure.[4]

In tandem mass spectrometry or mass spectrometry/mass spectrometry (MS/MS),[4-6] ions with given m/z are selected and their fragmentation reactions are observed directly. Namely, while the fragmentation mechanisms are deduced indirectly from the mass spectral pattern in the ordinary mass spectrometry, similar information is obtained through direct observation in MS/MS, hence removing any ambiguity on the reaction pathways.

s-Triazines are widely used as pre-emergence selective herbicides, simazine (I, 2-chloro-4,6-bis(ethylamino)-s-triazine), atrazine (II, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine), propazine (III, 2-chloro-4,6-bis-(isopropylamino)-s-

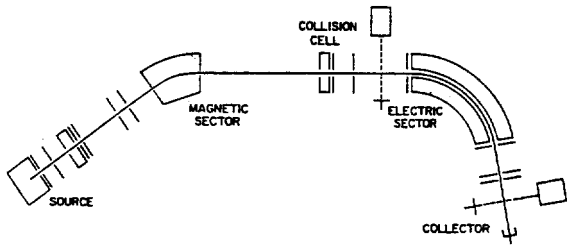
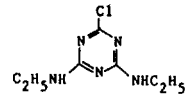
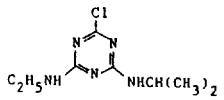


Figure 1. Schematic diagram of VG ZAB-E double focusing mass spectrometer with reversed geometry.

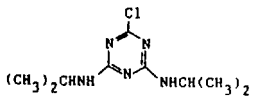
triazine) being the most important. In fact, atrazine is the most widely used herbicide.



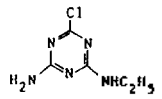
I



II



III



IV

Studies on mass spectral fragmentation pathways have been made,[7-10] prompted by the interest in identifying metabolites of these compounds.[11] However, the reliability of these studies based on the ordinary mass spectrometric methods is doubtful. In the present paper, we report the ion fragmentation pathways for these compounds investigated by MS/MS methods. MS/MS investigation on the ion fragmentation of 2-amino-4-chloro-6-ethylamino-s-triazine (IV) has been included for comparison.

Experimental

Instrument and Principle. The instrument used in this work was VG ZAB-E double focusing mass spectrometer with reversed geometry (Figure 1). With this instrument, ion fragmentation can be observed both in the first field free region (1st FFR) between the source and the magnetic sector and in the second field free region (2nd FFR) between the magnetic sector and the electric sector. In the mass-analyzed ion kinetic energy spectrometry (MIKES),[12] the parent ion (m_1^+) is selected by the magnetic sector. The daughter ion (m_2^+) formed by the fragmentation of m_1^+ in the 2nd FFR

$$m_1^+ \rightarrow m_2^+ + m_3 \quad (1)$$

has the translational energy

$$T(m_2^+) = (m_2/m_1) \times T(m_1^+) \quad (2)$$

Hence, by scanning the potential applied to the electric sector which is a translational energy analyzer all the daughter ions produced from m_1^+ can be detected. The parent ion may dissociate unimolecularly. Such a metastable ion (MI) decomposition is driven by the internal energy of m_1^+ acquired upon ion formation in the source. Since only the ions with narrow internal energy distribution dissociate in the 2nd FFR, ions with the same structure but from different origin tend to show similar MI spectral pattern.[13] Hence, MI spectrum becomes very characteristic of the ionic structure.

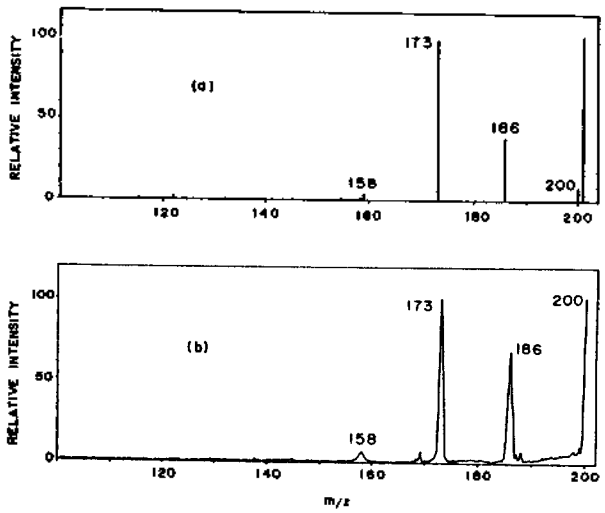


Figure 2. (a) B/E and (b) CA/MIKE spectra of the molecular ion (m/z 201) of simazine.

However, all the possible fragmentation products are not always observed in the MI spectrum because the internal energy of the metastable ions is only slightly (a few eV) larger than the critical energy of the least endoergic fragmentation channel. The most common method to provide further internal energy to the parent ion and hence to enhance the variety and the sensitivity of ion fragmentation is the collisional activation (CA).[6,14] In this method, the parent ion undergoes collision with the neutral gas introduced in the collision cell (Figure 1). Due to the ion/neutral collision some of the translational energy of the parent is converted to its internal energy, facilitating its fragmentation. It is known that CA of ions with keV translational energy deposits internal energy comparable to the ionic energy deposited by 70 eV EI.[15] Hence, CA spectra of molecular ions resemble 70 eV EI mass spectra closely. To observe ion fragmentation occurring in the 1st FFR, various linked scan techniques can be used.[6,16] For example, in the B/E linked scan technique, the magnetic and electric sector fields are scanned simultaneously with their ratio fixed. The information available from the B/E spectra is comparable to that from MIKES.

Experimental Details. Samples were introduced to the ion source using a direct insertion probe. Samples were ionized by 70 eV EI with trap current of 200 μA. Source temperature was 200 °C and accelerating voltage was 8 kV. Elemental compositions for all the ions to be discussed were determined by accurate mass measurement at 10,000 resolution. MIKE and B/E spectra were acquired using a data system (VG 11-250J) and signal averaging was performed when necessary. To obtain MIKE spectra enhanced by collisional activation (CA/MIKES), N_2 was used as the collision gas. The collision gas pressure was adjusted such that the parent ion intensity was attenuated by 30% to minimize multiple collisions.[17]

The s-triazine herbicides (I, II, and III) were analytical grade (Poly Science Co., U.S.A.) and were used without further purification. The compound IV was synthesized and purified according to the method described in the literature.[18,19] Its structure and purity was checked by thin layer chromatography, gas chromatography, and mass spectrometry.

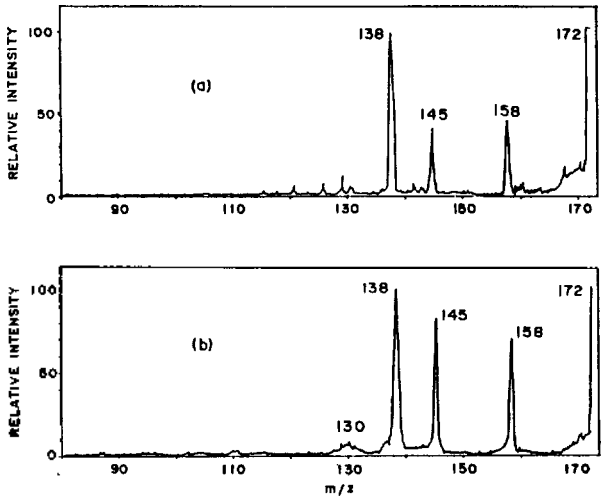


Figure 3. (a) MIKE and (b) CA/MIKE spectra of the *m/z* 173 ion of simazine.

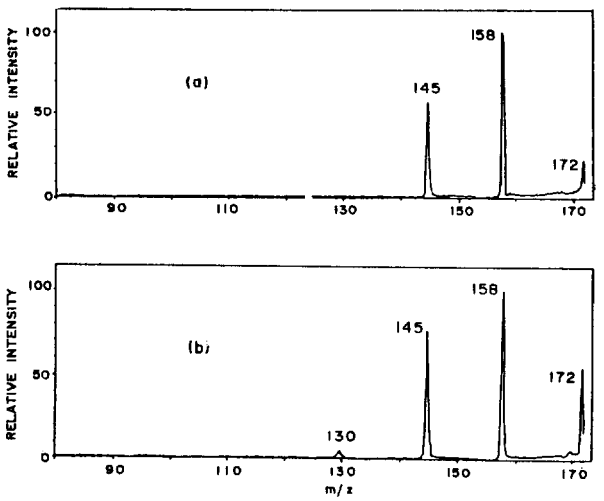
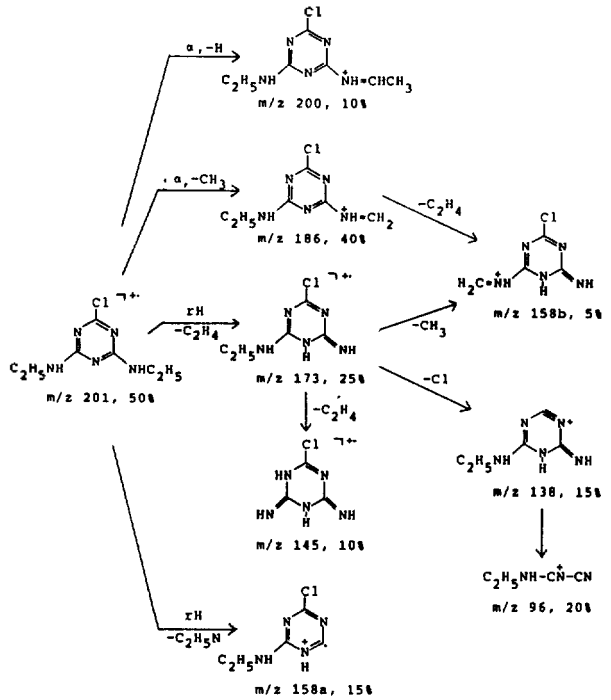


Figure 4. (a) MIKE and (b) CA/MIKE spectra of the *m/z* 173 ion of 2-amino-4-chloro-6-ethylamino-s-triazine (compound IV).

Results and Discussion

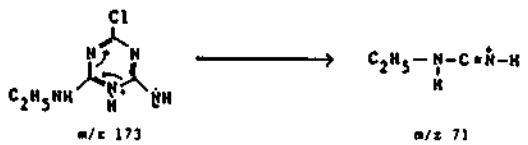
The EI mass spectra of s-triazine derivatives I–IV have been published[7,8,20] and will not be reproduced here. The major fragmentation pathways for the molecular ions of chlorinated s-triazines with alkylamino side chains are α-cleavages and McLafferty rearrangements[3] as proposed by Jörg *et al.*[7] and by Ross and Tweedy.[8] This can be confirmed readily by the MS/MS spectra of the molecular ion (*m/z* 201) of simazine shown in Figure 2. The daughter ions appearing at *m/z* 200 and at *m/z* 186 are α-cleavage products and *m/z* 173 arises from McLafferty rearrangement (Scheme A).



Scheme A

The minor peak at *m/z* 158 which will be designated 158a might arise by loss of an ethylamino side chain together with a hydrogen migration as proposed by Ross and Tweedy. For chlorinated s-triazines with two alkylamino side chains, Jörg *et al.* proposed consecutive reactions of McLafferty rearrangement and α-cleavage. This can be confirmed easily from the MS/MS spectra of the fragment ions. For example, in the MS/MS spectra of *m/z* 173 of simazine shown in Figure 3, the peak at *m/z* 158 which will be designated 158b is due to the methyl loss (α-cleavage) and the peak at *m/z* 145 arises from the ethylene loss (McLafferty rearrangement). The peak at *m/z* 138 is due to the chlorine loss as proposed by Ross and Tweedy. These major pathways are common to simazine (I), atrazine (II), and propazine (III). For atrazine, fragmentation displays more variety than the others due to the presence of dissimilar alkylamino side chains. The major fragmentation pathways which were proposed by previous workers and confirmed by the MS/MS method here are shown in scheme A with simazine as an example. The relative intensities of each ions in the 70 eV EI spectrum of simazine with respect to the base peak (*m/z* 44, $C_2H_5NH^+$) are also shown. The relative intensities of 158a ($C_5H_7N_4Cl$, *m/z* 158.0359) and 158b ($C_4H_5N_5Cl$, *m/z* 158.0233) are based on the intensities of *m/z* 158 doublet observed in the high resolution mass spectrum.

The *m/z* 173 ion from simazine is a tautomer of the molecular ion of 2-amino-4-chloro-6-ethylamino-s-triazine (compound IV). Jörg *et al.* postulated that the former ion underwent subsequent fragmentation like the latter ion with the implicit assumption of facile interconversion between two tautomers. Ross and Tweedy proposed that the *m/z* 71 ion present in the EI spectrum of simazine was formed from *m/z* 173 through a ring cleavage reaction,



(3)

This, together with the reaction $173^+ \rightarrow 138^+ \rightarrow 96^+$ (scheme A), was taken as an evidence for the presence of the tautomeric form of the *m/z* 173 ion shown in eq. (3). However, in our MS/MS spectra of the *m/z* 173 ion from simazine (Figure 3), the *m/z* 71 ion is completely missing indicating that the ring cleavage reaction of the type in eq. (3) is not

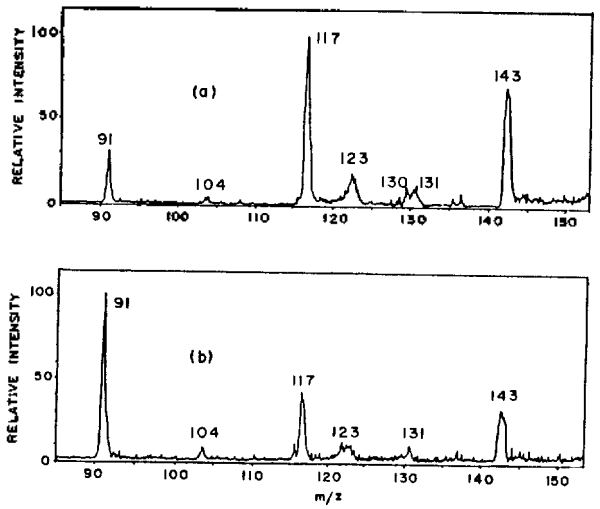


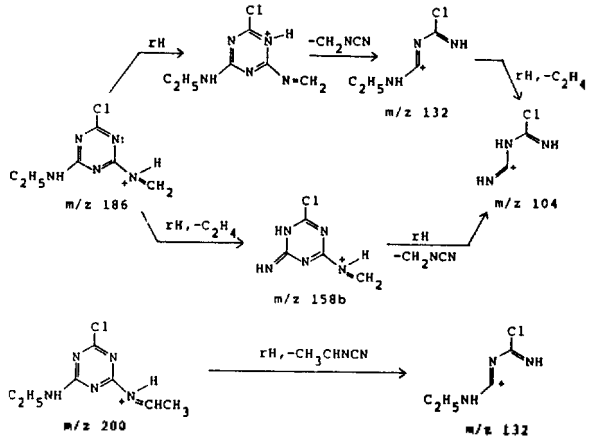
Figure 5. MIKE spectra for the *m/z* 158 ions from (a) simazine and (b) atrazine.

likely. To obtain structural information on the *m/z* 173 ion, MS/MS spectra of the molecular ion (*m/z* 173) of compound IV were obtained. These are shown in Figure 4. Both *m/z* 158 and 145 ions are present in the spectra. However, the *m/z* 138 ion which was the base peak in Figure 3 is completely missing both in the MIKE and the CA/MIKE spectra. Such a substantial difference in MS/MS spectra indicates that the tautomeric compositions of the *m/z* 173 ions from two different origins are widely different. Also, it is apparent from the CA/MIKE spectra that the interconversion between tautomers is not facilitated even when the ion internal energies are increased.

In addition to $173^+ \rightarrow 71^+$ reaction, Ross and Tweedy postulated three more ring cleavage reactions in simazine from precursor ions containing a chlorine atom, namely $200^+ \rightarrow 69^+$, $186^+ \rightarrow 55^+$, and $158a^+ \rightarrow 132^+$. None of these reactions are observed in the MS/MS spectra. The *m/z* 132 ion appears in the MS/MS spectra of the *m/z* 186 ion even though we can not rationalize its reaction mechanism at the moment. The *m/z* 69 and 55 ions must be formed by complicated consecutive reactions.

The fragmentation pathways for the *m/z* 158 ions of simazine are difficult to characterize by MS/MS because the parent peak is a mixture of two different ions, namely 158a and 158b. As mentioned earlier, a to b intensity ratio is approximately 3:1. Corresponding peaks appear at *m/z* 158 and 172 in the EI spectrum of atrazine with the a/b ratios 1:2 and 1:5, respectively. The peak at *m/z* 172 of propazine has the a/b ratio of 1:7. Hence, it is thought that the daughter ions from b-type ions will be more intense in the MS/MS spectra of these ions from atrazine and propazine than in that from simazine. In particular, since the *m/z* 158a and 158b ions from atrazine are thought to have the same structures as the corresponding ions from simazine, respectively, their MS/MS spectra are worth comparison. The MIKE spectra for the *m/z* 158 ions from simazine and atrazine are shown in Figure 5. It is apparent that the *m/z* 91 daughter ion which is formed through the loss of C_2NH_3 is stronger in the MIKE spectrum of *m/z* 158 from atrazine and hence is originated from 158b. On the other hand, the daughter ions 143 and 117, corresponding to the loss of CH_3 and C_2H_3N, respectively, should arise mostly from 158a. Similar neutral losses are observed in the fragmentation of the *m/z* 172 ion from propazine. These fragment ions appear with varying intensities (1-20%) in the EI spectrum of the above compounds. Hence, these ions are of some diagnostic value for the identification of s-triazines with alkylamino side chains.

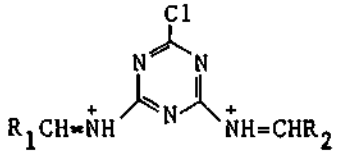
Using the MS/MS method, an important class of ring cleavage reactions which are very structure-specific for s-triazine herbicides have been found. Namely, in simazine, the ions with *m/z* 132 and 104 are formed from the *m/z* 186 ion through the consecutive loss of CH_2NCN and C_2H_4. These reactions form the base peaks in the MIKE spectra of *m/z* 186 and 132, respectively. Even though less efficient, the loss of C_2H_4 followed by the loss of CH_2NCN also occurs. Loss of CH_3CHNCN from *m/z* 200 which appears prominently in its MS/MS spectra is thought to occur through a similar mechanism. Mechanisms proposed to explain these ring cleavage reactions are shown in Scheme B.



Scheme B

Corresponding reactions also occur for atrazine and propazine. For example, $(M-H)^+$ of atrazine at *m/z* 214 loses $(CH_3)_2CNCN$ efficiently to form the *m/z* 132 ion. Similarly, the *m/z* 200 which corresponds to the *m/z* 186 ion from simazine follows two consecutive reaction channels shown in scheme B to form the *m/z* 132 and 104 ions. Intensities of the latter ions are ~15% in the EI spectrum of atrazine. Similar reactions also occur for 2-amino-4-chloro-6-ethylamino-s-triazine (compound IV). Ross and Tweedy postulated that the *m/z* 104 ion appearing with 30% relative intensity in the EI spectrum of this compound was formed by ring cleavage of *m/z* 130 which is equivalent to the *m/z* 158a ion of simazine. This reaction has not been observed in the MS/MS spectra of *m/z* 130, however. On the other hand, *m/z* 104 appears prominently in the MS/MS spectra of *m/z* 172 ($(M-H)^+$) and 158 ($(M-CH_3)^+$) which correspond to *m/z* 200 and 186 of simazine, respectively, through the mechanism shown in scheme B.

Finally, there appear doubly-charged ions with the structure in the EI spectra of the s-triazine herbicides with the relative intensities ~20%. These ions, reported for simazine by Jörg *et al.*, appear at half integer masses (85.5, 92.5, and



99.5 for simazine, atrazine, and propazine, respectively) and are of great analytical importance. The precursors for these ions could not be detected by the MS/MS method. Presumably, these ions are formed from the doubly-charged molecular ions through two α-cleavage reactions very rapidly inside the ion source. The extended conjugation for the above structure seems to counter-balance the strong Coulombic repulsion within the ion and stabilizes the structure.

Acknowledgement. This work was supported by Korea Science and Engineering Foundation.

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Application of BMPI/HOBT Reagent in Solid-Phase Peptide Synthesis

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The suitability of BMPI (2-bromo-N-methyl pyridinium iodide) for solid-phase peptide synthesis was investigated. The coupling rate of BMPI/HOBT procedure. BMPI/HOBT was superior to DCC/HOBT couplings using the solid-phase peptide bond formation proceeded to a greater degree of completion than DCC/HOBT method did. Double couplings with 2 equiv. of Boc-amino acids and 1.5 equiv. of BMPI and NEt_3 and 2 equiv. of HOBT in DMF/MC (1 : 1 v/v) gave the best result for the preparation of a model compound. Stepwise solid phase peptide synthesis using BMPI/HOBT procedure was successfully utilized for the preparation of (D-Ala)^2-dynorphine A. BMPI/HOBT procedure for the synthesis of (D-Ala)^2-dynorphine gave better yield (20%) than DCC/HOBT procedure did.

Introduction

The use of dicyclohexylcarbodiimide (DCC) proposed in 1955 by Sheehan and Hess[1] has since been widely adopted for peptide bond formation. However, this reagent could not be used without shortcomings[2] and side reactions such as racemization or intramolecular rearrangement of the O-acylisourea derivative have been reported during the activation step. Nevertheless, DCC has been widely used for peptide bond formation during Solid Phase Peptide Synthesis (SPPS).[3] Repetitive couplings are often required for the complete introduction of a residue in the peptide chain during SPPS, resulting in the consumption of protected amino acids and time consuming cycles of synthesis. Among the several coupling reagents suggested for the replacement of DCC in SPPS, benzotriazole-1-yl-oxy-tris (dimethyl amino) phosphonium hexafluorophosphate (BOP) was proposed by Fournier *et al.* in recent year.[4] BOP reagent has been used in SPPS for