Effect of Tautomerization on the Fast-Atom Bombardment Tandem Mass Spectra of Azo Dyes

Anthony J. Borgerding and Ronald A. Hites

School of Public and Environmental Affairs and Department of Chemistry, Indiana University, Bloomington, Indiana, USA

The effect of tautomerization on the collision-induced dissociation of negative ions produced from sulfonated azo dyes by fast-atom bombardment (FAB) was studied by analyzing the product ion spectra of several related compounds. The mechanisms by which azo dyes fragment were found to depend on the formation and stability of tautomers. The extent of tautomerization was affected by the number and location of hydroxy groups on the dye, as well as by the FAB matrix. Ions that retained a sodium were often inhibited from forming tautomers and gave different product ions. Substitution of deuterium for hydrogen on the hydroxy groups aided in the identification of ions having more than one possible structure and provided verification of proposed mechanisms. Mechanisms involving ions retaining a sodium were verified by substituting potassium for the sodium. (*J Am Soc Mass Spectrom* 1994, 5, 407–415)

About 150 million pounds of azo dyes are produced in the United States each year [1]. In the process of producing and applying these dyes, about 15% are discharged into waste waters [2]. Azo dyes are visible in surface waters at concentrations of less than 1 ppm, and normal aerobic degradation does not efficiently remove the color. In addition, some azo dyes and their metabolites are carcinogenic [2].

Most azo dyes are either too thermally labile or nonvolatile to be analyzed by gas chromatographic mass spectrometry; thus, mass spectrometry was not widely used for analyzing azo dyes until the advent of condensed-phase ionization techniques, such as fast-atom bombardment (FAB). Early studies by Monaghan et al. [3, 4] using FAB mass spectrometry showed that major fragment ions were formed as a result of cleavage at the azo linkage. A variety of other ionization techniques including thermospray [5–7], plasma desorption [8], atmospheric pressure ionization [9–11], and secondary ionization mass spectrometry [12] have also been used to analyze azo dyes. Many of these studies used tandem mass spectrometry (MS/MS) to provide additional information on structure, and certain fragmentations have been suggested for selectively screening for azo dyes [10, 11, 13, 14].

Preliminary studies of azo dyes in our laboratory using FAB and MS/MS suggested that many common fragmentations were dependent on the extent to which the compounds could tautomerize. Tautomerization of azo dyes has been studied extensively in solution, in

most cases using either NMR, ultra-violet, or visible spectrometry [15–17], but its effect on the mass spectrometric behavior of these compounds has not been studied. Mass spectrometry has been used, however, to study the effect of tautomerization on other classes of compounds, especially those involving keto-enol pairs [18]. Most of this previous work has focused on tautomerization in the gas phase after ionization by analyzing dissociations of cations produced by electron impact. For example, the difference between the keto-enol tautomer equilibrium in a series of simple ketone homologues has been proposed as an explanation of relative peak intensities in their mass spectra [19]. Mass spectrometry has also been used to characterize the tautomeric equilibrium of dimethyl phosphite and dimethyl phosphonate ions [20]. Studies of the tautomerization of gas-phase neutrals using neutralization-reionization mass spectrometry have shown that relative stabilities of neutral tautomers can be different than their corresponding ions [21, 22]. Neutral tautomers interconvert slowly in the gas phase, as shown by Masur et al. [23], who were able to separate three keto-enol tautomers by gas chromatography before analyzing the isomerically pure compounds with mass spectrometry.

Our strategy in studying the importance of tautomerization on the ion fragmentation of azo dyes was to compare the product-ion spectra of both $(M - Na)^-$ and $(M - H)^-$ ions from a group of azo dyes which differed primarily in the number and location of hydroxy substituents, groups which control the extent to which dyes can tautomerize. Our results show that fragmentation depends on the ability of the precursor ion to form a ketohydrazone tautomer.

Address reprint requests to Dr. Ronald A. Hites, School of Public and Environmental Affairs and Department of Chemistry, Indiana University, Bloomington, IN 47405.

Experimental

Materials

Glycerol, thioglycerol, ethylene glycol, triethanolamine, diethanolamine, oxalic acid, and all dyes were obtained from the Aldrich Chemical Co. (Milwaukee, WI). The dyes were 65–99% pure; liquid chromatography and FAB mass spectrometry analysis of the samples indicated that the impurities were simple salts such as NaCl and Na₂SO₄ and not organic impurities. Therefore, the dyes were used without further purification. Azobenzenesulfonic acid was synthesized in our laboratory by sulfonation of azobenzene which was purchased from Aldrich. Potassium chloride (analytical reagent grade) was obtained from Mallinkrodt (St. Louis, MO); and deuterium oxide (99.8% isotopic purity) was obtained from Cambridge Isotope Laboratories (Woburn, MA).

Instrumentation

All experiments were done on a VG 30-250 triple quadrupole mass spectrometer (Altrincham, UK) using FAB ionization. An Ion Tech FAB gun (Teddington, UK) was operated at 1 mA and 8 keV using xenon. The radiofrequency-only collision cell was held at 1.0 mtorr of SF₆ (as measured by a Baratron capacitance manometer, MKS Instruments, Burlington, MA), and the collision energy was 10 eV, unless noted otherwise. All collision energies are reported in the laboratory frame of reference. A product ion mass range from 30 to 400 mass units was scanned every 2 seconds. The spectra shown below are the average of 20 scans.

Sample Preparation

An amount corresponding to 10 mg of pure dye was weighed and dissolved in 1 mL of water, and 2 μ L of this solution was measured onto the probe tip, along with 1 μ L of the matrix: Glycerol was used for Acid Orange 7, while 3-nitrobenzyl alcohol gave better results for the other dyes. For the matrix comparison experiments, 50 mg of Acid Orange 7 were dissolved directly in 5 mL of the pure matrix. An additional sample of Acid Orange 7 was made using 50 mg of dye and 5 mL of glycerol that had been spiked with 100 μ L of aqueous 1 M oxalic acid. After allowing the analyte to dissolve in the matrix overnight, approximately 2 μ L was applied to the FAB probe tip for analysis.

For some experiments, the hydroxy groups of the dyes were deuterated. This was accomplished by dissolving the dyes in D₂O instead of regular water and adding that solution to the matrix as usual. This converted approximately 90% of the dye to the deuterated product. Potassium was substituted for sodium on the dyes by mixing 20 mg of KCl with 10 mg of dye prior to dissolution. The addition of KCl generally caused

incomplete dissolution, but it did not inhibit analysis. Over 80% of the sodium salt was changed to the potassium salt according to the mass spectral data.

Results and Discussion

The hydroxy group in almost all modern acid azo dyes is in the *ortho*-position relative to the azo linkage. In this position, hydrogen bonding makes the hydroxy group a much weaker acid than the *para*-isomer; the *ortho*-isomer is therefore less susceptible to color changes [24]. Having the hydroxy group in the *ortho* position also leads to the formation of ketohydrazone tautomers. While the formation of these tautomers does not affect the mass-to-charge ratio of the molecular anion formed from FAB ionization, it does affect the fragmentation of azo dyes under collision-induced dissociation (CID) conditions. For example, the product-ion spectrum of the (M - Na)⁻ ion from Acid Orange 7 (see Figure 1a) shows a C-N cleavage ion at *m/z* 156 and an azo cleavage ion at *m/z* 171 (see Scheme 1a). The latter ion includes a rearranged hydrogen, and it forms only by cleavage of the N-N bond of the ketohydrazone form of the dye, which can form only when an *ortho*-hydroxide group is present (see Scheme 1a). This concept was confirmed by deuterating the hydroxy group; the ketohydrazone cleavage product ion was shifted to *m/z* 172 (see Figure 1b). Acid

Figure 1. Product-ion spectra of the (M - Na)⁻ ions of (a) Acid Orange 7, (b) deuterated Acid Orange 7, (c) Acid Orange 8, and (d) azobenzenesulfonic acid sodium salt. See Scheme 1.

Orange 8, which is a methylated homologue of Acid Orange 7 (see Scheme Ia), gave a product-ion spectrum in which the azo cleavage ion was shifted 14 Da higher than that of Acid Orange 7; see m/z 185 in Figure 1c. Note that the C-N cleavage ion is also shifted by 14 Da to m/z 170. Clearly, similar fragmentation mechanisms are operative for both dyes, and the azo cleavage ions (at m/z 171 and 185) are formed from the ketohydrazone tautomer.

Azo dyes without an *ortho*-hydroxy or amino group cannot form the ketohydrazone tautomer, and thus the azo cleavage ion at m/z 171 is not formed. For example, the product-ion spectrum of azobenzenesulfonic acid (see Figure 1d), which has no *ortho*-hydroxy group, exhibits only a C-N cleavage to form an ion at m/z 156 and no N-N cleavage (see Scheme Ib).

The FAB mass spectra of sulfonated azo dyes also show ions from the loss of a proton instead of the loss of a sodium ion; these $(M - H)^-$ ions are 5-30% of the abundance of the $(M - Na)^-$ ions. The product-ion spectra of these $(M - H)^-$ ions often reveal information not seen in the $(M - Na)^-$ spectra. When a proton (rather than a sodium) is lost, the mechanisms by which fragmentations occur change, especially when the proton that is lost is the one that could shift to allow formation of a tautomer. This is usually the case, given the stability of the resulting phenoxide ions. For example, the $(M - H)^-$ ion of Acid Orange 7 cannot rearrange to form a ketohydrazone tautomer (see Scheme II). As a result, the product-ion spectrum of this ion (see Figure 2a) shows no sign of the typical C-N or azo cleavages at m/z 156 or 171, or other cleavage of a ketohydrazone structure. Instead, the $(M - H)^-$ ion loses N_2 to form an ion at m/z 321, followed by a loss of SO_2 to form an ion at m/z 257, which finally loses a sodium to form an ion at m/z 234 (see Scheme II). The ion at m/z 234 is probably stabilized by a rearrangement to form an extended *para*-quinone, because it occurs in high abundance.

Scheme I. Acid Orange 7 and 8.

Scheme II. Acid Orange 7 and 8.

Figure 2. Product-ion spectra of the $(M - H)^-$ ions of (a), Acid Orange 7, (b) the potassium salt of Acid Orange 7, and (c) Acid Orange 8. See Scheme II.

We studied the fragmentation of the $(M - H)^-$ ion of Acid Orange 7 more thoroughly by comparing the product-ion spectra over a range of collision energies (energy resolved mass spectrometry [25]). The results are shown in Figure 3 as the percent relative abundance of each ion versus collision energy. The data show that the ion at m/z 321 is preferred for collision energies less than about 8 eV. The formation of this ion requires that both C-N bonds be broken and that the aromatic rings become directly attached, perhaps via an ion-molecule complex intermediate [26]. At higher collision energies, the formation of this ion is less favorable. Instead, simple cleavage of the C-N bond to form the ion at m/z 156 is preferred. In addition, the abundances of the ions at m/z 257 and 234, relative to m/z 321, increase at energies between 5 and 15 eV, indicating that these product ions may form from the ion at m/z 321.

The loss of N_2 from negative ions of azo dyes has been previously postulated by Richardson et al. [13]. In the data they reported, only compounds which did not have an *ortho*-hydroxy group, and which were not capable of forming tautomers, lost N_2 . Dyes we have analyzed follow this trend as well. In compounds containing an *ortho*-hydroxy group, only the $(M - H)^-$ ions, which are incapable of forming tautomers, lost N_2 . This is more evidence that fragmentation using CID is dependent on the presence or absence of tautomers.

Our overall mechanism is supported by results from the analysis of the product-ion spectrum of Acid Orange 7 in which the sodium was replaced with potassium (see Figure 2b). Because potassium weighs 39 Da and Na weighs 23 Da, all of the potassium-containing ions are shifted by 16 Da relative to the sodium-containing ion, except for the ion at m/z 234, which does not contain the metal atom. The product-ion spectrum of the $(M - H)^-$ ion of Acid Orange 8 (Figure 2c) is very similar to that of Acid Orange 7, but all

of the ions are shifted 14 mass units due to the substitution of CH_3 for H.

Tautomerization of these dyes in the mass spectrometer and the subsequent dissociation of molecular anions in the collision cell are affected by several different processes. Our experiments have shown that the extent of fragmentation depends on the FAB matrix used; for example, the different ratios of the fragment ions at m/z 171 and 156 from Acid Orange 7 are given in Table 1. This matrix effect could be due to two different processes: (1) The internal energy of ions sputtered from different matrices could be different [27], which would change the extent to which the ion dissociates in the ion source or upon collisional activation. (2) In the FAB matrix solution, the dyes are in a tautomeric equilibrium that is influenced by external hydrogen bonding between the dye and the matrix. In basic matrices, the hydroxy group in the azohydroxy tautomer can form stronger hydrogen bonds than the imino group in the ketohydrazone tautomer; hence the azohydroxy form is stabilized. In acidic matrices, the basic imino group of the ketohydrazone form is stabilized and the equilibrium shifts in favor of this tautomer [28-30]. For example, phenylazo-1-naphthol is 85% in the azohydroxy form in pyridine but only 11% in acetic acid [30]. This solution-phase equilibrium may be retained after bombardment by the FAB beam, ionization into the gas phase, and transmission of ions to the collision cell. This latter idea is supported by the decrease in the m/z 171 to 156 ratio with increasing basicity of the matrix (see Table 1); the ion at m/z 171 forms exclusively from the ketohydrazone tautomer, while m/z 156 can form from either tautomer (see Scheme I). However, we cannot say that this is the only process by which the matrix affects the CID spectrum. Phenomenologically, the process by which the matrix effect is occurring is less important than the results of the process.

Another process that could affect the CID spectrum of these dyes is dissociation of molecular anions in the ion source region. If one of the tautomers is less stable toward dissociation, less of its molecular anion will reach the collision cell to be analyzed in the product-ion scan, unless the gas-phase equilibrium is fast enough to replenish it. However, it is unlikely that isomeriza-

Figure 3. Plot of relative product-ion abundance versus collision energy for the $(M - H)^-$ ion of Acid Orange 7. Each datum is based on 20 scans; the relative standard deviation of each measurement is about $\pm 5\%$. See Scheme II.

Table 1. Ratio of the m/z 171 to 156 in the product ion spectra of $(M - Na)^-$ from Acid Orange 7 ionized from various matrices^a

Matrix	Ratio
Oxalic acid, 0.02 M in glycerol	0.70
Thioglycerol	0.51
Glycerol	0.45
Ethylene glycol	0.45
Triethanolamine	0.27
Diethanolamine	0.23

^aListed in increasing order of basicities [31].

tion of this type would be fast, and indeed previous studies of keto-enol pairs have shown it to be slow [20]. Other relevant studies, again using keto-enol tautomers, have shown that ion mobility spectrometry can be used to separate the tautomers [32], indicating slow interconversion of the tautomeric ions. Our data show that unimolecular dissociation of this type does not cause preferential removal of either of the tautomers because evidence of both tautomers is present in the spectra. For example, the ion at m/z 171 from Acid Orange 7 comes only from the ketohydrazone tautomer; the ion at m/z 261 from azobenzenesulfonic acid comes only from the azohydroxy tautomer.

Tautomerization is evident in the CID spectrum of the $(M - Na)^-$ ion of Acid Orange 12 (see Figure 4a), an isomer of Acid Orange 7. Note that the structure of Acid Orange 12 differs from that of Acid Orange 7 only in that the SO_3 group is on the other side of the molecule (see Scheme III). As with Acid Orange 7, Acid Orange 12 shows an azo cleavage across the N-N bond of the ketohydrazone tautomer, in this case to form an ion at m/z 235. Product-ion scans of the ion at m/z 235, which also forms in the initial FAB ionization, indicate that the ion at m/z 207 was formed by a loss of 28 Da from this intermediate ion. In this case, we suggest that this ion represents the loss of CO from the ion at m/z 235 (see Scheme III). To support this scheme, we deuterated the hydroxy group. The CID spectrum of the deuterated ion at m/z 328 (see Figure 4b) also showed ions at m/z 235 and 207, indicating that the proton from the hydroxy group is *not* a part of the fragment ions, supporting the structures given in Scheme III. Further support of these structures is the loss of 26 Da, corresponding to CN, in the product-ion spectrum of the m/z 207 ion.

As with Acid Orange 7, the $(M - H)^-$ ion of Acid Orange 12 cannot form a ketohydrazone tautomer, and therefore it fragments differently than the $(M - Na)^-$ ion (see Figure 5a). The fragmentation occurs by two separate mechanisms. Like Acid Orange 7, it loses N_2 , SO_2 , and Na sequentially to form ions at m/z 321 and

Scheme III. Acid Orange 12.

234 (see Scheme IV), but these ions are low in abundance compared to the ion at m/z 221. This latter ion corresponds to cleavage of the C-N bond of the azo linkage, to form an ion at m/z 244, and subsequent loss of the sodium (see Scheme IV). As with the $(M - H)^-$ ion of Acid Orange 7, these ions may form via an ion-molecule complex intermediate [26]. The rearrangement of a hydrogen and ring formation may explain the high abundance of this ion at m/z 221. Such ring formation is not possible in Acid Orange 7 because the SO_3 group is not on the naphthalene moiety. In addition, the rearrangement to form an extended quinone, which stabilized the ion at m/z 234 in Acid Orange 7, cannot occur in Acid Orange 12, explaining the low abundance of this ion. More support of the structure of the ion at m/z 221 was obtained from its product-ion spectrum, which shows a loss of 28 Da, corresponding to CO. The presence of a metal in some of the fragment ions was verified by substituting potassium for sodium, and noting the proper shift in their masses (see Figure 5b). Note that the ions at m/z 221 and 234 do not shift because they do not contain a metal atom.

Figure 4. Product-ion spectra of the $(M - Na)^-$ ions of (a) Acid Orange 12 and (b) the deuterated Acid Orange 12. See Scheme III.

Figure 5. Product-ion spectra of the $(M - H)^-$ ions of (a) Acid Orange 12 and (b) potassium salt of Acid Orange 12. See Scheme IV.

Scheme IV. Acid Orange 12.

In compounds such as Acid Alizarin Violet N, which contain two *ortho*-hydroxy groups, three tautomers can be formed (see Scheme V). The CID spectrum of the $(M - Na)^+$ ion of this dye (see Figure 6a) shows several unusual ions that are stabilized by the second

hydroxy group on the molecule. For example, there is an abundant ion at m/z 186 resulting from the cleavage of the azo bond, indicating a fragmentation of the azohydroxy tautomer rather than of either of the keto-hydrazone forms, which would fragment to m/z 185 or m/z 187. The product-ion spectrum of the deuterated form (see Figure 6b) shows that the mass of this ion has shifted to m/z 187, verifying that this fragment contains one exchangeable proton. The product-ion spectrum of the m/z 186 ion, when formed directly by FAB ionization, shows an ion corresponding to HSO_3^- at m/z 81. This indicates that the proton of the hydroxy group *meta* to the sulfonate is transferred to the sulfonate, allowing formation of a four-membered ring and a resonance stabilized anion (see Scheme V, left).

The ion at m/z 263 is from the loss of SO_3 from the $(M - Na)^+$ ion; because it retains both labile protons, the mass of this ion shifts to m/z 265 upon deuteration (see Figure 6b). The ion at m/z 143 is due to the naphtholate ion. This is an unusual example of an ion formed from the side of the molecule not containing the original charged moiety. The deuterated compound fragments to form m/z 144 (see Figure 6b); thus, this ion forms from C-N cleavage of either the azohydroxy or the keto-hydrazone tautomer that retained the naphthol moiety, followed by transfer of an hydroxy proton. These processes may occur via an ion-molecule complex intermediate [26]. A loss of 28 Da, corresponding to CO, in the product-ion spectrum of this ion further confirms the naphtholate structure. Product-ion scans of the ion at m/z 263 indicate that the naphtholate ion forms from both the intact $(M - Na)^+$ ion and after it has lost SO_3 . Formation of the

Scheme V. Acid Alizarin Violet N.

Figure 6. Product-ion spectra of the $(M - Na)^+$ ions of (a) Acid Alizarin Violet N, (b) deuterated Acid Alizarin Violet N, and (c) Palatine Chrome Black. See Scheme V.

naphtholate ion in the CID mass spectra of azo dyes has not been reported previously; it is formed from $(M - Na)^+$ ions only in compounds containing two hydroxy groups *ortho* to the same azo bond.

Analysis of the product-ion spectrum of the deuterated dye revealed that the ions at m/z 171 and 121 did not shift by 1 mass unit; therefore, they do not contain any exchangeable protons. This indicates that both m/z 171 and 121 were formed from the ketohydrazone tautomer (see Scheme V, right). The ion at m/z 121 is another example of the second hydroxy group stabilizing an anion by allowing resonance forms. It forms from the loss of SO_2 after cleavage of the azo bond in one of the possible ketohydrazone forms. The stability of this ion explains why the sulfonated intermediate is not observed. The ion at m/z 171 forms from the cleavage of a C-N double bond of a ketohydrazone form, forming an epoxide. While the ring strain of this structure makes formation of this ion difficult, the only alternative ion with no labile protons would contain two radical electrons in addition to the charge retained by the sulfonate. Of the two, the epoxide is much more likely.

All of these same fragmentations are seen in the product-ion spectrum of Palatine Chrome Black, the dinaphthalene homologue of Acid Alizarin Violet N (see Figure 6c). Note that the ions corresponding to fragments of the sulfonated part of the molecule (at m/z 121, 171, 186, and 263) are shifted up 50 Da, corresponding to the difference between the benzene and the naphthalene groups.

The fragmentation of the $(M - Na)^+$ ion of Acid Alizarin Violet N over a range of collision energies gives more information about its fragmentation pro-

cesses. Figure 7 shows that the relative abundance of the ion at m/z 186 decreases with increasing energy, with a corresponding increase in the abundance of the HSO_3^- ion, indicating further dissociation of the m/z 186 ion at higher energies. The ions at m/z 263 and 171 also show lower stabilities at higher energies. The naphtholate ion at m/z 143 is the most abundant fragment ion at collision energies above 15 eV.

Because Acid Alizarin Violet N has two *ortho*-hydroxy groups, it can form two tautomers even in ions containing sodium (see Scheme VI). Therefore, the product-ion spectrum of the $(M - H)^+$ ion has some features in common with that of the $(M - Na)^+$ ion; namely, the ions at m/z 171, 143, and 121 (see Figure 8a). However, the presence of the sodium clearly has an effect on the ion chemistry. For example, the product-ion spectrum of the $(M - H)^+$ ion shows no evidence of cleavage across the azo linkage to form the ion at m/z 186, as occurred for the $(M - Na)^+$ ion. In this ion, the proton transfer that allowed the resonance stabilized structure to form cannot occur. Instead, the naphtholate ion at m/z 143 dominates the spectrum; note that this ion shifts to m/z 144 upon deuteration (see Figure 8b). Because none of these product ions contains sodium, only the $(M - H)^+$ ion shifts upon treatment with potassium (see Figure 8c).

Conclusions

We have shown that azo-hydrazone tautomerization has a large effect on the fragmentation of azo dyes in MS/MS. The number and location of hydroxy substituents controls the extent to which azo dyes can tautomerize and controls the mechanisms by which the ions dissociate. Furthermore, the fragmentation process is dependent on the presence or absence of a metal atom in the ion, because this often inhibits formation of ketohydrazone tautomers. Because tautomerization is common in almost all dyes, this con-

Figure 7. Plot of relative product-ion abundance versus collision energy for the $(M - Na)^+$ ion of Acid Alizarin Violet N. Each datum is based on 20 scans; the relative standard deviation of each measurement is about $\pm 5\%$. See Scheme V.

Scheme VI. Acid Alizarin Violet N.

cept may be applicable to the interpretation of tandem mass spectra of dyes in general. Unusual patterns in the fragmentation of Acid Alizarin Violet N suggest that the presence of substituents containing electronegative atoms on the same aromatic group as the sulfonate may allow the formation of resonance stabilized anions. Clearly, more information about the structure of these compounds can be obtained by analyzing the product-ion spectra of *both* the $(M - Na)^-$ and $(M - H)^-$ ions. Shimanskas et al. [33] have suggested removing the metal ions from dyes prior to analysis to

simplify the spectra and increase sensitivity. Clearly, this procedure would eliminate a potential source of information on the structure of the dye.

Acknowledgment

We thank the U. S. Department of Energy (grant no. 87ER-60530) for support.

References

1. Text. Chem. Col. **1992**, 24, 6.
2. Zollinger, H. *Color Chemistry: Synthesis, Properties and Applications of Organic Dyes and Pigments*; VCH: New York, 1987; pp 321-324.
3. Monaghan, J.; Barber, M.; Bordoli, R.; Sedgwick, R.; Tyler, A. *Org. Mass Spectrom.* **1982**, 18, 75.
4. Monaghan, J.; Barber, M.; Bordoli, R.; Sedgwick, R.; Tyler, A. *Org. Mass Spectrom.* **1982**, 17, 569.
5. Voyksner, R. *Anal. Chem.* **1985**, 57, 2600.
6. McLean, M.; Freas, R. *Anal. Chem.* **1989**, 61, 2054.
7. Yinon, J.; Jones, T.; Betowski, L. *Biomed. Environ. Mass Spectrom.* **1989**, 18, 445.
8. Pannell, L.; Sokoloske, E.; Fales, H.; Tate, R. *Anal. Chem.* **1985**, 57, 1060.
9. Bruins, A.; Weidolf, L.; Henion, J.; Budde, W. *Anal. Chem.* **1987**, 59, 2647.
10. Edlund, P.; Lee, E.; Henion, J.; Budde, W. *Biomed. Environ. Mass Spectrom.* **1989**, 18, 233.
11. Lee, E.; Muck, W.; Henion, J.; Covey, T. *Biomed. Environ. Mass Spectrom.* **1989**, 18, 253.
12. Scheifers, S.; Verma, S.; Cooks, R. *Anal. Chem.* **1983**, 55, 2260.
13. Richardson, S.; McGuire, J.; Thruston, A.; Baughman, G. *Org. Mass Spectrom.*, **1992**, 27, 289.
14. Monaghan, J.; Morden, W. *Rapid Commun. Mass Spectrom.*, **1990**, 4, 436.
15. Hsieh, B.; Desilets, D.; Kazmaier, P. *Dyes and Pigments* **1990**, 14, 165.
16. Tawarah, K.; Abu-Shamleh, H. *Dyes and Pigments* **1991**, 16, 241.
17. Yamamoto, K.; Nakai, K.; Kawaguchi, T. *Dyes and Pigments* **1989**, 11, 173-177.

Figure 8. Product-ion spectra of the $(M - H)^-$ ions of (a) Acid Alizarin Violet N, (b) deuterated Acid Alizarin Violet N, and (c) the potassium salt of Acid Alizarin Violet N. See Scheme VI.

18. Schwartz, H. *Adv. Mass Spectrom.* **1985**, 13-34.
19. Hrusak, J.; Tkaczyk, M. *Org. Mass Spectrom.* **1990**, 25, 214.
20. Kenttamaa, H.; Cooks, R. *J. Am. Chem. Soc.* **1985**, 107, 1881.
21. Wong, T.; Terlouw, J.; Keck, H.; Kuchen, W.; Tommes, P. *J. Am. Chem. Soc.* **1992**, 114, 8208.
22. Wesdemiotis, C.; McLafferty, F. *J. Am. Chem. Soc.* **1987**, 109, 4760.
23. Masur, M.; Grutzmacher, H.; Munster, H.; Budzikiewicz, H. *Org. Mass Spectrom.* **1987**, 22, 493.
24. Zollinger, H. *Color Chemistry: Synthesis, Properties, and Applications of Organic Dyes and Pigments*; VCH: New York, 1987; pp 107-109.
25. Busch, K.; Glish, G.; McLuckey, S. *Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry*; VCH: New York, 1988; pp 78-82.
26. McAdoo, D. *Mass Spectrom. Rev.* **1988**, 7, 363.
27. Cooks, R.; Busch, K. *Int. J. Mass Spectrom. Ion Phys.* **1983**, 53, 111.
28. Barnes, A.; Majid, M.; Stuckey, M.; Gregory, P.; Stead, C. *Spectrochimica Acta* **1985**, 41A, 629.
29. Suzuki, S.; Das, R.; Harada, K.; Stoyanov, S. *Bull. Chem. Soc. Jpn.* **1980**, 53, 2666.
30. Kishimoto, S.; Kitahara, S.; Manabe, O.; Hiyama, H. *J. Org. Chem.* **1978**, 43, 3882.
31. Cook, K.; Todd, P.; Friar, D. *Biomed. Environ. Mass Spectrom.* **1989**, 18, 492.
32. Karpas, Z.; Stimac, R.; Rappaport, Z. *Int. J. Mass Spectrom. Ion Proc.* **1988**, 83, 163.
33. Shimanskas, C.; Ng, K.; Karliner, J. *Rapid Commun. Mass Spectrom.* **1989**, 3, 300.