GC–MS Analysis of Bromoxynil Esters: Anomalous Results Explained

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Analytical confirmation of the presence of pesticide residues in environmental samples routinely requires the use of gas chromatography–mass spectrometry (GC–MS). While the electron impact (EI) mass spectra of bromoxynil octanoate and the free phenol, bromoxynil, in the NIST/EPA/NIH library are essentially the same, the mass spectra of bromoxynil esters are, in fact, substantially different from the spectrum of bromoxynil itself: an acylium ion is the major diagnostic species for the esters. Mass spectral libraries therefore need to be amended to reflect this information. The direct determination of bromoxynil ester residues by GC–MS is more difficult than that of bromoxynil itself. The fragment ions seen for the esters are explained.

INTRODUCTION

GC–MS Systems. Gas chromatography–mass spectrometry (GC–MS) has become a routine analytical method in laboratories that carry out pesticide residue analysis. The coupling of the separation power of capillary GC with the characterization made possible by molecular fragmentation has yielded an analytical method that almost every pesticide analytical laboratory knows it now must have to be scientifically credible.

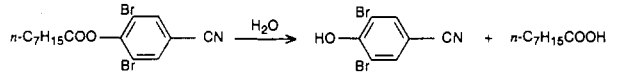
Electron ionization mass spectrometry (EI-MS) is now the basis of the pesticide residue monitoring used in regulatory systems throughout the world. Other ionization techniques also contribute; however, the EI technique enjoys wide acceptance.

Bromoxynil Esters. The herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), used to control broadleafed weeds in cereal crops (wheat, oats, rye, and barley) in the Canadian prairie provinces, is often formulated as the octanoate ester and is present as an active ingredient in Buctril M and Hoegrass II (Worthing and Hance, 1991). It was also commercially available as the heptanoate and butanoate esters, although these formulations have now been discontinued in Canada.

Bromoxynil esters have been reported (Smith, 1980) to hydrolyze readily in many soils to yield the free phenol (Scheme I). Analytical methods used to monitor environmental matrices, e.g., soil and water, for bromoxynil and bromoxynil esters include electron capture GC, ultraviolet high-pressure liquid chromatography (UV-HPLC), and GC–MS (Bruns et al.,1991; Muir et al., 1991; Krawchuk and Webster, 1987; Brown et al., 1984). Both GC and HPLC methods are capable of detecting residues of both the esters and the free phenol. Confirmation of residue identity characteristically relies on GC–MS techniques.

In the case of bromoxynil octanoate, mass spectral libraries do not allow an easy distinction between the ester and free bromoxynil. The current NIST/EPA/NIH (1992) library lists much the same mass spectrum for each (Figure 1). The EI mass spectrum of bromoxynil octanoate differs remarkably from that in the NIST/EPA/NIH database and from that of bromoxynil itself, leading to the potential for false negative results for detection of bromoxynil

Scheme I



octanoate from methodology based on GC–MS analysis plus library search techniques. Muir et al. have indicated that the mass spectra "were dominated by four and eight carbon fragments" and that "no parent ions were observed". The nature of the mass spectra obtained was not described further except that these authors developed a selected ion monitoring program for bromoxynil esters using m/z 127, 248, and 277 (Muir et al., 1991). No discussion of the library spectrum of the octanoate was included. We report the complete EI mass spectra of four bromoxynil esters and recommend that mass spectral libraries be corrected to reflect this information.

EXPERIMENTAL PROCEDURES

Chemicals. Reference standards included bromoxynil and bromoxynil octanoate (Riedel-de Haën AG, Seelze, Germany) and bromoxynil heptanoate and bromoxynil butanoate (supplied by Canada Department of Fisheries and Oceans, Winnipeg, MB). Solvents used were of distilled-in-glass quality (Caledon Laboratories, Georgetown, ON).

GC–MS Analysis. All four compounds, dissolved in toluene (~10 ng/μL), were analyzed by capillary column GC–EI-MS in which the column, or a capillary transfer line, transported the eluates directly into the ion sources of the mass spectrometers. Bromoxynil octanoate was also examined by gas chromatography–negative ion chemical ionization mass spectrometry (GC–NICI-MS).

Quadrupole Mass Spectrometry. Analysis was performed by EI-MS on a Hewlett-Packard 5790 mass selective detector (MSD) instrument. Samples were introduced through a Hewlett-Packard HP5890 GC operated in the splitless mode, with a 30-m DB-5 capillary column of 0.25-mm i.d. and a 0.25-μm film thickness, a helium carrier gas flow rate of 1.2 mL/min, and a transfer line temperature of 280 °C. The injector port temperature was 280 °C, and the oven program was as follows: 70 °C (2 min), 10 °C/min, 250 °C (10 min). The ion source temperature was 250 °C.

Ion Trap Mass Spectrometry. Analysis was performed by EI-MS, on a Finnigan Model 801 ion trap detector (ITD) supported by an IBM PS2/30 personal computer. Samples were introduced through an HP5890 GC, operating in the splitless mode, with a 60-m DB-5 column of 0.25-mm i.d. and a 0.1-μm film thickness, a He carrier gas flow rate of 1.7 mL/min, and a transfer line temperature of 280 °C. The injector port temperature was 200 °C, and the oven program was as follows: 100 °C

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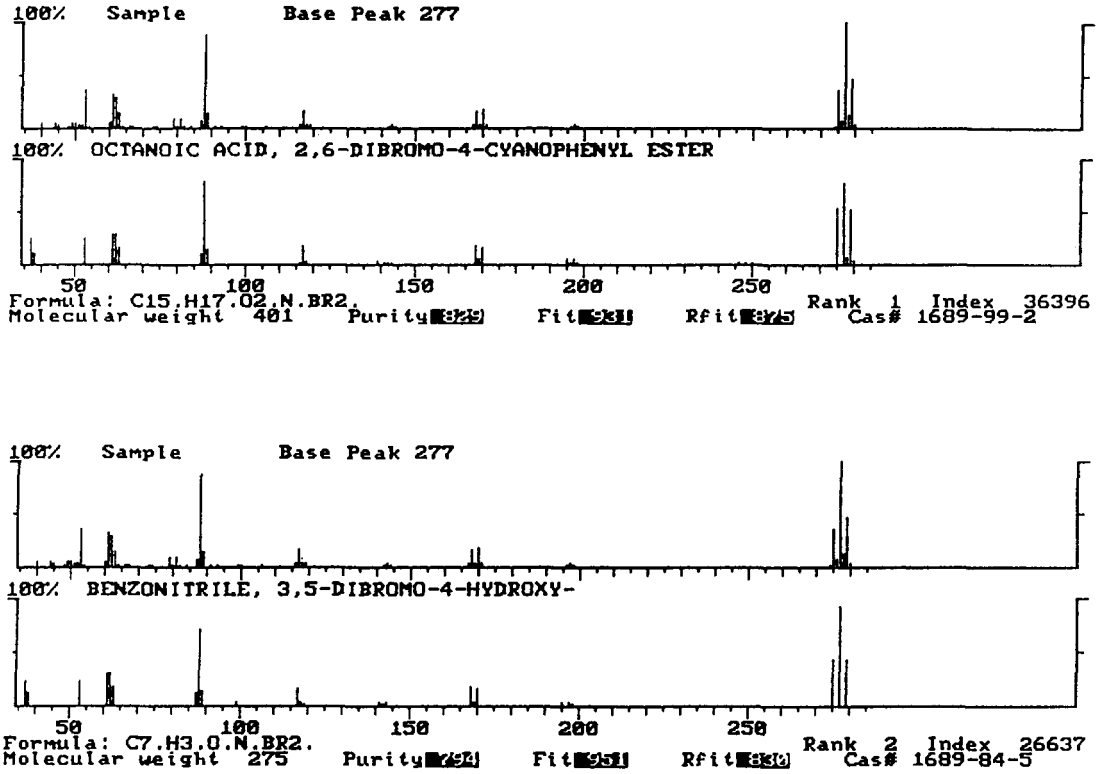


Figure 1. Experimental EI mass spectrum of a bromoxynil sample compared to those of bromoxynil and bromoxynil octanoate retrieved by the NIST/EPA/NIH library installed on the data system of the ion trap mass spectrometer.

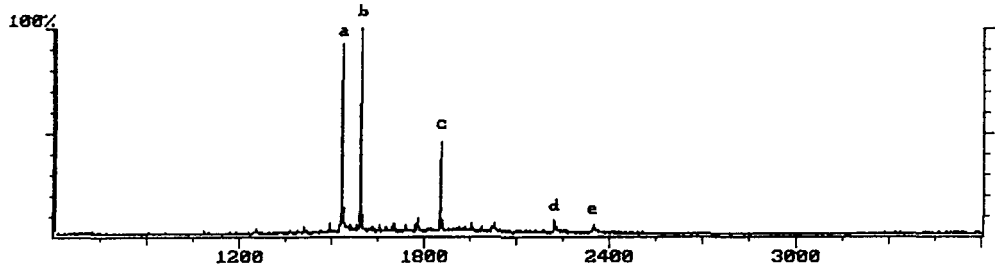


Figure 2. Total ion chromatogram for a toluene solution containing bromoxynil (a) and its acetate (b), butanoate (c), heptanoate (d), and octanoate (e) esters.

(3 min), 5 °C/min, 250 °C (6 min), 2 °C/min, 280 °C (3 min). The ion source temperature was 250 °C.

Sector-Field Mass Spectrometry. Analysis was performed on a VG 7070E-HF double-focusing (EB geometry) mass spectrometer, supported by an 11-250J data system. Samples were introduced through an HP 5890A GC, operating in the splitless mode, with a 60-m DB-5 column of 0.25-mm i.d. and 0.25-μm film thickness, a helium flow rate of 20 cm/s (1.2 mL/min), and a transfer line temperature of 250 °C. The injector port temperature was 265 °C, and the oven program was as follows: 80 °C (1 min), 30 °C/min, 210 °C (1 min), 10 °C/min, 265 °C (20 min). For EI-MS the ion source temperature was 250 °C. In the NICI-GC mode the ion source temperature was 100 °C and nitrogen was used to thermalize the electrons. Fast atom bombardment (FAB) mass spectrometry was performed by bombardment of bromoxynil octanoate in glycerol or 3-nitrobenzyl alcohol matrices with 8 keV of xenon atoms.

RESULTS AND DISCUSSION

Initial indication that the EI mass spectrum of bromoxynil octanoate, generated by the HP GC–MSD, was not consistent with library mass spectra was observed during routine analysis of residues of bromoxynil octanoate and bromoxynil in samples of runoff water from treated plots (Kenny et al., 1992). In this case, the presence of the octanoate had been demonstrated by capillary GC with electron capture detection.

The NIST/EPA/NIH library EI mass spectrum of bromoxynil is shown in Figure 1. Similar spectra were generated by our GC–MSD, GC-ITD, and GC–sector-field-MS instruments. The molecular ion, $[CNC_6H_2Br_2OH]^{\cdot+}$, is clearly visible as the bromine-containing group of peaks at m/z 275/277/279. Fragment ions are present at m/z 195/197, 168/170, and 88.

When analyzed by GC–MS, bromoxynil and its esters gave well-defined total ion current peaks (Figure 2) at the retention times expected from their GC electron capture responses. The mass spectrum of bromoxynil was as expected. However, the mass spectra of the esters (Figure 3) do not show a molecular ion; a bromoxynil fragment is barely detectable in the ITD mass spectra, but there are clear, though small, peaks (m/z 275/277/279) in the spectra obtained with the sector-field instrument. Instead, for bromoxynil octanoate, there is a prominent m/z 127 ion, which is consistent with $[C_7H_{15}CO]^+$ expected from the octanoyl residue. Similarly, the heptanoate gives a prominent m/z 113 ion, $[C_6H_{13}CO]^+$, consistent with the presence of the heptanoyl group; the butanoate gives m/z 71, $[C_3H_7CO]^+$, characteristic of the butanoyl group, and the acetate gives m/z 43, $[CH_3CO]^+$, from the acetyl group. It should be noted that we confirmed the integrity of the bromoxynil octanoate used for these experiments by obtaining its FAB mass spectrum, which showed the expected $[M + 1]^+$ molecular ion cluster at m/z 402/404/406.

Since positive ion EI mass spectra do not give unequiv-

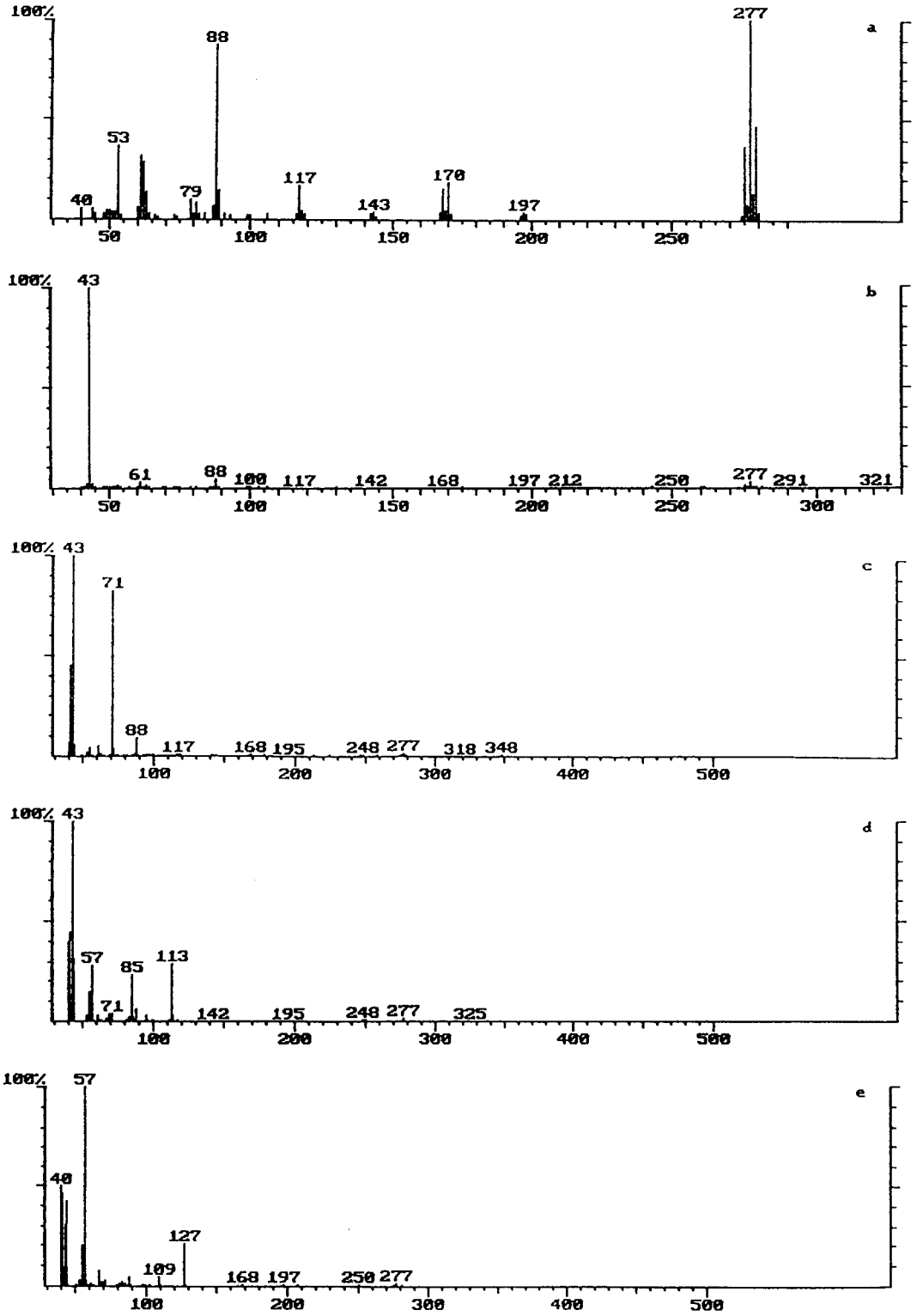


Figure 3. EI mass spectra of bromoxynil and its esters: (a) bromoxynil; (b) acetate; (c) butanoate; (d) heptanoate; (e) octanoate.

ocal confirmation of the elution of undecomposed esters, we examined bromoxynil octanoate by GC–NICI-MS. Again, the molecular ion was absent; the base peak (m/z 274/276/278) corresponded to $[CNC_6H_2Br_2O]^-$, from the bromoxynil moiety. Nevertheless, this result confirms that undecomposed bromoxynil octanoate elutes from the GC column because the negative ion detected is complementary to the ion detected in the EI positive spectrum.

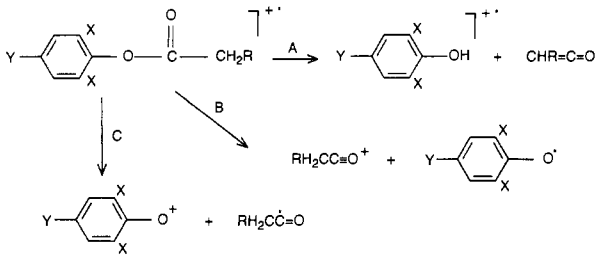
To understand the typical EI induced fragmentations of substituted phenyl acylates, we selected appropriate compounds (Table I) from the NIST/EPA/NIH mass spectral library. In each case, the spectra are dominated by ions resulting from scission of the ester C–O bond, but the simple scission denoted by pathway C (Scheme II) is suppressed in favor of the scission accompanied by rearrangement of a hydrogen atom from the acyl group to give an ionized phenol (pathway A). Pathway B yields an acylium ion plus a phenoxy radical. The relative importances of pathways A and B are strongly influenced by the electronic properties of the substituents attached to the aromatic ring. Such effects on mass spectra are well-known. The compounds in Table I are arranged in order of these properties, as given by their Hammett σ or σ^+ constants (March, 1985). (Since the σ^+ constants were introduced for reactions in which an electron-releasing group interacts with a developing positive charge in the

Table I. Influence of Electronic Properties[a] of the Substituents on Relative Abundances of Phenol and Acylium Fragment Ions of Substituted Phenyl Acylates

compound	σ	σ^+	phenol ion [m/z (RA%)] (pathway A)	acylium ion [m/z (RA%)] (pathway B)
bromoxynil octanoate	*b*	*b*	275/277/279 (277 = 1.8%)	127 (100)
bromoxynil heptanoate	*b*	*b*	275/277/279 (277 = 1.6%)	113 (100)
bromoxynil butanoate	*b*	*b*	275/277/279 (277 = 2.0%)	71 (100)
bromoxynil acetate	*b*	*b*	275/277/279 (277 = 3.4%)	43 (100)
4-methylphenyl acetate	−0.14	−0.31	108 (100)	43 (3.6)
3-methylphenyl acetate	−0.06	−0.10	108 (100)	43 (46)
phenyl acetate	0	0	94 (100)	43 (55)
phenyl propanoate	0	0	94 (100)	57 (40)
4-chlorophenyl acetate	0.24	0.11	128 (100)	43 (26)
4-bromophenyl acetate	0.26	0.15	172/174[c] (100)	43 (48)
2-bromophenyl acetate	*b*	*b*	172/174 (100)	43 (41)
3,5-dichlorophenyl acetate	0.74[d]	0.80[d]	162 (40)	43 (100)
4-nitrophenyl acetate	0.81	0.79	139 (73)	43 (100)

[a] Hammett constants for meta or para substituents, as appropriate. [b] See text. [c] Probable m/z values (error in database). [d] Sum of Hammett constants.

Scheme II



transition state, they may well be the better indicator to use in the present discussion.) It can be clearly seen that electron-releasing substituents promote fragmentation by pathway A, while electron-withdrawing substituents promote fragmentation by pathway B. This is in accord with Stevenson's rule (Stevenson, 1951) whereby, in bond scission, the positive charge preferentially locates on the fragment of lower ionization energy.

The electronic effects of ortho substituents are often not well-described by the Hammett equation, so that the effects of the bromo substituents of bromoxynil esters are not amenable to prediction from Hammett constants. It is noteworthy that the mass spectrum of 2-bromophenyl acetate is very similar to that of 4-bromophenyl acetate, both of which are dominated by the respective bromophenol ions. Consequently, the strongly electron-withdrawing nitrile substituent (σ_p = 0.70, σ_p^+ = 0.66) is expected to promote fragmentation by pathway B, so that the failure to observe the diagnostic fragment for bromoxynil in mass spectra generated by any of our mass spectrometers is now not surprising.

In summary, these results demonstrate that the fragmentation of bromoxynil octanoate on EI is consistent with the mass spectral behavior of related compounds and that its spectrum in the current NIST/EPA/NIH mass spectral library is in error and should be corrected as shown above. Analytical methods based on diagnostic library searching for bromoxynil octanoate will present some difficulties if the standard GC–MS procedures are used, particularly by monitoring laboratories where the instrument technician may be trained to recognize peak patterns rather than to look for reasonable mass spectra or to realize whether a credible mass spectrum has been obtained.

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