Determination of *s*-Triazine Metabolites: A Mass Spectrometric Investigation

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The *s*-triazine herbicides have highly polar metabolites that often cannot be analyzed directly by gas chromatography−mass spectrometry (GC-MS). Chemical derivatization may be necessary to improve identification and quantitative analytical methods. The analysis of eight suspected metabolites of two *s*-triazine herbicides, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] and ametryn [2-(methylthio)-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], was performed in the electron-impact mode by direct inlet system−mass spectrometry (DIS-MS) and by GC-MS, after sample derivatization. So far, previous derivatization procedures were either difficult or yielded a mixture of derivatives. By using *N*-methylbis[trifluoroacetamide] (MBTFA), we obtained only one derivative of each metabolite, and the method easily detected microbial degradation-derived metabolites. Moreover, we identified some frequently present ions that can be used in the analysis of complex samples.

Keywords: *Atrazine; ametryn; herbicides; s-triazine metabolites; analysis*

INTRODUCTION

The herbicidal activity of *s*-triazines was discovered in 1952 by a J. R. Geigy S.A. research group in Basel, Switzerland (Knuesli et al., 1969). These herbicides are used mainly on corn, sorghum, and sugar cane. Residues of triazines are frequently found in environmental samples of soil and water, because of their extensive use and relatively high persistence and solubility in water. Therefore, it is important not only to know how much of the parent compound remains in the environment, but also to identify and quantify the metabolites (photolyic, microbial, chemical). Studies on the fragmentation patterns of triazines have either been limited to relatively few *s*-triazine metabolites or have been incomplete (Ross and Tweedy, 1970; Hapeman-Somich et al., 1992). Gas chromatography−mass spectrometry (GC-MS) is often used on the parent compounds (Davoli et al., 1987) because of its high sensitivity, but most *s*-triazine metabolites are highly polar and often cannot be analyzed directly by this method. Chemical derivatization may be necessary to improve identification and quantitative analytical methods (Benfenati et al., 1990). Previous derivatization procedures were either difficult or yielded a mixture of derivatives (Khan et al., 1975; Lusby and Kearney, 1978). Moreover, a combination of different mass spectrometric methods gives integrated information for analytical characterization of the organic molecules (Benfenati et al., 1994). Different triazines lead to the same metabolites, and the structure of unknown products can be hypothesized by the identification of well-known fragmentation.

The aims of this work were twofold. First, to aid in the identification of *s*-triazine metabolites in water or biological samples by presenting a new GC-MS method of analysis of eight suspected metabolites of two *s*-triazine herbicides, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] and ametryn [2-(methylthio)-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], after a very simple derivatization. The second goal was to suggest some characteristic fragmentations, which are obtained from the easily interpretable trifluoroacetylated mass spectra and some direct inlet system (DIS) mass spectra. The eight metabolites are: Cl-deethyl-atrazine [2-chloro-4-amino-6-(isopropylamino)-*s*-triazine], deisopropyl-atrazine [2-chloro-4-(ethylamino)-6-amino-*s*-triazine], Cl-diamino-atrazine [2-chloro-4-amino-6-amino-*s*-triazine], OH-atrazine, OH-deethyl-atrazine, OH-deisopropyl-atrazine, OH-diamino-atrazine, and deethyl-ametryn [2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine]. By using *N*-methylbis[trifluoroacetamide] (MBTFA) we obtained only one derivative of each metabolite. Moreover, we present some preliminary results on the application of our derivatization method in the analysis of some bacterial samples collected to study the metabolism of atrazine.

EXPERIMENTAL PROCEDURES

Materials. Atrazine and ametryn (98%) were purchased from Supelco Inc. (Bellefonte, PA); Cl-deethyl-atrazine, Cl-deisopropyl-atrazine, Cl-diamino-atrazine, OH-atrazine, OH-deethyl-atrazine, OH-deisopropyl-atrazine, and OH-diamino-atrazine were purchased from Labor Dr Ehrenstonfer (Ausburg, West Germany); deethyl-ametryn was a product of bacterial metabolism studies in our laboratory (unpublished results). Each standard solution was kept in methanol obtained from Merck (Darmstadt, West Germany). MBTFA was purchased from Pierce (Rockford, IL).

Preparation of Bacterial Samples. Mixed bacterial strains collected from soil were cultured both in liquid and agar-solidified media, using atrazine or ametryn (0.05%) as the carbon source. Metabolites from solid agar plates and from supernatants of liquid media were extracted by methanol. Purification by thin-layer chromatography (TLC) was performed on silica gel (UV_{254nm}), with dichloromethane:methanol:formic acid:water (90:15:3:2) as solvent. *s*-Triazine metabolites were identified by GC-MS and by DIS-MS, before and after sample derivatization by MBTFA.

Derivatization. The trifluoroacetyl derivatives were prepared by placing the samples in a glass microreaction vial with MBTFA, capping the vial, heating the solution at 110 °C for 2 h.

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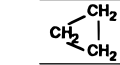
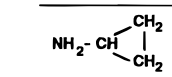
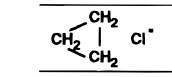
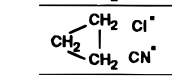
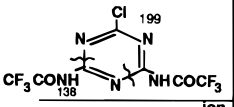
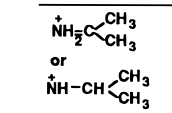
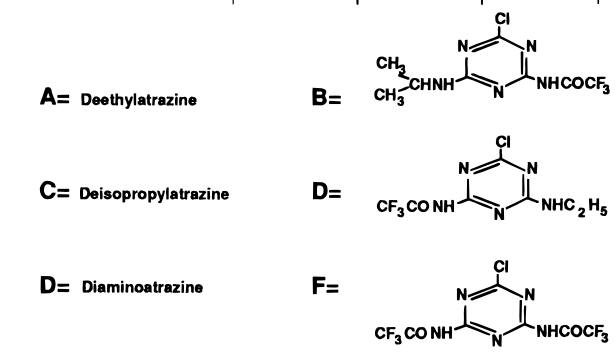
Table 1. Some Cl-Atrazine Metabolites and Their Main Fragments Determined by GC-MS in the EI Mode before and after Trifluoroacetylation by MBTFA

	A	B	C	D	E	F
Molecular ion	187	283	173	269	145	337
Fragment						
$CH_3^{\bullet}$	172	268	158	254		
$CH_2 = CH_2$			145	241		
$Cl^{\bullet}$					110	
	145	241				
C_2H_5N			130			
	130	226				
$C_2H_4, Cl^{\bullet}$			110	206		
CF_3						268
$C_2H_5N, CN^{\bullet}$			104			
	110	206				
	104	200				
$COCF_3$		186		172		
						198 139
ion $CH_3CH{=}\overset{+}{N}HCN$	69	69	69	69		
ion $\overset{+}{C}F_3$						69
$\overset{+}{N}H{\equiv}C - NHCN$	68	68	68	68		
ion 	58	58				
ion $CH_2{=}\overset{+}{N}HCN$			55	55		



Instruments. A VG TS-250 mass spectrometer coupled directly (without molecular separator) to a gas chromatograph HP 5890 equipped with a split-splitless injector was used. The capillary column was a CP Sil 8 CB (length, 25 m; i.d., 0.32 mm; film thickness, 0.12 μm; Chrompack, Middelburg, The Netherlands). The chromatographic conditions were as follows: injection temperature, 240 °C; oven temperature program, from 80 to 300 °C at 20 °C/min; carrier gas, helium; head pressure, 31 kPa. The parameters of the mass spectrometer were as follows: electron-impact (EI) electron energy, 70 eV; source temperature, 200 °C; and trap current, 500 mA.

RESULTS AND DISCUSSION

2-Cl-*s*-Triazine Metabolites. The mass spectral analyses of Cl-deethylatrazine, Cl-deisopropylatrazine, and Cl-diaminoatrazine, before and after derivatization by MBTFA, are shown in Table 1. The initial loss of the chlorine atom occurs only in the Cl-diamino-atrazine metabolite, and is a very simple fragmentation process, as already reported (Ross and Tweedy, 1970). The structures of the ions at m/z 145 and at m/z 130, which are common to Cl-deethyl- and Cl-deisopropyl-atrazine, respectively, and the structure of the ion at m/z 110, which is common to the three underivatized molecules, are shown in Figure 1. Trifluoroacetylation in these compounds occurs on the NH_2 moiety. The mass spectra of the three trifluoroacetylated compounds are shown in Figure 2. The monoacetylated derivatives follow almost the same fragmentation patterns as the underivatized ones ([M − CH_3], [M − C_2H_4 (or C_3H_6)], [M − C_2H_4 (or C_3H_6) − Cl], etc.), with some exceptions; that is, the relative abundance of [M] and [M − CH_3] is very high with respect to that of the other fragments, which is <25%, and there are ions present at m/z [M − $COCF_3$]. In the trifluoroacetylated Cl-diaminoatrazine metabolite there is the loss of the CF_3 group, but not of the $COCF_3$ fragment.

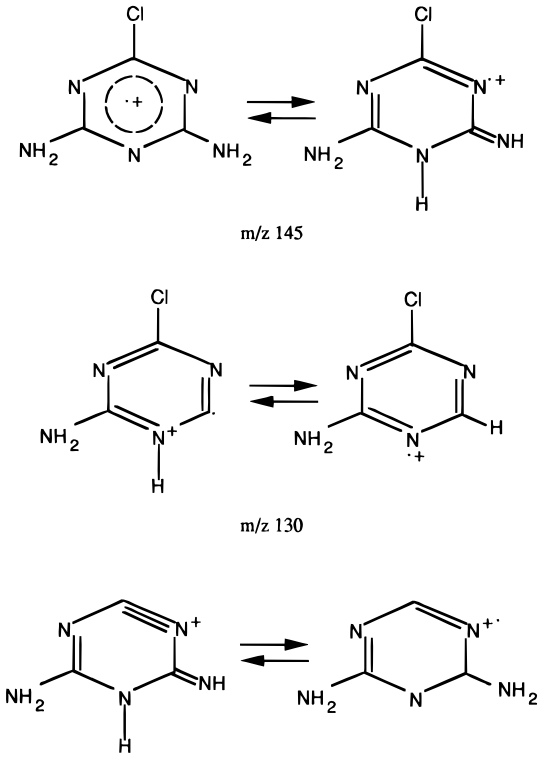


Figure 1. Structures of ions at m/z 145 and 130, which are common to 2-Cl-4-amino-6-(isopropylamino)-*s*-triazine and 2-Cl-4-(ethylamino)-6-amino-*s*-triazine, respectively, and at m/z 110, which is also common to 2-Cl-4-amino-6-amino-*s*-triazine.

2-OH-*s*-Triazine Metabolites. The mass spectral analyses of 2-OH-, 2-OH-deethyl-, 2-OH-deisopropyl-, and 2-OH-diaminoatrazine, before and after derivatizatization by MBTFA, are shown in Table 2. Some fragmentations of these metabolites are similar to their 2-Cl- analogues, and others are characteristic of the hydroxylic group on the *s*-triazine ring. The DIS mass spectra of 2-OH-deethyl-, 2-OH-deisopropyl-, and 2-OH-diaminoatrazine are shown in Figure 3. 2-OH-Diaminoatrazine has a very simple fragmentation, probably due to its symmetry: the base peak is at m/z 126 [M − H]; then there is the ion at m/z 98, derived from it by the loss of CO; at m/z 86 [$^{+}$OH=CNCNH_2]; at m/z 110, which has a very low abundance referred to its ana-

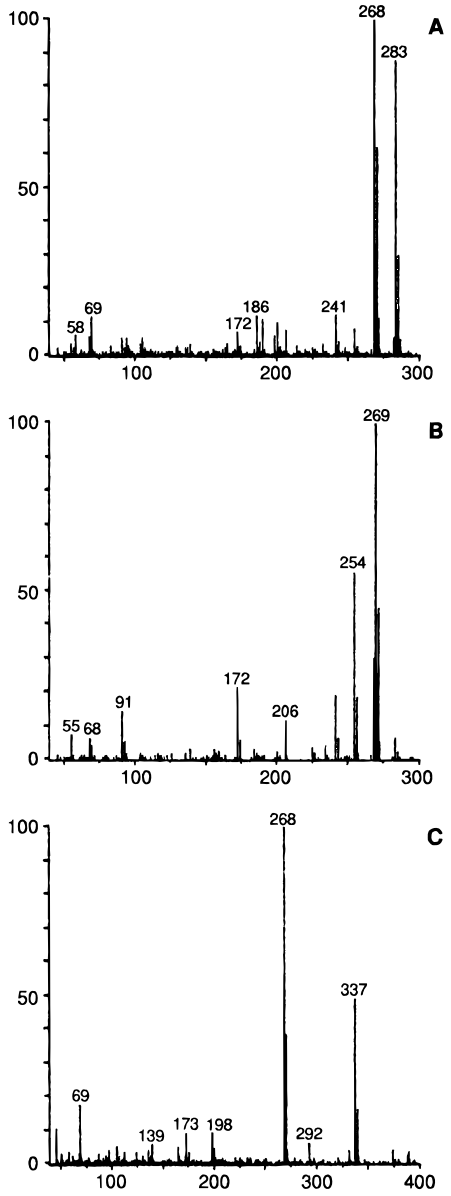


Figure 2. Mass spectra of the three trifluoroacetylated 2-Cl-metabolites: (A) 2-Cl-4-amino-6-(isopropylamino)-*s*-triazine; (B) 2-Cl-4-(ethylamino)-6-amino-*s*-triazine; and (C) 2-Cl-4-amino-6-amino-*s*-triazine.

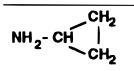
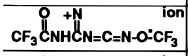
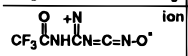
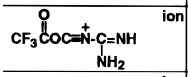
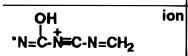
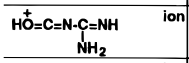
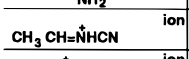
logues in the Cl-substituted metabolites, at *m*/*z* 68 [NH_2CN^+CN], at *m*/*z* 56 [$+N{=}C{=}NO^{\bullet}$].

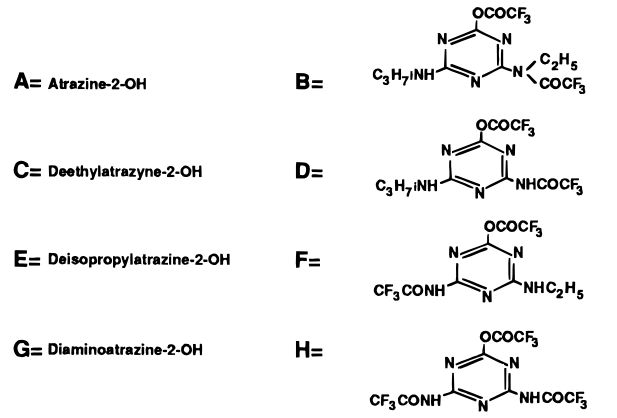
Two proposed noteworthy fragmentation patterns due to the presence of the hydroxylic group are shown in Figure 4. The ion at *m*/*z* 127, which is the molecular ion in 2-OH-diamino-atrazine, in the 2-OH-deethyl- and 2-OH-deisopropyl-atrazine mass spectra is due to the loss of C_3H_6 and C_2H_4, respectively. This ion is fragmented to the ion at *m*/*z* 57 either by subsequent loss of the OH radical, HCN, and the CN radical, as proposed in pathway a, or by loss of CO, the NH_2 radical, and the CN radical, as proposed in pathway b.

The GC-MS spectra of 2-OH-, 2-OH-deethyl-, 2-OH-deisopropyl-, and 2-OH-diamino-atrazine, after derivatization by MBTFA, are shown in Figure 5. The OH moiety was always trifluoroacetylated. Trifluoroacetylation also occurs on the NHC_2H_5 group in 2-OH atrazine and on the NH_2 moieties in 2-OH-deethyl-, 2-OH-deisopropyl-, and 2-OH-diamino-atrazine.

Deethyl-ametryn. The GC-MS mass spectrum of deethylametryn is shown in Figure 6. Some ions are common to the atrazine metabolites; they are, the ions

Table 2. Some OH-Atrazine Metabolites and Their Main Fragments Determined by GC-MS in the EI Mode before and after Trifluoroacetylation by MBTFA

	A	B	C	D	E	F	G	H
Molecular ion	197	389	169	361	155	347	127	415
Fragment								
$H^{\bullet}$							126	
$CH_3^{\bullet}$	182	374	154	346		332		
$CH_2 = CH_2$	169				127			
$H^{\bullet}$, CO							98	
	155	347		319				
	140	331		303				
CF_3		320		292		278		
$COCF_3$		292		264		250		
 ion								263
 ion								194
 ion		182		182		182		
$COCF_3^+$ ion		97		97		97		
 ion			97		97			
 ion							86	
 ion			69		69			
$NH_2C{\equiv}\overset{+}{N}CN$ ion	68						68	
$+N{=}C{=}N{-}\dot{O}$ ion							56	56



at *m*/*z* 58 [$(CH_3)_2CHNH^+$], at *m*/*z* 68 [NH_2CN^+CN], at *m*/*z* 69 [CH_3CHNH^+CN], at *m*/*z* 83 [$(CH_3)_2CNH^+CN$], and at *m*/*z* 110.

The mass spectrum of diamino-atrazine extracted from bacterial samples, after derivatization by MBTFA, is shown in Figure 7. We can identify this metabolite by some characteristic peaks and by the same retention time as the derivatized standard, despite the presence of a high background.

The examined *s*-triazine metabolites were easily analyzed by DIS-MS and by GC-MS after our derivatization method. The combination of these two MS methods has been used to obtain a good analytical characterization of the molecules studied and of their main fragmentation pathways. The characterization of some common fragmentations of *s*-triazines can help in rapidly identifying these compounds and unknown metabolites. The yield of only one trifluoroacetylated derivative of each considered metabolite by using MB-

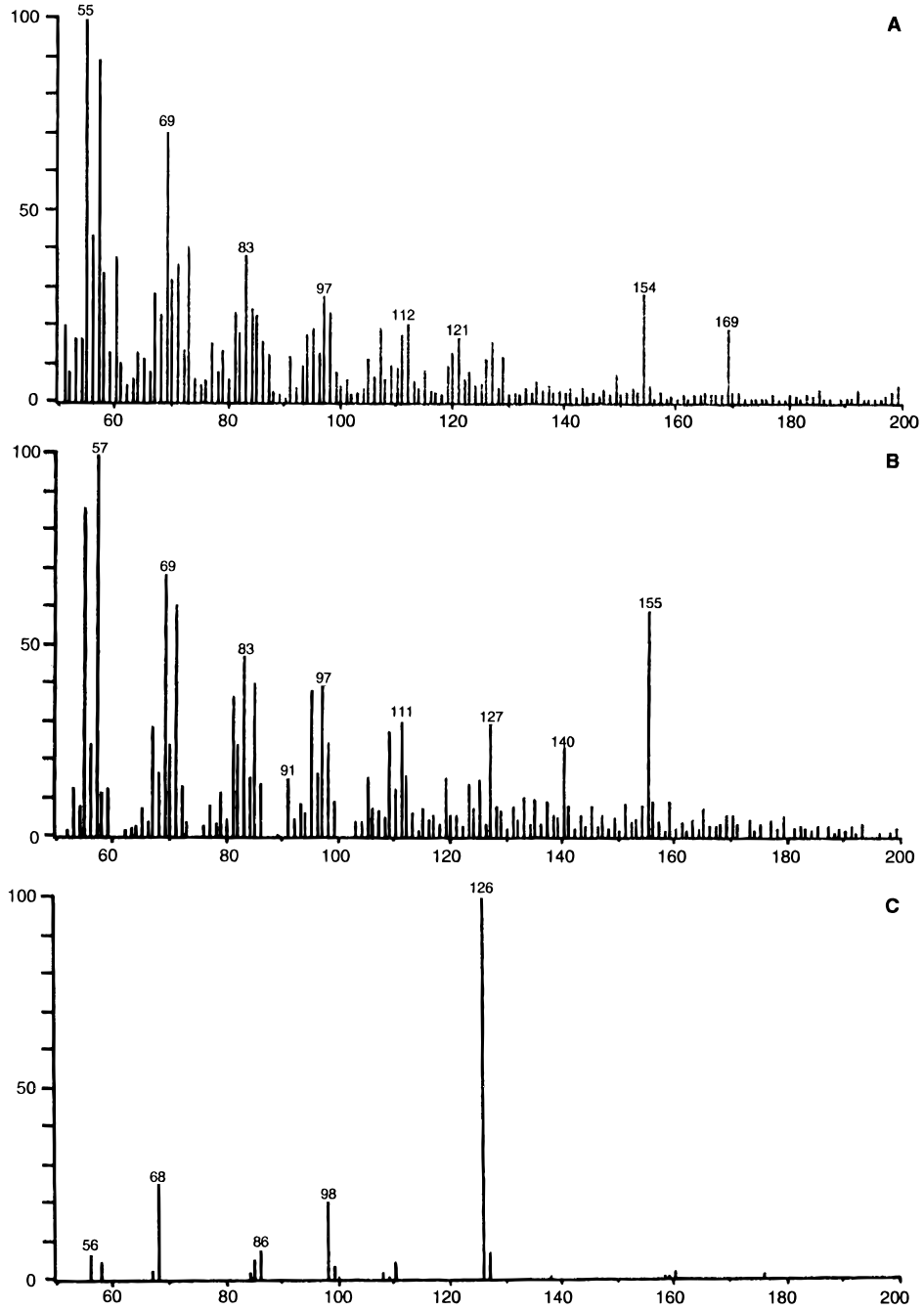


Figure 3. DIS mass spectra of (A) 2-OH-4-amino-6-(isopropylamino)-*s*-triazine, (B), 2-OH-4-(ethylamino)-6-amino-*s*-triazine, and (C) 2-OH-4-amino-6-amino-*s*-triazine.

TFA will allow us to easily quantify this class of compounds by GC-MS in environmental and biological matrices.

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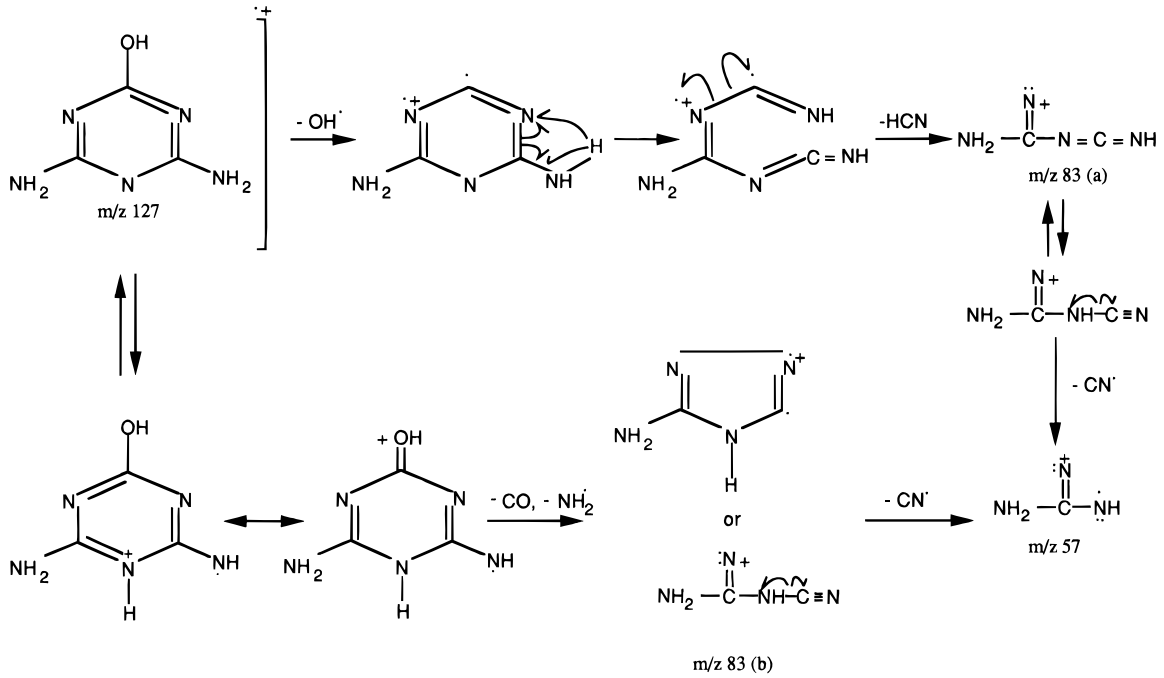

Figure 4. Two proposed noteworthy fragmentation patterns of 2-OH-4-amino-6-(isopropylamino)-*s*-triazine and 2-OH-4-(ethylamino)-6-amino-*s*-triazine.

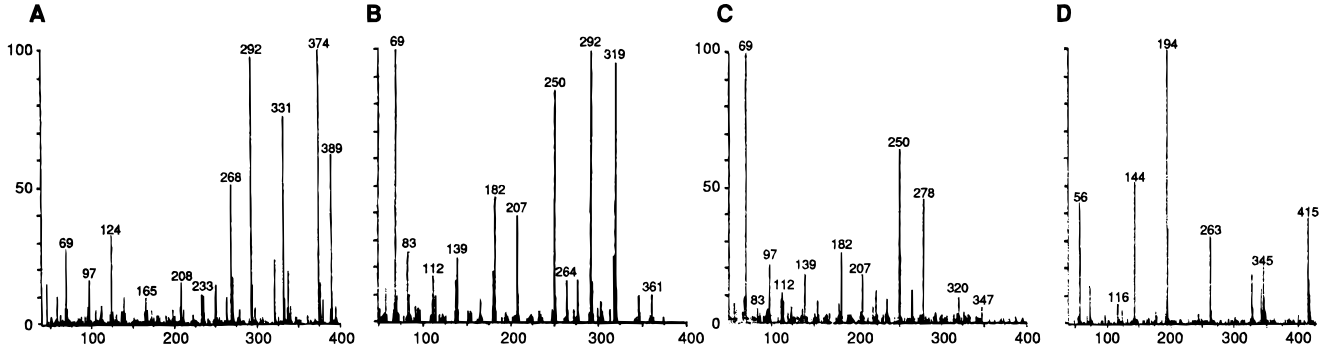

Figure 5. Mass spectra of the four trifluoroacetylated 2-OH- metabolites: (A) 2-OH-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; (B) 2-OH-4-amino-6-(isopropylamino)-*s*-triazine; (C) 2-OH-4-(ethylamino)-6-amino-*s*-triazine; and (D) 2-OH-4-amino-6-amino-*s*-triazine.

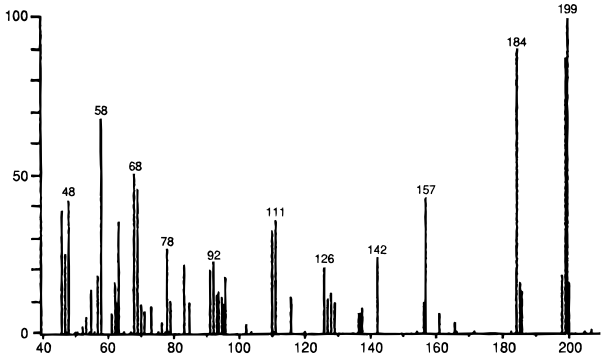

Figure 6. GC-MS mass spectrum of 2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine.

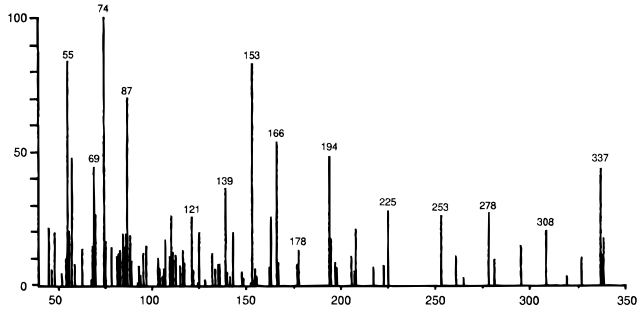

Figure 7. GC-MS spectrum of derivatized 2-Cl-4-amino-6-amino-*s*-triazine in bacterial samples.

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