As exemplified by the GC profile (Figure 1) a DB-1 capillary column allows for an effective resolution of the quinolizidine alkaloids. Initially, a packed column (3% apiezon L) was used that allowed for the detection of four poorly resolved peaks. With use of the capillary column we were able to identify two previously undetected peaks.

Keeler (1973a) reported that lupines with an anagyrine plant concentration of over 1.44 g/kg were generally teratogenic when fed to pregnant cows. Davis and Stout (1986) reported that *L. latifolius* Agardh. contained anagyrine at 6.04 g/kg dry plant weight and 26% of the total alkaloids. The *L. latifolius* from Trinity County contained anagyrine at 11.43 g/kg dry plant weight and 86% of the total alkaloids. Both values are remarkably high relative to other reported values for lupines in general. Keeler (1973a) reported finding anagyrine levels up to 7.31 g/kg dry plant weight and 50% of the total alkaloids in *Lupinus caudatus* and 6.76 g/kg and 37% in *Lupinus sericeus*. He also reported finding anagyrine at 10.41 g/kg dry plant weight and 52% of the total alkaloids in an unidentified species from central Idaho. Davis (1982) reported finding anagyrine values ranging from 1.35 to 9.83 g/kg of dry plant weight for *L. caudatus* harvested in May 1978 while *L. sericeus* showed ranges of 3.54–5.76 g/kg for the same period. Davis and Stout (1986) also reported finding anagyrine at 10.27 g/kg dry plant weight and 53% of the total alkaloids in *Lupinus montigenus*.

Kilgore et al. (1981) point out the possibility of anagyrine producing the human deformity is circumstantial, and adequate proof may be long in coming. The presence of anagyrine at a very high percentage (86%) of the total alkaloids and plant dry weight (1.14%) supports their hypothesis. It is reasonable that a goat foraging on the plant investigated in this study could be exposed to a relatively high level of anagyrine before the acute toxic effects resulting from the total alkaloid consumption become apparent. This would allow for a higher concentration of anagyrine in goats milk relative to alkaloid profiles from lupines containing a lesser percentage of anagyrine. Currently, we are investigating the transfer of anagyrine into goats milk to obtain information on the level humans may incur.

ACKNOWLEDGMENT

We thank Clay Reece for mass spectral analysis and Dr. Takayuki Shibamoto for capillary gas chromatography consultation. This project was supported by NIH ES-07059-07 and the U.S. Department of Agriculture, CSRS, CA-D-ETX-3739-AH.

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Received for review August 19, 1986. Accepted February 4, 1987.

Identification of Several New Metabolites from Pentachloronitrobenzene by Gas Chromatography/Mass Spectrometry

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Four new metabolites derived from pentachloronitrobenzene have been detected and identified in parsnips: a tetrachlorophenyl methyl sulfoxide, two isomeric tetrachlorophenyl methyl sulfones, and a trichlorophenyl methyl sulfone. Their occurrence has been tentatively determined to have taken place in the soil over a long period after initial application with subsequent translocation to newly planted crop foliage.

The primary use of pentachloronitrobenzene (PCNB) as a soil fungicide to control *Rhizoctonia* in cotton (*Gossypium hirsutum*) and *Sclerotium* in peanut (*Arachis hypogaea*) has been widespread for some time (U.S. EPA, 1976). Definitive metabolism studies of PCNB in the peanut have been reported by Lamoureux et al. (1980, 1981) and Rusness and Lamoureux (1980). These authors concluded that three main competing reactions were responsible for the observed metabolic pathway: (1) aryl nitro reduction; (2) nucleophilic displacement of the nitro group; (3) nucleophilic displacement of a chloro group. The major product via aryl nitro reduction was also identified as pentachloroaniline (PCA), with pentachlorothioanisole (PCTA) being identified as a minor product through catabolic reaction via *S*-(pentachlorophenyl)cysteine. Translocation of PCNB and its primary

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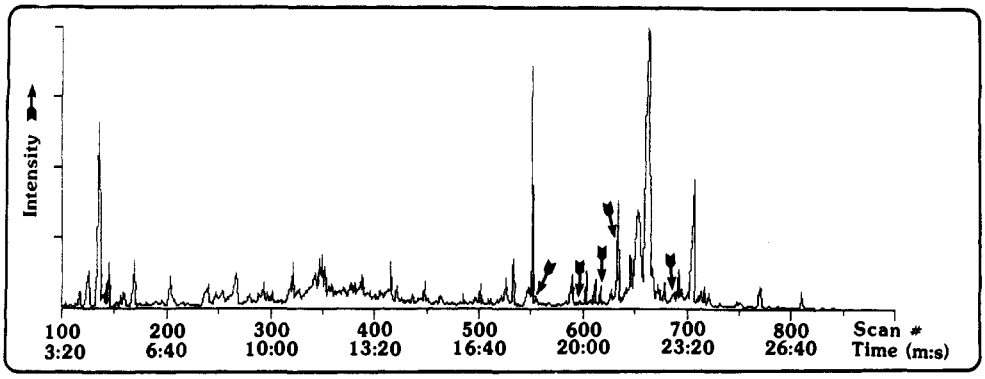


Figure 1. Reconstructed total ion current (m/z 40–500) for parsnips extract on capillary column indicating the location of the five compounds (A–E) considered to be unidentified analytical responses (UARs) at m/z 554, 597, 616, 628, and 684 respectively.

metabolites from the roots to the foliar tissue of the peanut plant was determined to be very restrictive. However, soil residues were quite common in treated soils (Heikes, 1980). More recently, however, the occurrence of PCNB, PCA, and PCTA in spinach leaves (Cairns et al., 1983) was tentatively attributed to spraying the crop rather than translocation from the soil after metabolism.

This paper now reports the occurrence of four new metabolites in parsnips, namely the sulfoxides and sulfones derived from trichloro- and tetrachlorothioanisoles. Originally detected by gas chromatography as unidentified analytical responses (UARs), molecular structures were elucidated by electron impact (EI) mass spectrometry and confirmed via synthetic preparation of reference standards to declare such identifications unambiguous.

MATERIALS AND METHODS

Sample Preparation. The sample (20 g) was extracted with acetone and partitioned into methylene chloride/petroleum ether as previously described (Luke et al., 1975, 1981) and then concentrated to 100 μL with dry nitrogen; 3 μL was injected into the gas chromatograph/mass spectrometer system.

Mass Spectrometry. Mass spectra were reported on a Finnigan Model 45A triple-stage quadrupole mass spectrometer equipped with an electron impact source and Incos data system. Operating conditions for residue sample and synthetic reference standards were as follows: 30-m DB-5 capillary column programmed at 10 °C/min from 40 to 250 °C; 1 μL of solution injected in the splitless mode. Typical instrument parameters used: extractor −7 V, lens −150 V, electron energy 100 V, quad entrance −27 V, source pressure 300 mTorr, electron multiplier −1050 V, source 170 °C.

Synthesis of Reference Standards of Metabolites. Oxidations of the commercially available thioanisoles, namely 2,4,5-trichlorothioanisole, 2,3,5,6-tetrachlorothioanisole, and pentachlorothioanisole to the corresponding sulfoxides and sulfones were carried out by dissolving 1–2 mg in 5 mL of methylene chloride, adding 10 mg of 3-chloroperoxybenzoic acid, and shaking the solution to dissolve the reagent according to the method of Blau and King (1977). This solution was then allowed to stand for 40 min before 2 mL of 0.1 N NaOH was added to neutralize the excess reagent. The methylene chloride layer was evaporated to dryness and diluted with hexane to about 50 ng/mL of oxidized product. Oxidized products were used without further purification and contained mixtures of the corresponding sulfoxide and sulfone.

RESULTS AND DISCUSSION

The multiresidue procedure (Luke et al., 1975, 1981) adopted for routine use in this laboratory in the pesticide monitoring program on fruits and vegetables uses a Hall electrolytic conductivity detector (HECD) for organohalogen, organonitrogen, and organosulfur and a flame photometric detector (FPD-P) for organophosphorus pesticides. The sensitivities of these detectors are now routinely in the subnanogram range. In the absence of a sample cleanup step, the background peaks are kept to a minimum through the use of these element-sensitive detectors. Two major unknown halogenated compounds of interest in the parsnip extract had relative retention times (to chloropyrifos) of 1.15 and 1.29 on 3% OV-101 and 1.7 and 2.2 on 3% OV-17 (both operated isothermally at 200 °C).

With the availability of this relative retention data, a translation can be made to gas chromatography/mass spectrometry (GS/MS) from HECD-X. However, the wealth of data usually gathered by a typical total ion current (TIC) chromatogram rather than the highly specific GC detector can give an entirely different elution profile. This dramatic change in elution profile (Figure 1) can cause some problems. First, the transition from a packed column employed isothermally by GC to a capillary column used in GC/MS via temperature programming necessitates the employment of two additional reference standards with retention times that bracket such UAR responses. Such an approach has been useful in locating the precise retention window that contains the UARs of interest. Second, the ability of the GC/MS system to detect all eluting compounds rather than just those containing a halogen requires detailed handling of the acquired data in the TIC mode to sort out the mass spectrum of the compound of interst. In many cases, the UARs are often obscured by a coeluting compound in the extract. Examination of the resultant TIC chromatogram produced under EI conditions (Figure 1) revealed that gross interferences by coeluting hydrocarbons were presented. In spite of these coeluting hydrocarbons, the UARs of interest were located (arrowed positions in Figure 1) by careful data profile analysis and subtraction techniques to have the mass spectral characteristics displayed in Figure 2.

It would appear at first that the potential molecular weights of the five UARs were 258, 276, 292, 292, and 310 (assuming the highest ions recorded under EI conditions represented the molecular ions) and contained three, four, four, four, and five chlorine atoms, respectively (derived from the isotopic cluster patterns).

For compound A, the fragmentation pathway indicated the initial loss of a methyl group to yield m/z 243. Loss of 62 amu from the potential molecular ion yielded the ion at m/z 196. Since this ion was then able to lose an additional 16 amu to give the trichlorinated ion at m/z 179, the presence of an oxygen atom in the structure was strongly indicated. Since the overall mass spectral char-

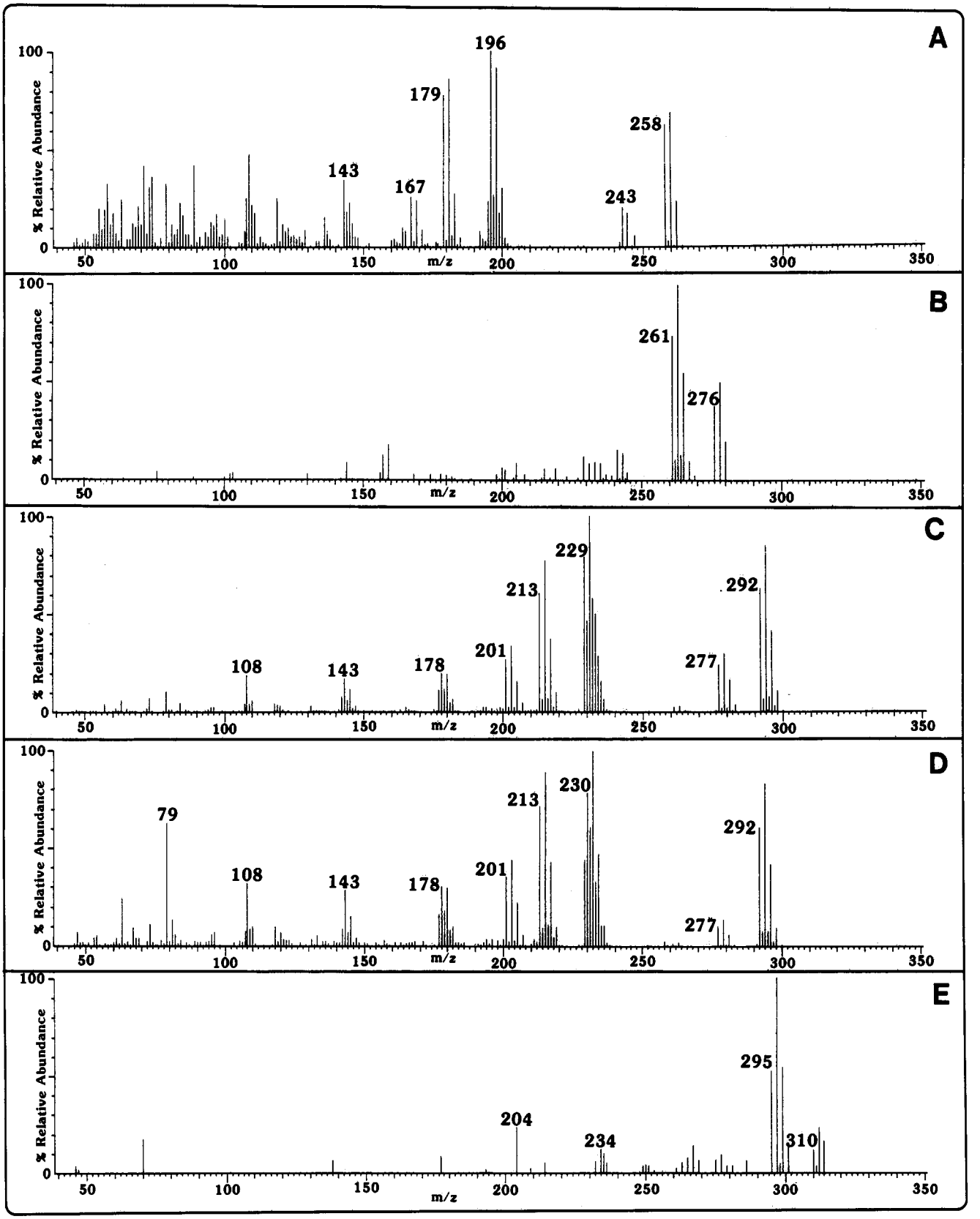


Figure 2. Mass spectra under EI conditions of five compounds extracted from chromatogram in order of increasing retention time.

acteristics were indicative of an aromatic compound (empirical formula for m/z 179 could be $C_6H_2Cl_3$ if no other heteroatoms present), the ion at m/z 179 was determined to have the structure of a trichlorophenyl ion (actual distribution of chlorine atoms unknown). This determination in turn inferred the structure of the ion at m/z 196 to be that of a trichlorophenol. The appearance of an ion cluster at m/z 195 (loss of an additional hydrogen, i.e. 63 amu) of much weaker intensity than the cluster at m/z 196 also added supporting evidence that the ion had the structure of a trichlorophenol. Since the mass losses from the ion at m/z 258 to give m/z 196 and 195 were 62 and 63 amu, respectively, the presence of sulfur was suspected. Confirmation of the presence of sulfur was provided by HECD-S. With the presence of sulfur confirmed, the loss of 63 amu from m/z 258 (M^+) was determined to involve

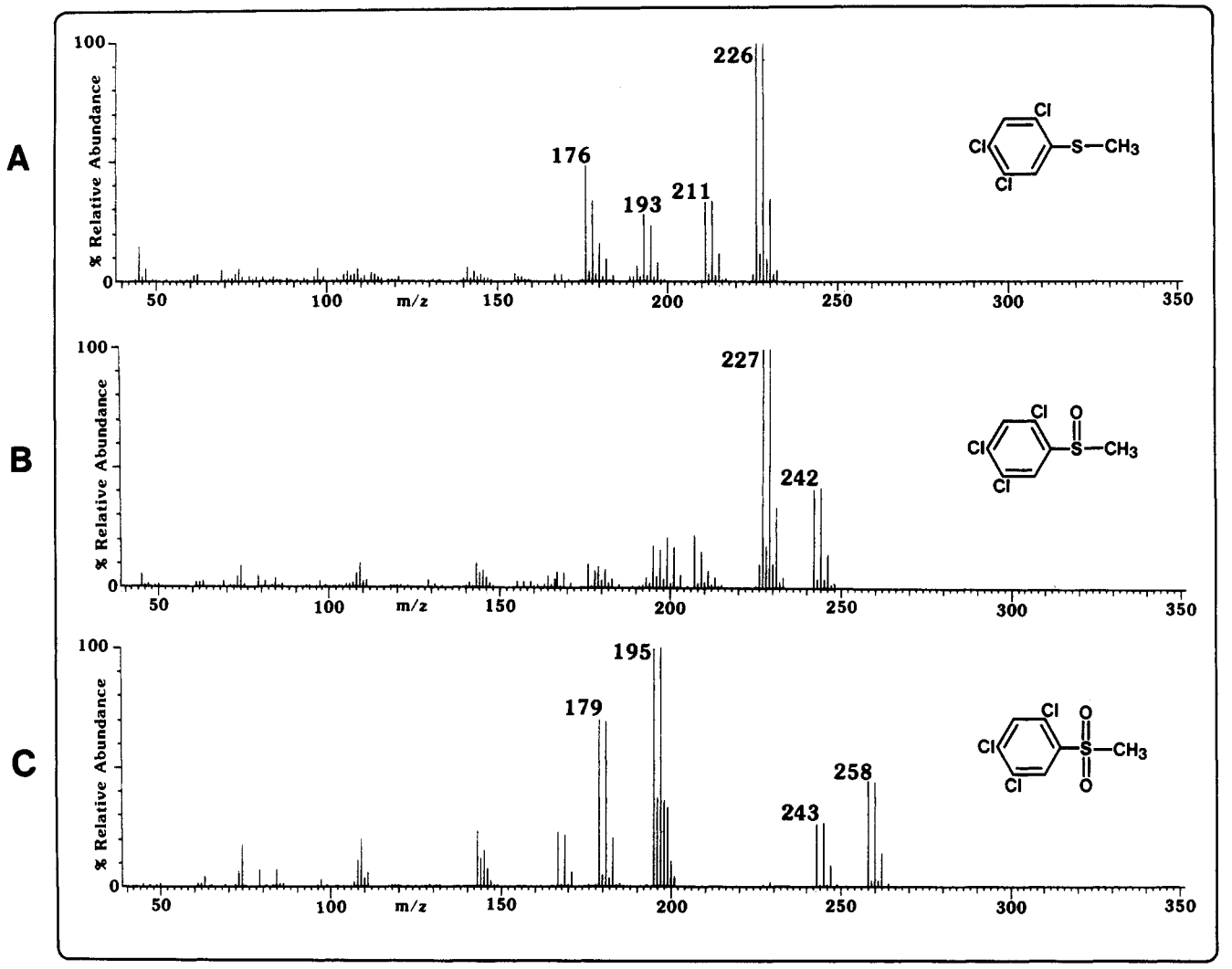


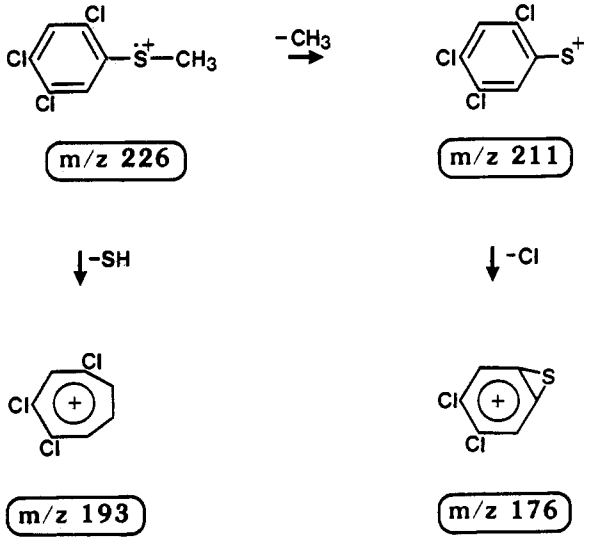
Figure 3. Mass spectra under EI conditions: (A) 2,4,5-trichlorothioanisole; (B) 2,4,5-trichlorophenyl methyl sulfoxide; (C) 2,4,5-trichlorophenyl methyl sulfone.

the loss of $SOCH_3$. With the structural fragments identified, the identity of compound A was then thought to be a trichlorophenyl methyl sulfone. The similarity of the mass fragmentation of compounds C and D (Figure 2) relative to that of compound A, but shifted up 34 amu (i.e. tetrachloro rather than trichloro), was taken as an indication that these compounds were isomeric tetrachlorophenyl methyl sulfones. Since compound B had a potential molecular weight 16 amu less than the tetrachlorophenyl methyl sulfones suspected to be compounds C and D, it seemed logical to suspect the identity as the corresponding sulfoxide. Compound E then by extrapolation was suspected to be the pentachlorophenyl methyl sulfoxide.

These five related compounds appeared to be derived from a major metabolite, pentachlorothioanisole (PTA), of PCNB. To provide unambiguous proof of presence in the parsnips extract, representative trichloro-, tetrachloro-, and pentachlorothioanisoles were oxidized to their corresponding sulfoxides and sulfones as reference standards for further study and comparison. The mass spectral data for the parent thioanisoles and their corresponding sulfoxides and sulfones are presented in Figures 3–5. For discussion purposes, the trichloro compounds have been used to illustrate the salient features exhibited by the generic class.

In the case of thioanisoles, the fragmentation can be characterized by detailed reference to trichlorothioanisole

Scheme I. Proposed Fragmentation Pathway for Chlorinated Phenylthioanisoles



(Figure 3A). Three major fragmentation pathways are evident (Scheme I). Loss of the methyl group occurs in all three anisoles studied. The second major loss of SH infers a rearrangement process to give the trichlorotropylium ion at m/z 193. Finally, the loss of Cl from the phenyl ring presumably from m/z 211 must involve a

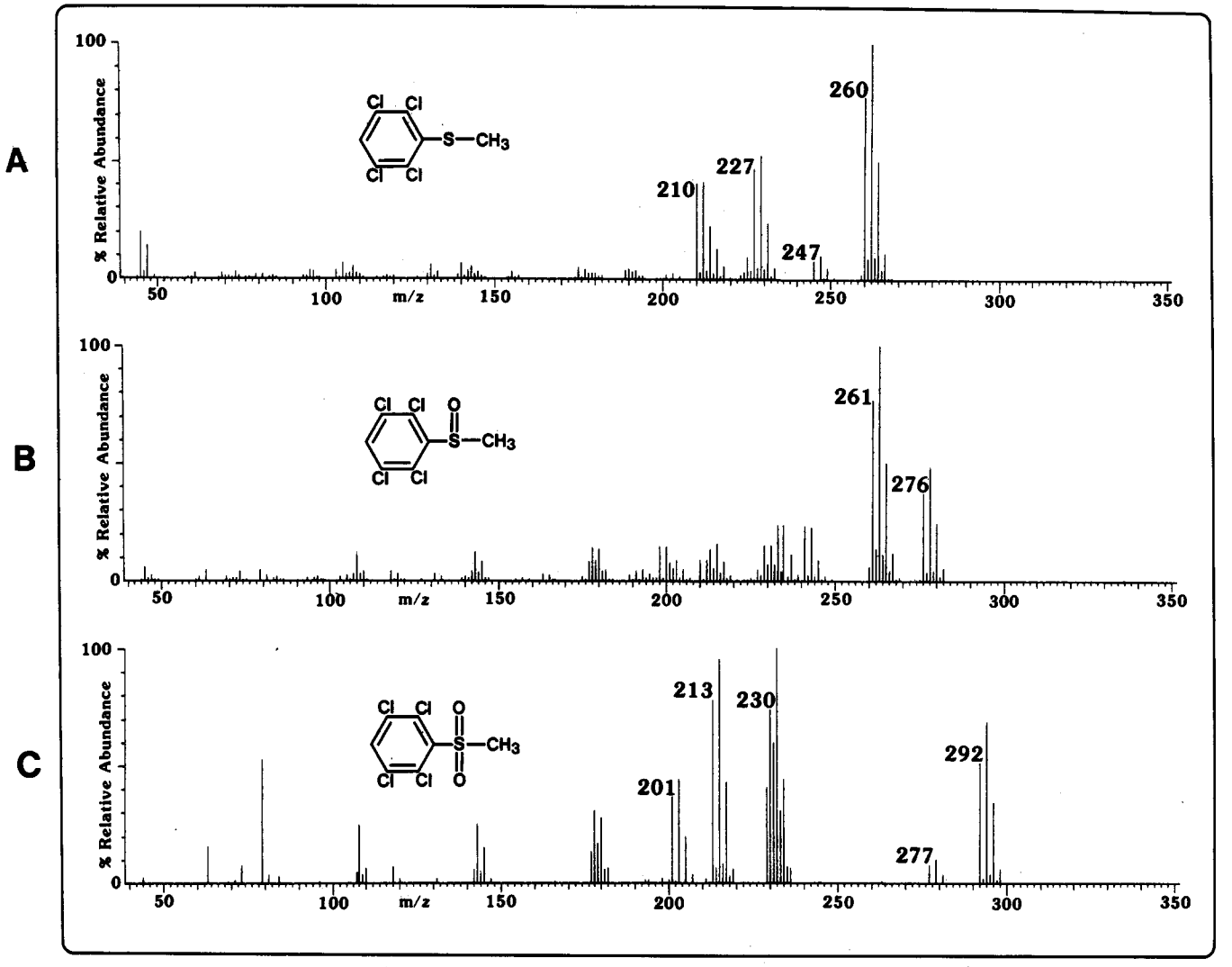
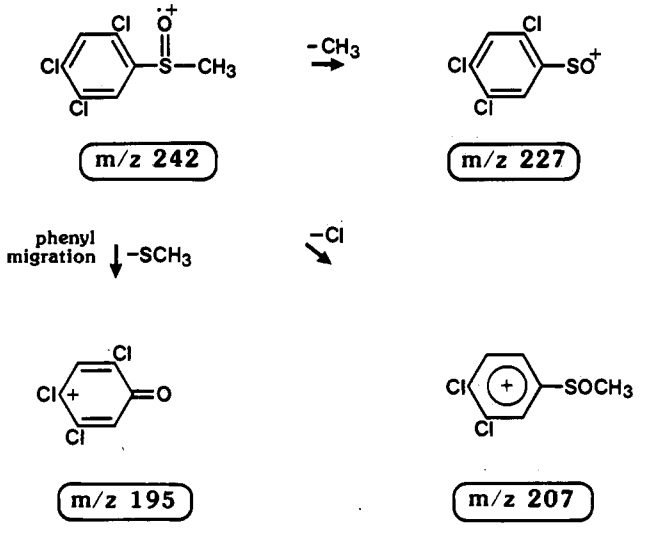
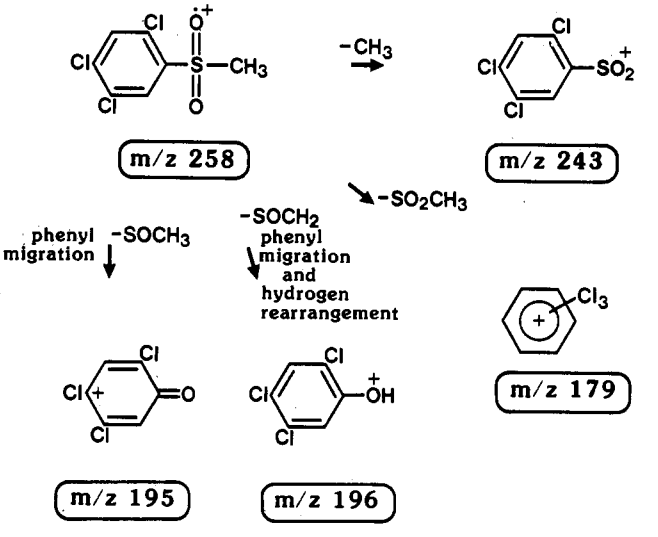


Figure 4. Mass spectra under EI conditions: (A) 2,3,5,6-tetrachlorothioanisole; (B) 2,3,5,6-tetrachlorophenyl methyl sulfoxide; (C) 2,3,5,6-tetrachlorophenyl methyl sulfone.

Scheme II. Proposed Fragmentation Pathway for Chlorinated Phenyl Methyl Sulfoxides



Scheme III. Proposed Fragmentation Pathway for Chlorinated Phenyl Methyl Sulfones



resonance-stabilized carbonium ion as illustrated in Scheme I. The other chlorinated thioanisoles behaved in a similar fashion.

In the case of the trichlorophenyl methyl sulfoxide (Figure 3B), the initial loss of a methyl group to give the ion at m/z 227 was the dominant fragmentation pathway (Scheme II). Two other fragmentation pathways were observed. Perhaps the most interesting fragmentation pathway was that in producing the ion at m/z 195. To accommodate the loss of SCH_3, it is necessary to envisage the migration of the phenyl ring to the oxygen before fragmentation. Phenyl migration has already been invoked (Pratanata et al., 1974) to explain the mass spectral characteristics of aryl methyl sulfones. This phenomenon

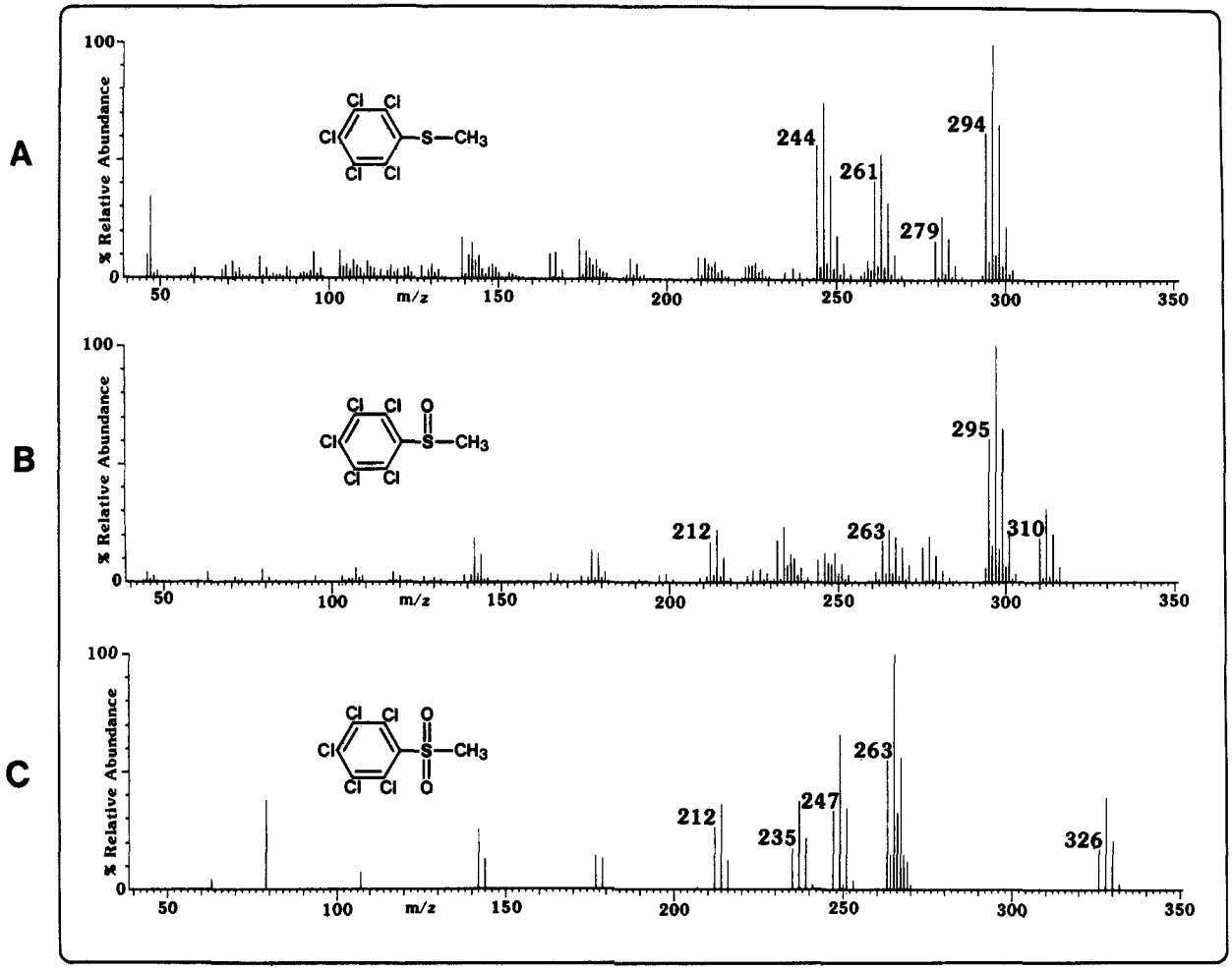


Figure 5. Mass spectra under EI conditions: (A) pentachlorothioanisole; (B) pentachlorophenyl methyl sulfoxide; (C) pentachlorophenyl methyl sulfone.

can now be extended to include aryl methyl sulfoxides. The remaining ion of some importance was at m/z 207 representing the loss of Cl. Independent of the number of chlorines in the phenyl nucleus, these general fragmentation pathways were observed.

Finally, in the case of trichlorophenyl methyl sulfone (Figure 3C), an additional fragmentation pathway not observed for the thioanisoles or sulfoxides was observed (Scheme III). While losses of CH_3, SO_2CH_3, and $SOCH_3$ were operative, the appearance of an ion cluster at m/z 196 indicated the loss of $SOCH_2$ to form the trichlorophenol ion. The overlap with the ion cluster at m/z 195 (Figure 3C) was clearly evident. All sulfones examined exhibited this same behavior pattern.

While these model compounds did confirm the identity of the five UARs in the parsnip extract, the exact location of the chlorines in the tri- and tetrachlorinated metabolites was not determined at this time. The location of the chlorine atoms in the aromatic nucleus is thought to play an important role in resonance stabilization of ion at m/z 195 and 196. Detailed analysis with various isomers should assist in the final structural determination of the trichlorophenyl methyl sulfone detected in the parsnips extract.

Metabolic Implications. The detection of five sulfoxides and sulfones closely related to pentachlorothioanisole (PCTA) in the parsnip extract implies that such compounds are additional metabolites derived from PCNB formed by catabolism of the *S*-(pentachlorophenyl)cysteine to PCTA (Lamoureux et al., 1981). Pentachlorophenyl methyl sulfoxide had previously been detected by Rusness and Lamoureux (1980) as a metabolite in peanut roots. These authors proposed the formation of pentachlorophenyl methyl sulfoxide from the oxidation of PCTA. It would now appear that additional oxidation and dechlorination can take place in the soil to account for the appearance of the detected sulfoxides and sulfones now reported. To date, the dechlorination pathway to tetrachloro compounds had only been observed in metabolism studies conducted with goats and sheep (Ashbacker and Feil, 1983). The fact that these compounds were detected in parsnips would strongly suggest that translocation from the soil due to the high polarity of the compounds can result in such residue findings. It would appear, therefore, a tentative conclusion to suggest that such metabolites might be produced in the soil long after the initial application and new crops grown in that soil translocate these polar metabolites to their foliage. The apparent absence of the parent compound, PCNB, and its main metabolities in this sample extract would lend support to the theory of oxidation and dechlorination occurring long after the initial application of PCNB as a soil fungicide.

Registry No. Tetrachlorophenyl methyl sulfoxide, 107409-52-9; tetrachlorophenyl methyl sulfone, 107409-53-0; trichlorophenyl methyl sulfone, 4163-80-8.

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Received for review June 2, 1986. Accepted December 1, 1986.

Decomposition of Aspartame Caused by Heat in the Acidified and Dried State

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L-Aspartyl-L-phenylalanine methyl ester (aspartame) forms a variety of degradation products when heated in an acidified–lyophilized state at 110 °C for 24 h in vacuo. In addition to the well-characterized degradation products of aspartame, our results suggest that aspartame can also dehydrate upon heating and that oligopeptides can be generated containing higher ratios of aspartic acid to phenylalanine than the starting material. Aspartame dehydrates to form an anhydro derivative (MW 276) as determined by fast atom bombardment mass spectrometry. Photoacoustic infrared spectroscopy results suggest an anhydride can occur between the β-carboxyl group and the peptide linkage upon heating.

L-Aspartyl-L-phenylalanine methyl ester (aspartame) is used today widely as an artificial sweetener in a variety of food products and beverages, but it is not commonly used as an additive in cooking or baking because of its instability at high temperatures (FDA, 1981). Decomposition products are known to form by heating aspartame in solution or in the dry state. In solution, its stability depends on the pH and temperature (Holmer, 1984; Harper, 1975; Mazur, 1976; Furda et al., 1975; Prudel et al., 1986). A diketopiperazine and products derived from hydrolysis of the peptide and ester bonds have been identified. Recently, it has been shown that extensive racemization of bound aspartic acid occurs at 100 °C in a couple of days at pH 7.0 in the diketopiperazine product (Boehm and Bada, 1984). At pH 4.0, less racemization takes place, and it occurs in intact aspartame. In the dry state, the stability of aspartame is considered reasonably good. Mazur (1976) states that only 5% of the diketopiperazine is formed after heating for 70 h at 105 °C.

In a recent report, Luo et al. (1987) found that aspartyl- and asparaginylglycine-containing peptides are sensitive to heat in the dry state. A cyclic imide, proven by fast atom bombardment mass spectrometry and Fourier transform infrared photoacoustic spectroscopy, occurs when peptides are acidified, lyophilized, and then heated in vacuo. Because of these effects, we decided to examine further the stability of aspartame in the dry state. The results reported herein show that aspartame is not very stable at 90 °C or above if it is acidified and lyophilized before heating. A number of new products are formed and identified by HPLC, amino acid analysis, FAB–mass spectrometry, and photoacoustic infrared spectroscopy. An anhydride containing aspartic acid and phenylalanine methyl ester is suggested to form upon heating. Peptide products with higher ratios of aspartic acid to phenylalanine than aspartame are indicated.

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MATERIALS AND METHODS

L-Aspartyl-L-phenylalanine methyl ester (aspartame) was purchased from Sigma Co. All other chemicals and reagents were of high-quality analytical grades.

Heating Procedures. Aspartame was dissolved in distilled water to a concentration of 5 mg/mL. Aliquots of this solution were adjusted to pH 2.0, 3.0, or 7.0 with 0.1 M HCl or 0.1 M ammonium hydroxide. Each aliquot was freeze-dried in a hydrolysis tube (Pierce Chemical Co.). The tubes were sealed under vacuum and heated at various temperatures for 24 h.

Paper Electrophoresis. The heated aspartame was analyzed by high-voltage paper electrophoresis. Sample (1–2 μL) was applied to the middle of a 20-cm electrophoresis paper. Electrophoresis was performed in a buffer of 2.4% pyridine and 0.6% acetic acid (pH 5.6), in water at 2000 V for 1 h. Aspartame and thermal degradation products were identified by reaction with fluorescamine.

HPLC Separation. Degradation products were separated on a Microsorb C_{18} reversed-phase HPLC column (4.6-mm i.d. × 25-cm length, Rainin Instruments Co.). Buffer A consisted of 0.1% trifluoroacetic acid in water. Buffer B was 90% acetonitrile, 10% water, and 0.1% trifluoracetic acid. Elution was performed at 1 mL/min with a 38-min linear gradient of 0–60% buffer B, and the products were detected at 260 nm, collected, pooled, and lyophilized to remove solvent.

FTIR-PAS. Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) is useful for determining the structure of peptides and proteins (Renugopalakrishnan and Bhatnagar, 1984). In this study, all IR spectra were measured with a Perkin-Elmer Model 1800 FTIR spectrometer equipped with a METC Model 100 photoacoustic cell. Sample spectra were normalized by dividing the sample spectrum by a carbon black spectrum.

Aspartame and its degradation products were applied on a thin layer of collodion membrane as a water solution.

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