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ARTICLE *in* ANALYTICAL CHEMISTRY · JANUARY 1996

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Anal. Chem. **1995**, *67*, 4349–4353

Tandem Mass Spectrometry of Herbicide Residues in Lipid-Rich Tissue

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A tandem mass spectrometry procedure, originally developed for bacterial biofilms was adapted for the identification of herbicide residues in lipid-rich tissue of amphipods collected from microcosms in a prairie wetland. For this application, the amounts of tissue employed (less than 1 mg wet weight), and detection of target analytes at picogram levels, were similar to the values reported for bacterial biofilms. Described is an application of the technique for the identification of residues of the herbicide *S*-2,3,3-trichloroallyl diisopropyl thiocarbamate (triallate; trade name Avadex-BW). For amphipods collected from microcosms exposed to the herbicide 2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid methyl ester (diclofop-methyl, trade name Hoe Grass), there were detectable levels of only the hydrolysis product, diclofop acid, in the lipid-rich tissue. Other transformation products reported for bacterial biofilms were not observed in the amphipods.

Detection of herbicides in aquatic biota requires appreciable amounts of tissue; typically 10 g dry weight. For larger organisms such as fish, this poses little problem. However, for common invertebrates such as amphipods, there can be a significant time factor involved in obtaining enough individuals (up to 2000 amphipods, in the case of *Hyalella azteca*) to achieve the necessary biomass.

This problem was encountered in our laboratory, for an investigation of the distribution of contaminants within a population of amphipods exposed to pesticide residues in wetland environments. For this investigation, an analytical method was required for (a) the confirmation of the presence of contaminants in the individual amphipods and (b) the assessment of the distribution of contaminants within the lipid reserves of the amphipod. To meet these objectives, the utility of tandem mass spectrometry (MS/MS) was evaluated for specific application to the limited amounts (less than 1 mg weight) of lipid-rich tissue. This development was anticipated to be of potential application to a broad range of aquatic environments, because many of the most persistent contaminants in aquatic systems are lipophilic. Lipophilic contaminants dissolved in the lipid tissues[1] provide a more concentrated contaminant source compared to the matrix represented by the entire organism.

We describe an application of a MS/MS technique for wetland environments, based on procedures originally developed for the characterization of microbial biofilms[2] cultured under laboratory conditions in continuous-flow cells.[3] The application presented in this work capitalizes on the elevated contaminant levels found in lipid-rich tissues while at the same time markedly reduces the number of organisms required for detection of pesticides. This tandem mass spectrometry procedure was applied to field environments in which a series of microcosms were inoculated with triallate and diclofop-methyl to derive field data on the detection limit of the technique under real-life conditions in a eutrophic prairie pond. This application is considered to be complementary to earlier laboratory studies[2] and provides an extension of the technique to more complex natural systems.

Triallate is the common name for the herbicide with the Chemical Abstracts Service (CAS) and IUPAC name *S*-2,3,3-trichloroallyl diisopropyl thiocarbamate. It is an amber oil with a molecular formula of $C_{10}H_{16}Cl_3NOS$ (see chemical structure given in Figure 1). Triallate was introduced into Canada in 1962 by Monsanto Agricultural Co. and is currently marketed under the trade names Avadex-BW and Fortress. Triallate is a popular, moderately lipophilic (log octanol/water partition coefficient; K_{ow} = 4.6), preemergence, herbicide recommended for control of wild oats in barley, lentils, durum wheat, spring wheat, and dry peas.[4] It is also recommended for wild oat control on rapeseed, flax, sugarbeets, and mustard.[5]

Diclofop-methyl [IUPAC name 2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid methyl ester] is used for weed control in agricultural crop production[6,7] (see chemical structure given in Figure 1). Specifically, diclofop-methyl is used to control graminaceous annual weeds.[6,7] At application rates ranging from 57 to 132 kg/km^2 active ingredient there is strong adsorption of the herbicide to soil and low potential for volatilization.[8,9] Under field conditions, diclofop-methyl is known to undergo hydrolysis to its corresponding acid, with formation of trace levels of various metabolites.[2]

Environmental effects have been reported for both herbicides in the Canadian prairies. Triallate can persist from several weeks

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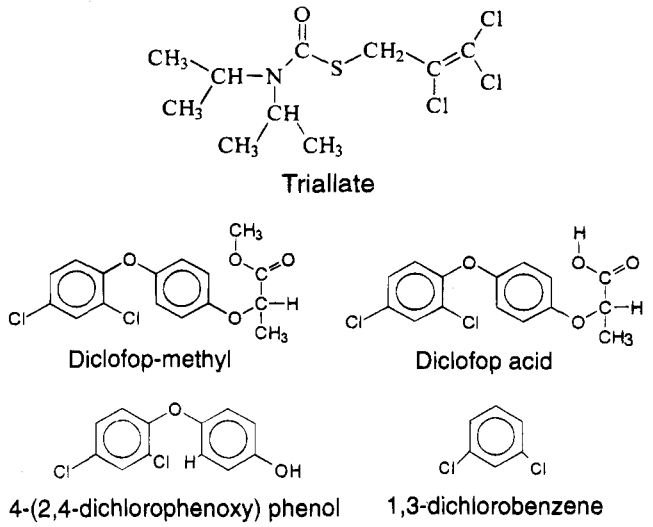

Figure 1. Chemical structures of herbicides and herbicide residues.

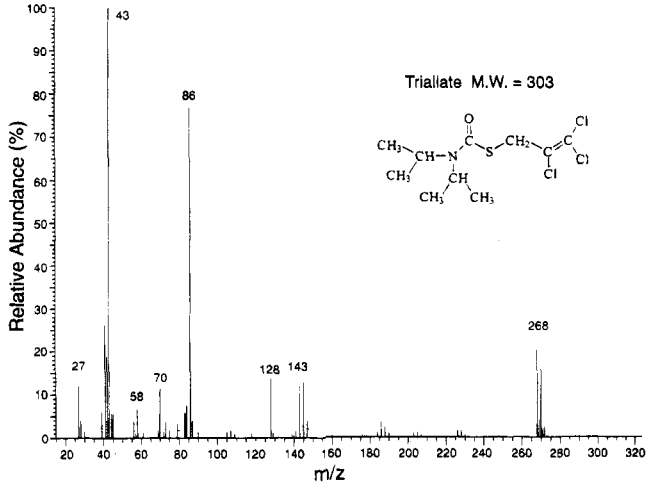

Figure 2. Example of EI mass spectrum of an authentic standard, triallate.

to several months in soils, depending on the organic matter content.[10] Trace residues of triallate in aquatic systems may be acutely toxic to some aquatic invertebrates[11-13] which in turn are critical for ducks, especially during their reproductive period.[14] Likewise, residues of diclofop-methyl have been detected in shallow groundwater, surface waters, and air samples.[15]

EXPERIMENTAL SECTION

The tandem mass spectrometry procedure described in this work is intended to be a broad spectrum screening technique[16,17] complementary to conventional analytical methods that require solvent extractions, preconcentration, or derivatization steps.[18-21]

Materials. Authentic standards were obtained from Monsanto and Hoechst Canada for triallate and diclofop-methyl, respectively. These standards were used to obtain library spectra for confirmation of the identity of the herbicides and possible transformation products in the amphipod tissue.

Procedure. The design, construction, and operation of the field microcosms have been reported earlier.[1] In brief, microcosms were composed of a wooden frame and polyethylene side walls enclosing a volume of 1 m^3. The microcosms were embedded in the sediment but were open to the atmosphere. A total of seven microcosms were deployed. Three of the microcosms were spiked with triallate (Avadex-BW) and three with diclofop-methyl (Hoe grass) in a log series (1, 10, and 100 μg/L active ingredient). One of the microcosms served as a control.

Two hundred adult amphipods (*Gammarus lacustris* Sars) were added to each enclosure, subsequently removed in batches of 10 at days 1, 3, 15, and 30 and stored frozen (−75 °C) in the dark. The frozen amphipods were cut in half longitudinally with a sterile razor blade prior to MS analyses and stained with the neutral-lipid specific stain Nile Red (0.25 mg in 10 mL of acetone) to reveal lipid-rich tissues. Nile Red has been shown to bind specifically to tissues rich in neutral lipid such as triacylglycerol.[22]

For laboratory experiments, lipid-rich tissue (~2 μL) was transferred, via the needle of a hypodermic syringe, directly to quartz glass containers for subsequent MS analysis, using the direct insertion probe (DIP). Lipid-rich tissue was chosen as the matrix to study rather than the whole specimen for two main reasons: (a) many environmental contaminants are lipophilic and concentrate in the fatty regions of amphipods leading to enhanced detection during analysis, and (b) lipid reserves are naturally increased in spring and fall, coinciding with agricultural pesticide applications.[1] Introduction of the entire matrix of an amphipod directly into the mass spectrometer system would have similar effects on the instrument with respect to source contamination as only introducing lipid-rich tissue. However, this would negate the advantage of the enrichment of the lipophilic compounds, result in a dilution of the analytes, and likely introduce additional matrix interference. The lipid tissue samples were subjected directly to instrumental analysis, using the procedure developed for bacterial biofilms.[2] The procedure required no sample extraction, cleanup, or preconcentration steps. For analysis performed in succession, 30−50 samples could be analyzed before it was necessary to clean the source. To evaluate the technique, amphipods from microcosms spiked with triallate were studied in detail. For the purpose of demonstrating whether the technique could be readily adapted to the determination of transformation products in lipid-rich tissue, studies were also conducted for two of the microcosms spiked with diclofop-methyl.

To facilitate comparisons with the results obtained for bacterial biofilms,[2] instrumental conditions similar to those employed in earlier work[2] were utilized in the present study. In brief, MS experiments were conducted using a Fisons AutospecQ mass

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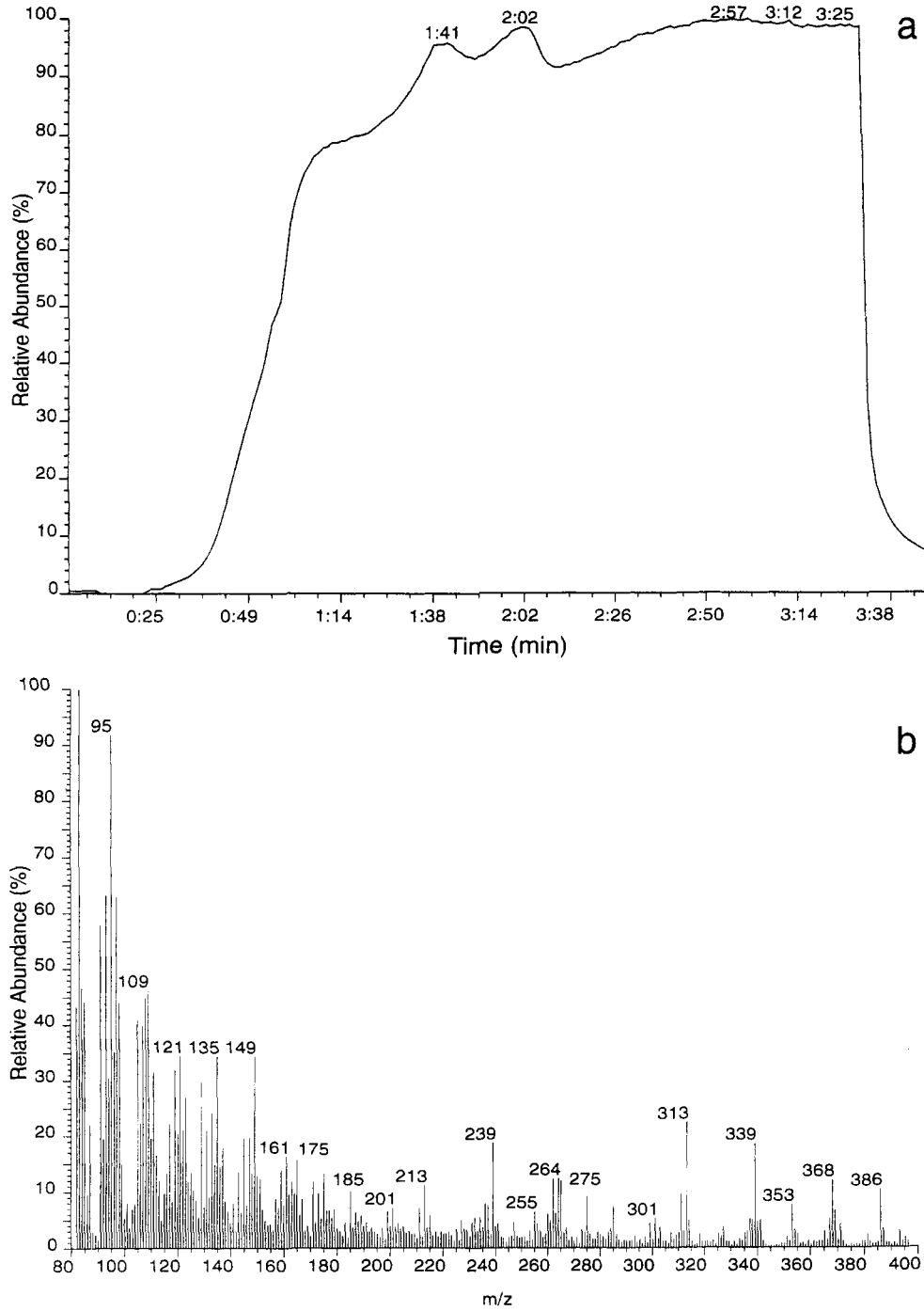

Figure 3. (a) Total ion chromatogram (a) and full-scan mass spectrum (b) of amphipod tissue collected from microcosm exposed to 10 μg/L triallate.

spectrometer, equipped with a 4000-60 VAX data system, Digital Equipment Co., and Opus 3.1x Software. Samples were placed in shallow specimen holders of the direct insertion probe for 30 min at room temperature (~23 °C) and ambient room pressure, prior to introduction to the ion source. This was necessary to reduce the moisture content of the samples and avoid tripping the vacuum protection system (set at 5×10^{-5} Torr) of the mass spectrometer. The direct insertion probe was water-cooled, and heating was limited to the radiant heat from the ion source with no additional heat supplied by the probe heaters. The ion source was operated under electron impact conditions at 70 eV, 250 °C, trap current 250 μA, and the mass spectrometer was operated at 1300 resolution, with a scan speed 1 s/decade, and mass range 50–600 daltons.

For the MS/MS experiments, the electronvolts in the ion source were adjusted to optimize the abundances of the precursor ions (35–40 eV). Prior to selecting the precursor ions manually, the ion beam was reduced using xenon as the collision gas to 50% transmission while transmitting the m/z 331 ion of perfluorokerosene. The collision cell was located between the second electrostatic analyzer (E) and the quadrupole analyzer (Q), corresponding to the fourth field free region of the mass spectrometer (EBEQ geometry; B denotes the magnet mass analyzer). Experiments were performed for low-energy collisions in which the collision cell was held at 20–30 and 12–20 eV (laboratory frame of reference), for studies of triallate and diclofop-methyl, respectively. Product ions were detected by scanning the

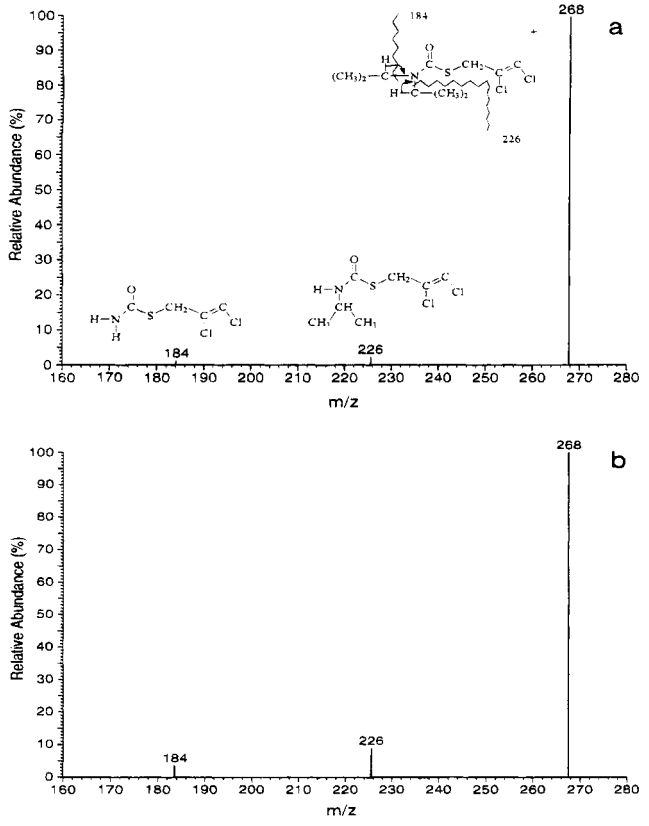

Figure 4. Example of a product ion spectrum: (a) authentic triallate standard and (b) field amphipod tissue containing triallate.

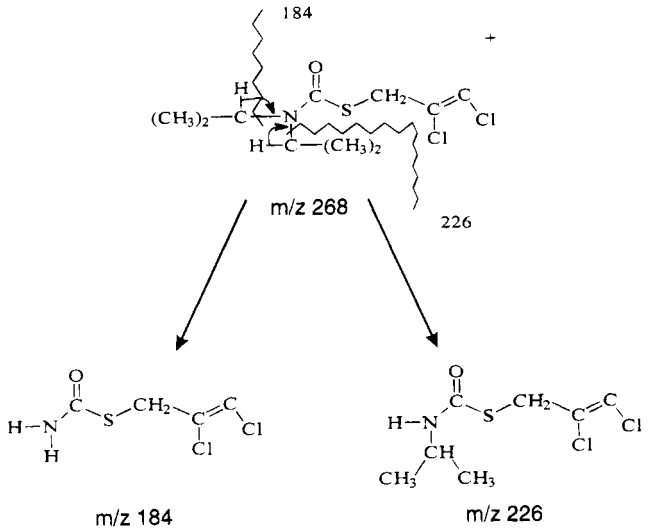

Figure 5. Formation of precursor ions arising from collision-induced dissociation for triallate precursor ion *m/z* 268.

quadrupole in the mass range 30–350 daltons at unit resolution.[23]

RESULTS AND DISCUSSION

MS. The electron impact mass spectra of authentic standards of the herbicides (an example of triallate mass spectrum is given in Figure 2) were first examined to select diagnostic ions suitable for evaluating the presence of the target analytes in the full-scan mass spectra of amphipod tissue. The selected diagnostic ions for triallate and diclofop-methyl were *m/z* 268, 270 and 340, 353,

Table 1. Summary of the Detection of Triallate Residues in Lipid-Rich Tissue of Amphipods Confirmed Using Tandem Mass Spectrometry[a]

		initial triallate concn (μg/L)		
day	control	1	10	100
1	N	Y	Y	Y
3	N	T	Y	Y
15	N	N	Y	Y
30	N	N	Y	Y

[a] Y denotes positive detection, precursor and both product ions; T denotes trace detection, precursor and one product ion; and N denotes no detection. All values indicated are based on duplicate analyses.

respectively. Criteria for the selection of these ions were based on (a) their occurrence at relatively high mass, (b) their relative abundances, and (c) the low probability of formation from fragmentation or rearrangement in the ion source.[2,24] Other diagnostic ion candidates, such as *m/z* 86 and 43 for triallate, were rejected on the basis that they would be more prone to matrix interference in actual samples relative to the higher mass ions. The determination of possible transformation products of diclofop-methyl was based on reference spectra previously reported[2] for diclofop acid, 4-(2,4-diclorophenoxy)phenol, and 1,3-dichlorobenzene (chemical structures given in Figure 1).

Examples of the total ion chromatogram and corresponding mass spectrum for tissue samples from the microcosms exposed to triallate are illustrated in Figure 3. Figure 3b was obtained using an average of scans from the time 0.50–3.25 min in the total ion chromatogram (Figure 3a). The scans in the region 0–0.50 and 3.25–3.50 min (Figure 3a) were used for background subtraction.

MS/MS. As described for the application of the tandem MS technique to biofilm,[2] the product ion spectra were used to (a) verify that the selected extracted ions were diagnostic for the respective analytes and (b) enhance the sensitivity of the method for identification of the herbicides, relative to the MS full-scan procedure.

Product Ion Spectra of Triallate and Diclofop-methyl. A product ion spectrum obtained for an authentic standard of triallate from the precursor ion at *m/z* 268 is given in Figure 4 with corresponding fragmentation from collision-induced dissociations given in Figure 5. Product ion abundances of the precursor *m/z* 268 for triallate could be enhanced using higher collision gas pressures for applications requiring quantification. The relative abundances of the product ions *m/z* 226:184 of the authentic standard triallate compared with the actual sample are very similar and thus are suitable for confirmation of the herbicide. The product ion spectra for diclofop-methyl and transformation products have been previously reported.[2]

Tissue Samples. The results, derived from duplicate analyses, of the product ion scans from tissue samples investigated for evaluation of the technique are summarized in Table 1. These results provide confirmation of triallate residues in the individual amphipods. The results also indicate that triallate could still be detected after 30 days in the 10 μg/L treatment but was not detected in the control microcosm. Preliminary data obtained for diclofop-methyl also confirmed the presence of the herbicide,

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present only as the hydrolysis product, with no evidence of other transformation products present in the lipid-rich tissue of the amphipods. This distribution was very dissimilar to the wide range of transformation products observed for degradative biofilms.[2] The latter were grown in the laboratory and consisted of a bacterial biofilm (capable of degrading diclofop-methyl as the sole carbon source), contained on a large quantity of supporting matrix composed of exopolysaccharide (EPS) and polypeptide polymers.[2] For the field microcosms, there was no detectable change in the distribution of the transformation products observed for tissue collected at day 3 and following 15 days of exposure to diclofop-methyl. Apart from diclofop-acid, other transformation products were below the detection limit of the procedure (~20 pg). This may indicate that little or no degradation of the herbicide occurred within the lipid-rich tissue, in contrast to the process observed for bacterial biofilms.[2]

The detection limit of the tandem mass spectrometry technique was sufficiently low for the confirmation of herbicides in the individual amphipods and served as a complementary tool to conventional analyses of herbicide residues in the water and sediment compartments (Arts, M. T.; Headley, J. V.; Peru, K. M., unpublished work). For this work, detection limits were obtained similar to those reported for bacterial biofilm materials.[2] Good product ion spectra were obtained for tissue spiked with 20 pg of the herbicides. In contrast, this spike level was masked by chemical noise in the MS prescreening experiments. This level of performance, however, was subject to cleaning the inner ion source following two or three days of analysis, corresponding to ~30−50 samples run in succession. Maintenance and cleaning was only necessary for the inner source region due to the fact that most of the nonvolatile matrix remained in the quartz glass containers. Thus, the MS/MS procedure can be considered to be a viable analytical technique for the analysis of a series of samples in a very short time period.

This application demonstrates that the tandem mass spectrometry technique, originally developed for bacterial biofilms cultured in the laboratory, can be extended to more complex tissue samples from field environments.

CONCLUSION

Application of the tandem MS procedure provided identification and confirmation of triallate and the hydrolysis product of diclofop-methyl in small volumes (~2 μL) of tissue materials at picogram levels with no sample extraction or preconcentration steps. Field experiments revealed that triallate could still be detected in a single amphipod 30 days after a one-time addition of 10 μg/L triallate. For diclofop-methyl, there was evidence for little or no degradation occurring within lipid-rich tissue, in contrast to results reported for bacterial biofilms. Further studies are warranted to determine whether this observation is generally applicable to other contaminants.

ACKNOWLEDGMENT

Technical assistance was provided by Brenda Headley and Mary Ferguson.

Received for review May 9, 1995. Accepted August 28, 1995.[⊗]

AC950441E

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1995.