Formation of Fluorophores from Nitrogenous Pesticides by Photolysis and Reaction with OPA-2-Mercaptoethanol for Fluorescence Detection in Liquid Chromatography[§]

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Postcolumn UV photolysis followed by reaction with *o*-phthalaldehyde-2-mercaptoethanol (OPA-MERC) and fluorescence detection was investigated for analysis of several classes of nitrogenous pesticides. Typical reversed-phase mobile phases, water, mixtures of methanol/water (1:1) and acetonitrile/water (1:1), and methanol, were evaluated as the solvents. Acetone, acetophenone, and surfactant Triton X-100 were assessed as photosensitizers to enhance photolysis and fluorescence responses. Liquid chromatographic separations of several pesticides were established that were followed by UV photolysis and reaction with OPA-MERC reagent for fluorescence detection. The mobile-phase solvents as well as photosensitizing agents greatly influenced photolysis reaction products and fluorescence responses. Photochemical transformation products of some of the pesticides are proposed. Analytical figures of merit were determined. This detection technique is applicable to pesticides at microgram per kilogram concentrations.

Trace analysis of pesticide residues in complex matrices with the required high sensitivity and selectivity is generally performed employing high-performance liquid chromatography (HPLC) and gas chromatography (GC). A variety of detectors have been developed for the HPLC and GC techniques that enable such trace analysis. Fluorometric and electrochemical are two of the more sensitive and selective HPLC detectors. Postcolumn derivatization can often be employed to improve the selectivity and sensitivity of detection for an analyte during HPLC assay (Frei, 1981). Postcolumn reaction detectors allow the chromatographer to modify analyte chemical structure in-line and are popular as a means of improving sensitivity and selectivity. Moye et al. (1979) were the first to report a postcolumn reaction detector that employed alkaline hydrolysis of *N*-methylcarbamate and carbamoyl oxime pesticides to methylamine followed by condensation with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (MERC) to produce a highly fluorescent isoindole. This approach was refined and formed the basis of U.S. EPA Method 531 (1985), as well as one of the official AOAC methods for foods (Krause, 1980). It has been demonstrated recently that the introduction of a UV lamp in-line allows photoactivation of many pesticide compounds prior to electrochemical (Krull and LaCourse, 1986) or fluorescence detection (Luchtefeld, 1985; Miles and Moye, 1987, 1988).

The photochemical transformation of pesticide chemicals in pure water occurs only by a direct photolysis mechanism in which the compound absorbs the incident radiation directly. In the indirect photolysis processes, an additional solute is needed to act as a chromophore and to transfer absorbed energy or generate an active oxidizing species (Zepp and Cline, 1977). Such materials are referred to as "photosensitizers". The photochemical degradation of pesticides has been reviewed extensively in the literature (Crosby and Li, 1969; Plimmer, 1971; Marcheterre et al., 1988). Photodecomposition of several pesticide chemicals exposed to UV is accelerated by photosensitizing agents. Organic photosensitizers are useful in accelerating many photochemical reactions (Ivie and Casida, 1971). Matthews (1986) has shown that aqueous suspensions of TiO_2 illuminated with UV light initiate both reductive and oxidative chemical reactions. Reduction increases the likelihood that primary amines would be formed from some of the nitrogenous compounds chosen for this study.

We constructed a postcolumn photolysis/fluorescence detector and extensively examined several classes of nitrogenous pesticides for fluorescence after UV photolysis followed by derivatization reaction with the OPA-MERC reagent. Water, methanol, and 1:1 mixtures of methanol/water and acetonitrile/water were evaluated as the photolytic mobile-phase solvents. Acetone, acetophenone, and surfactant Triton X-100 were assessed as photosensitizers to enhance photolysis and fluorescence responses. Liquid chromatographic separations followed by UV photolysis and reaction with OPA-MERC were evaluated for fluorescence detection and photoconductivity detection of several pesticides. Analytical figures of merit for several analytes were determined, and the data are presented for analysis of several pesticides in water with this technique.

EXPERIMENTAL SECTION

Apparatus. A schematic diagram of the HPLC postcolumn UV photolysis/OPA-MERC reaction fluorescence detection instrumental system is shown in Figure 1. It is similar to that described earlier (Miles and Moye, 1987) except for the knitted Teflon reaction coil and an ABI Analytical Kratos Division Spectroflow 980 programmable fluorescence detector that replaced a Model FS 970 detector. A Tracor Model 965 photoconductivity detector was also incorporated in series with the fluorescence detector along with a Soltec Model 261 chart recorder for the detector response evaluation.

A Perkin-Elmer 3 × 3 (0.46 × 3.3 cm; 3 μm) C_{18} column (room temperature) and an acetonitrile/water gradient were used for all separations with 0.4-mL sample injections of water samples in order to detect nanogram amounts of pesticides. The solvent program used was as follows: 1.0 mL/min, linear gradient of 95:5 water/acetonitrile to 50:50 water/acetonitrile in 10 min; hold at 50:50 for 5 min, step gradient to 95:5 water/acetonitrile; equilibrate for 8 min.

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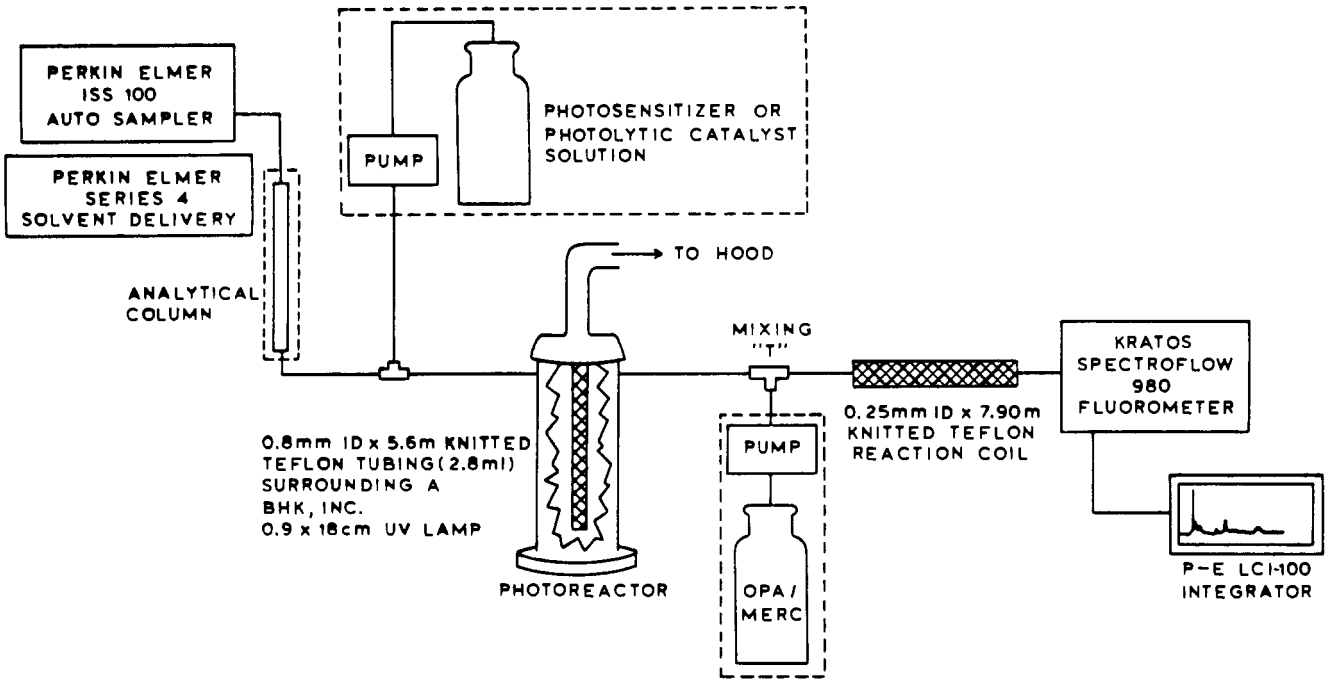

Figure 1. Schematic diagram of the HPLC postcolumn UV photolysis—OPA–MERC reaction fluorescence detection instrumental system. Optional components are enclosed with dashed lines.

All initial photolysis studies were performed employing flow injection analysis (FIA) to eliminate chromatographic problems and to allow rapid screening. The solvent program used was 1 mL/min of the solvent/photosensitizer systems.

The OPA–MERC reagent was metered into the LC column effluent with a mixing tee (Rainin No. 200-22) at 0.5 mL/min. Reaction of the OPA–MERC reagent with analyte was achieved in a 7.9 m × 0.25 mm (i.d.) × 1.5 mm (o.d.) knitted Teflon reaction coil woven as described by Engelhardt and Neue (1982).

Fluorescence was measured at wavelengths greater than 418 nm after excitation at 235 nm with a deuterium source. The fluorescence signals were integrated by the Perkin-Elmer LCI-100 integrator and printed on thermal paper. Fluorescence signals were also printed on a Soltec Model 261 strip chart recorder.

Reagents. Acetonitrile, acetone, and water were all HPLC grade, and methanol, Optima grade, and surfactant Triton X-100 were obtained from Fisher Scientific Co.

Chemicals. Chlorsulfuron, chlorimuron ethyl, metsulfuron methyl, and sulfometuron methyl were obtained from Du Pont (Wilmington, DE) while fenoxycarb was obtained from Maag Agrochemicals (Vero Beach, FL). The remainder of the pesticide standards came from the U.S. Environmental Protection Agency, Pesticides and Industrial Chemicals Repository (Research Triangle Park, NC). The purities ranged from 95% to 100%. Solutions of each pesticide (1 mg/mL) were prepared in methanol or water and diluted such that a 1-μL injection provided from 0.1 to 10 nmol. Injections of 1 μL were made in the initial screening to minimize the effects of the pesticide solution solvent on the photolysis/fluorescence responses.

Measurement Procedure. Relative fluorescence response measurements were carried out initially by FIA without an HPLC column in place, thus permitting rapid screening. Each of the solvent systems, water, methanol, and 1:1 mixtures of methanol/water and acetonitrile/water, were evaluated as the mobile phases. The solvent systems evaluated with the photosensitizers acetone, acetophenone, and Triton X-100 were comprised of the following: water, 1:1 mixtures of methanol/water, and acetonitrile/water each with 0.5% acetone; 1:1 mixtures of methanol/water each with 0.5% acetophenone and 0.02% Triton X-100. One-microliter injections of each of the nitrogenous pesticide solutions were used for each of these mobile phases. The fluorescence measurements were performed with the instrumental setup in two modes of operation: (1) no UV, OPA–MERC (analyte and/or associated material reaction with OPA–MERC); (2) UV, OPA–MERC (reaction of photodegradation products with OPA–MERC reagent). All measurements were carried out in duplicate. Relative fluorescence intensities (relative to an equimolar amount of methylamine standard) for UV photolysis followed by OPA–MERC reaction were evaluated from these measurements for each analyte and every solvent system. The fluorescence response of the analyte is the difference between the average of duplicate measurements with the UV lamp on and the UV lamp off. The fluorescence improvements were also evaluated for each analyte in the UV photolysis followed by OPA–MERC reaction mode for the derivatization reaction of photodegradation products with the OPA–MERC reagent. The fluorescence improvement in this case is the ratio of fluorescence intensity of photolyzed and derivatized chemical to fluorescence intensity of untreated chemical.

HPLC separations were performed using the reversed-phase solvents listed above, and measurements were carried out similarly, for several of those analytes showing very high fluorescence responses. Fluorescence responses, relative fluorescences, and analytical figures of merit were evaluated for those analytes.

The photosensitizers acetone, acetophenone, and surfactant Triton X-100 were added directly to the typical reversed-phase solvents to produce the photosensitization, thus eliminating the need for an additional pump.

RESULTS AND DISCUSSION

Fluorescence Detection of Pesticides. All of the 37 nitrogenous pesticide chemicals representing several classes of compounds were evaluated for fluorescence after UV irradiation followed by OPA–MERC reagent derivatization reaction with each compound. The pesticides are arbitrarily divided in classes according to a major functional group. Obviously, polyfunctional compounds could belong to several groups. The pesticide classes studied include amines, phenols, carbamates, triazines, amides, anilides, etc. The listings of pesticides are grouped by class, with each class being identified in the tables. The highest fluorescence responses obtained for each compound from among all of the analytes screened and the nine solvent/photosensitizer systems evaluated are summarized in Table I. The fluorescence signal intensities after UV photolysis followed by OPA–MERC reaction (with UV lamp on), fluorescence, if any, with OPA–MERC reagent only (UV lamp off), net fluorescence response per nanomole of each pesticide, and relative flu-

Table I. Most Intense Fluorescence Observed from among Several Solvent/Photosensitizer Systems upon UV Photolysis and OPA–MERC Reagent Derivatization of Nitrogenous Pesticides

pesticide name[a]	fluorescence signal for 1 μg analyte (total area × 10^6) UV + OPA (UV on)	OPA only (UV off)	fluorescence resp/nmol[b]	rel fluorescence[c]	solvent/photosensitizer syst	fluorescence improvement[d]
diphenamid (Enide) (1)	6.429	0.025	1.533	0.385	1:1 H_2O/CH_3OH	257.2
EPTC (Eptam) (2)	4.014	0.019	0.757	0.192	1:1 H_2O/CH_3CN	211.3
thiobencarb (Bolero) (2)	4.990	0.027	1.280	0.324	1:1 H_2O/CH_3CN	184.8
molinate (Ordram) (2)	0.775	0.014	0.143	0.036	1:1 H_2O/CH_3OH	55.4
paraquat (Gramoxone) (3)	3.865	0.022	0.988	0.249	1:1 H_2O/CH_3OH	175.7
cyanazine (Bladex) (4)	0.662	0.021	0.154	0.053	100% H_2O	31.5
dinoseb (DNBP) (5)	1.421	0.045	0.352	0.139	1:1 H_2O/CH_3OH + 0.5% acetone	31.6
metribuzin (Lexone) (4)	0.135	0.013	0.026	0.007	1:1 H_2O/CH_3OH	10.4
daminozide (Alar) (6)	4.127	0.034	0.656	0.166	1:1 H_2O/CH_3CN	121.4
chlorpyrifos (Dursban) (7)	0.302	0.060	0.085	0.014	1:1 H_2O/CH_3OH	5.0
aldicarb (Temik) (6)[e]	0.895	0.082	3.095	0.514	1:1 H_2O/CH_3OH	10.9
ferbam (Knockmate) (2)[e]	0.584	0.091	4.108	0.683	1:1 H_2O/CH_3OH	6.4
1,1-dimethylhydrazine (1)	6.406	0.176	0.375	0.094	1:1 H_2O/CH_3OH	36.4
methomyl (Lannate) (6)[f]	2.146	0.048	3.403	0.566	1:1 H_2O/CH_3OH	44.7
N-methylformamide (1)	0.323	0.190	0.008	0.002	1:1 H_2O/CH_3CN	1.7

[a] Class: (1) amide; (2) thiocarbamate; (3) bipyridylium; (4) triazine; (5) dinitrophenol; (6) carbamate; (7) organophosphorus. [b] Fluorescence response of the pesticide is the difference between average of duplicate measurements with UV lamp on the UV lamp off. [c] Relative fluorescence is the ratio of the fluorescence response of pesticide to an equimolar amount of methylamine. [d] Fluorescence improvement = fluorescence intensity of photolyzed and derivatized chemical/fluorescence intensity of untreated chemical. [e] 0.5 μg. [f] 0.1 μg.

orescence are presented for each compound. Also included are the fluorescence improvement factors for each of the pesticides showing positive response to the UV photolysis and subsequent reaction with the OPA–MERC reagent. The optimal solvent systems that produced the highest fluorescence improvement factors for each analyte are also indicated in the table. These results indicate that a little more than one-third (40%) of all the nitrogenous pesticides studied show significant fluorescence responses after UV photolysis followed by OPA–MERC reaction, i.e., relative fluorescence greater than 1% and/or fluorescence improvement factors larger than 3. Further, the solvent type significantly affects the fluorescence responses. Various solvent systems produced the highest responses for various pesticides. No one solvent system produced the best responses for all the compounds screened. Other investigators also have similarly observed that solvent type affects the fluorescence responses of phototransformation products (Scholten and Frei, 1979; Scholten et al., 1980). The relative fluorescence values in Table I are not corrected for the responses observed (if at all) for UV photolysis only since the effect of the OPA–MERC reagent on the photoinduced or native fluorescence of these compounds is unknown.

Addition of acetone, a photosensitizer, to the 1:1 water/methanol solvent system very significantly enhanced the OPA–MERC reaction and greatly increased relative fluorescence (percent photolysis efficiency) of dinoseb from 0.1% without acetone to 13.9% with acetone. Presence of acetone in the solvent system 1:1 water/acetonitrile upon UV photolysis appears to produce large amounts of primary amine as evidenced by the OPA–MERC reagent derivatization reaction that produced very high fluorescence background. Fluorescence measurements, therefore, could not be carried out with this solvent system in the UV photolysis followed by OPA–MERC reaction mode.

Relative fluorescence was very dependent upon the solvents used as noted earlier, showing that the solute environment was important in the photolysis mechanism. It has been reported that the presence and/or absence of oxygen affects the rate and products formed from some pesticides (Freeman and McCarthy, 1984; Freeman and Ndip, 1984). Even though the solvents used in this investigation were purged with helium to avoid bubble formation during chromatography, the oxygen permeability of Teflon (Poulsen et al., 1986) prevented deoxygenation from being achieved during the entire photolysis reaction. Therefore, the role of oxygen in the photolysis of these pesticides is undetermined. Further, UV irradiation of Teflon produces protons and fluoride ions (Batley, 1984), which may affect phototransformation products. During the course of these studies it was observed that Teflon tubing used for knitting the photoreaction coil lost its integrity and developed leaks in the presence of the surfactant Triton X-100 (0.02%) with the 1:1 H_2O/CH_3OH system during the UV photolysis followed by OPA–MERC derivatization reaction mode. The reactor coil damage occurred repeatedly and reproducibly (five times) within 40–60-min exposure to UV irradiation. This could probably be due to photosensitized reaction and deformation/degradation of the Teflon tubing material with UV irradiation at the critical micelle concentration (cmc; 0.02% of the surfactant Triton X-100 used in this work). The cmc of the surfactant Triton X-100 reported in the literature is 0.02% (Mukerjee and Mysels, 1971). It is well-known that at and above the cmc the surfactant micelles have unique properties and they enhance solubilization. Further, the ability of the micellar structure to partition analyte from the aqueous phase into the organic phase increases very significantly.

Aldicarb and methomyl have been shown to produce significant amounts of methylamine after photolysis in acetonitrile solutions (Freeman and McCarthy, 1984; Freeman and Ndip, 1984). Luchtefeld (1985) proposed that several phenylurea herbicides produce methylamine upon photolysis. Photodegraded solutions of several of the test pesticides have also been shown by Miles and Moye (1987, 1988) to produce methylamine and other primary amines during photolysis. These primary amines react with the OPA–MERC reagent forming strongly fluorescing compounds having the isoindole structures. The pesticides evaluated in the present study include nitrogenous compounds with substituted aromatic moieties and with *N,N*-dialkylamide structures that may photolytically cleave to produce primary amines and thus react with the OPA–MERC reagent. Some of these pesticides are from the EPA Pilot Groundwater Study list because of increasing interest in them.

Table II. Fluorescence Improvements Observed upon UV Photolysis Followed by OPA–MERC Derivatization of Several Pesticides in Various Solvent Systems

	fluorescence improvement[b]						
pesticide name[a]	H_2O	CH_3OH	1:1 H_2O/CH_3OH	1:1 H_2O/CH_3CN	H_2O + 0.5% acetone	1:1 H_2O/CH_3OH + 0.5% acetone	1:1 H_2O/CH_3OH + 0.5% acetophenone
trifluralin (1)	5.4	5.1	8.4	15.6	2.2	20.1	2.1
carbendazim (2)	1.7	16.5	6.5	12.7	1	0.6	1.1
carbetamide (3)	4.5	14.4	4.1	8	1.8	2.2	1
carboxin (3)	21.4	67.4	42.5	14.9	2.8	16.8	1.3
alachlor (4)	3.5	73	20.4	18.2	2	2.9	1.5
butachlor (4)	3.2	34.6	18.5	13.4	1.8	2.1	1.3
avermectin (5)	1.7	3.3	1.5	1.3	1.9	0.8	0.6
paraquat (6)	45	80.7	175.7	127	1	10.6	1.5
chloramben (7)	18.4	46	82.5	40.8	1.8	73.5	19.3
fenamiphos (8)	2.9	5.8	16.8	5.7	1.4	2.3	0
metolachlor (4)	6	20.6	13.5	12.7	1.8	3.2	1.6
metribuzin (9)	1	1.5	10.4	1.2	1.7	4.1	1.7
cyanazine (9)	31.5	3.5	10.8	7.8	1.7	2.3	0.9
terbacil (9)	2.3	5.5	1.1	1.7	1.2	0.4	1.1
cyromazine (9)	0.8	1.4	1.2	0.9	1.1	1	1.5
dinoseb (10)	0.8	0.8	0.5	0.8	0.8	32.6	3.3
daminozide (11)	7	10.4	5	121.4	1.7	5.9	2.9
molinate (11)	16.5	24.8	55.4	21.8	1.3	0.7	0.9
thiobencarb (11)	208.6	26.3	217.8	184.8	1.6	1	1.7
EPTC (11)	74.2	37.2	70.7	211.3	1.3	1.8	1.2
diphenamid (3)	242.4	85.7	257.2	210.3	1.6	12.2	0.9

[a] Class: (1) dinitroaniline; (2) carbamic acid; (3) amide; (4) acetanilide; (5) lactone macromolecule; (6) bipyridylium; (7) amine; (8) organophosphorus; (9) triazine; (10) dinitrophenol; (11) carbamate. [b] Fluorescence improvement = fluorescence intensity of photolyzed and derivatized chemical/fluorescence intensity of untreated chemical. 1:1 H_2O/CH_3CN + 0.5% acetone system: very high fluorescence background. 1:1 H_2O/CH_3OH + 0.02% Triton X-100 system: reaction with and damage to Teflon reaction coil.

The UV photolysis followed by OPA–MERC reagent reactions of several pesticide chemicals investigated in the present study was carried out with use of several compounds as possible photosensitizing agents, and the phototransformed products were monitored for fluorescence. Fluorescence improvements for several pesticide chemicals in various solvent systems are summarized in Table II. The fluorescence responses of several of the pesticide structures are viewed in the following discussion in light of available information in the literature pointing to possible phototransformation products responsible for the observed fluorescence improvements.

Paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) showed no fluorescence without UV photolysis. UV photolysis of paraquat produced a strong fluorescence response, and 1:1 water/methanol provided the highest fluorescence improvement. Acetone produced no noticeable effect. It was interesting to note that paraquat readily reacted with OPA–MERC derivatization reagent after UV photolysis to various extents in various solvent systems. The net fluorescence improvement for the OPA (total fluorescence improvement with UV and OPA reaction—fluorescence improvement with UV photolysis) with 1:1 water/acetonitrile system was highest followed by that in 1:1 water/methanol. Photochemical degradation of paraquat has been studied extensively (Slade, 1965; Funderburk and Bozarth, 1967; Kearney et al., 1985). It has been shown (Slade, 1965) that dilute aqueous solutions of paraquat are rapidly photochemically degraded by UV light to 1-methyl-4-carboxypyridinium ion (**1**), methylamine hydrochloride (**2**), and methylamine as the final product (see Figure 2). Further, small amounts of a mono- and dipyridone are also reported as the degradation products that are highly fluorescent (Kearney et al., 1985). As seen from the data in Table II, UV photolysis of paraquat in the solvents 1:1 water/methanol and 100% methanol produces very high fluorescence improvements compared to the 100% water and 1:1 water/acetonitrile solvent systems. The percent decomposition of paraquat varies with solvent composition. It seems that the presence of methanol in the water produces more of the 1-methyl-4-carboxypyridinium ion, monopyridone, and dipyridone having the aromatic structures that are responsible for the very high fluorescence response. Paraquat responds well with UV photolysis followed by OPA–MERC reagent derivatization and produces greatly enhanced fluorescence responses. The total fluorescence in the UV + OPA mode seems to be due to sums of compounds **1** + **2** (see Figure 2) and small amounts of the pyridones. As seen from the data on net fluorescence improvement upon UV photolysis + OPA–MERC reaction, the 1:1 water/acetonitrile system yields the highest amounts of methylamine hydrochloride (**2**), followed by 1:1 water/methanol, methanol, and water alone. The 1:1 water/acetonitrile solvent system is best suited, therefore, for UV photolysis followed by OPA–MERC reaction for fluorescence detection of paraquat with the highest sensitivity and the lowest limit of detection (see below). The fact that paraquat fluoresces upon UV photolysis is very important. It is a pesticide that is difficult to detect by GC because, being a bipyridyl, it is nonvolatile. However, it is now often analyzed by GC using a procedure that involves reduction to a neutral species, which then can be analyzed quite easily by GC. The typical method of analysis for paraquat is reduction by dithionite followed by colorimetric assay of the blue free-radical product formed. Paraquat is also typically analyzed by HPLC with UV detection but with moderate sensitivity (5 μg/kg). In addition, small, positively charged organics seldom fluoresce. Therefore, it is probably being reduced in some manner to a neutral or an ionic species. Finally, it fluoresces quite strongly compared to quinine sulfate, making the UV photolytic–fluorescence approach a very good candidate for HPLC analysis.

The photochemistry of the important group of triazine pesticides (atrazine, cyanazine, cyromazine, melamine, metribuzin) has not been thoroughly studied. They are, however, comparatively stable to light and require low wavelengths to disrupt the molecules. Earlier investigations confirm that radiation of short wavelengths is nec-

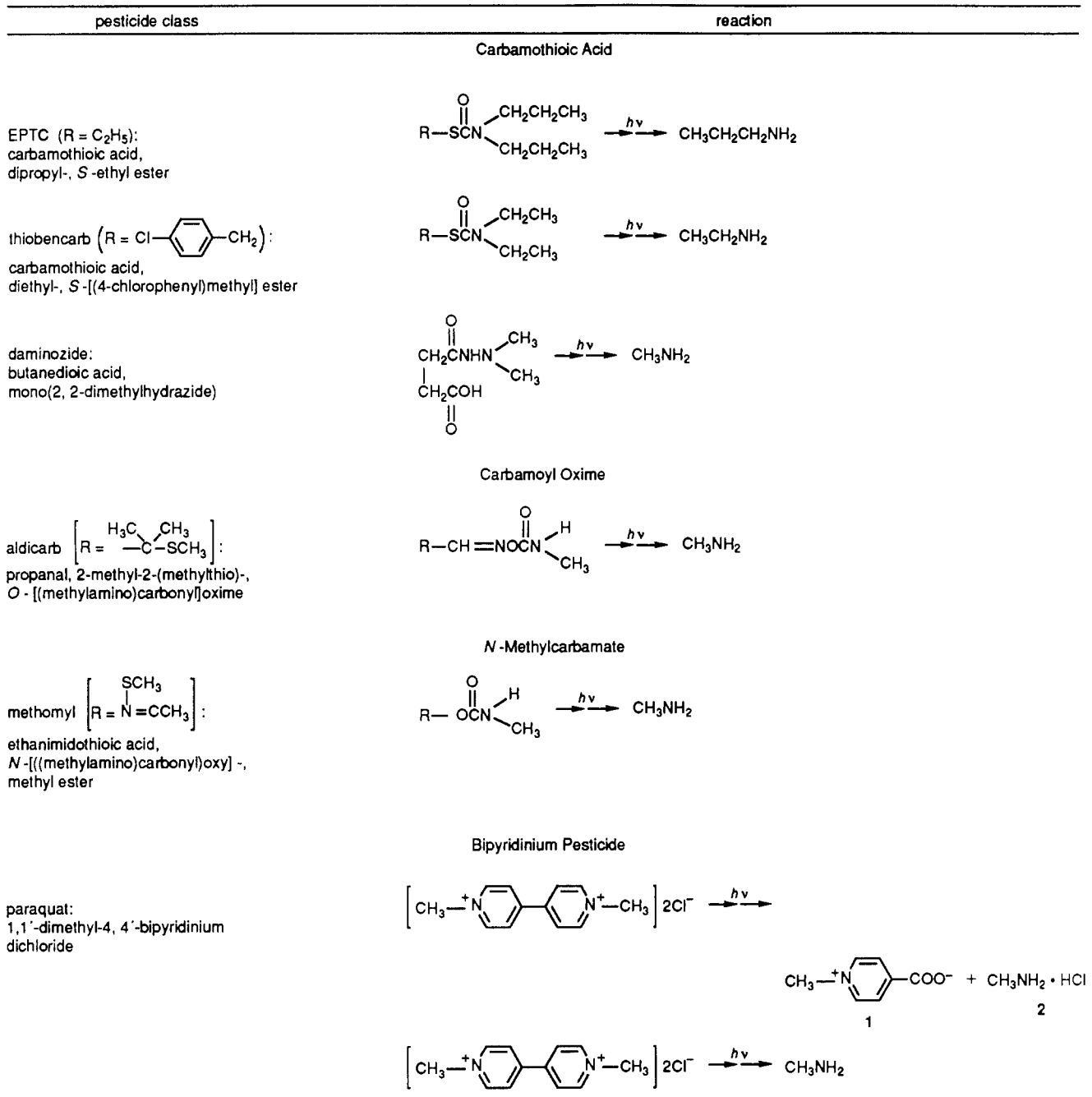

Figure 2. UV photolysis reactions of several pesticide chemicals.

essary to affect the (dialkylamino)-*s*-triazines and light of 220 nm was necessary to bring about a photochemical reaction (Plimmer, 1971). In the present study, the highest fluorescence improvements obtained upon UV photolysis were small though, in the range of 3.5–9.4 for these analytes. Metribuzin and cyromazine showed the best fluorescence improvements of 9.4 and 6.7, respectively, with 1:1 water/acetonitrile and acetone systems. The photosensitizer acetone greatly enhanced photolysis of these pesticides. UV photolysis followed by OPA–MERC derivatization provided the highest fluorescence improvements only for metribuzin with 1:1 water/methanol solvent and for cyanazine with 100% water system. However, melamine, atrazine, and cyromazine did not show any reaction with the OPA–MERC reagent. It appears that UV photolysis in the presence of solvents such as water, water/methanol, and water/acetonitrile leads to photochemical degradation forming a number of products. The chloro group is displaced as would be predicted. Replacement of chlorine by a methoxyl group, leading to formation of methylated products, also is possible and is reported in the literature (Crosby and Li, 1969; Plimmer, 1971; Kearney and Helling, 1969). Use of the lower wavelength radiation 214-nm zinc lamp should lead to enhanced photolysis and improved fluorescence responses for the triazine pesticides. Such studies are in progress.

Dinoseb (2-*sec*-butyl-4,6-dinitrophenol) showed no native fluorescence without UV photolysis. Upon UV irradiation it produced the highest fluorescence improvement only with 1:1 water/acetonitrile. Acetone enhanced fluorescence response to some extent. However, dinoseb reacted very well upon UV photolysis followed by OPA–MERC derivatization, and the best fluorescence improvement was seen with 1:1 water/methanol with acetone. Dinitrophenols and their esters are extensively used as pesticides. The photochemistry of dinitrophenolic pesticide chemicals is poorly understood. Photodegrada-

Table III. Analytical Figures of Merit for Several Pesticides with the UV Photolysis Followed by OPA-MERC Derivatization Fluorescence Detection Technique

pesticide name[a]	solvent system with or without photosensitizer	fluorescence response/nmol of analyte	rel fluorescence w/methylamine std	photolysis efficiency, %	anal. range, ng	limit of detection,[b] 10^{-9} g
dinoseb (1)	50% H_2O + 50% methanol with 0.5% acetone	1.01	0.079	7.9	5–1000	5
EPTC (2)	50% H_2O + 50% acetonitrile	6.25	0.397	39.7	0.4–100	0.4
daminozide (2)	50% H_2O + 50% acetonitrile	3.50	0.245	24.5	1–100	1
thiobencarb (2)	50% H_2O + 50% acetonitrile	15.1	0.995	99.5	0.3–100	0.3
trifluralin (3)	50% H_2O + 50% methanol	1.72	0.066	6.6	2–1000	2
molinate (2)	50% H_2O + 50% methanol	2.53	0.098	9.8	1–1000	1
paraquat (4)	50% H_2O + 50% methanol	9.12	0.343	34.3	0.7–300	0.7
diphenamid (5)	50% H_2O + 50% methanol	24.4	1.007	100.7	0.2–100	0.2

[a] Class: (1) dinitrophenol; (2) carbamate; (3) amine; (4) bipyridinium; (5) amide. [b] Limit of detection from signal peak height and background peak-to-peak noise for S/N = 3.

tion of dinoseb under UV light in oxygenated methanol solution was reported to yield many degradation products by hydrolysis, reduction of the *o*-nitro group, oxidation of the *sec*-butyl side chain, and sensitized photoalteration, primarily involving polymerization (Kearney and Helling, 1969; Matsuo and Casida, 1970; Bandal and Casida, 1972). It seems UV photolysis of dinoseb in 1:1 water/methanol with acetone produces reduction by abstraction of hydrogen atoms from the medium, and either or both NO_2 groups are reduced to the NH_2 group. This phototransformation product then reacts with the OPA–MERC reagent leading to high fluorescence response. Photoreduction of one of the nitro groups to the amine of the 2,4-dinitro-6-methylphenol was observed; partial photoreduction of aromatic nitro compounds to the corresponding nitrosobenzenes has long been known (Plimmer, 1971). Photodealkylation of other alkylanilines is also well-known. Photolysis of dinoseb and trifluralin as well as its close relatives may be expected to produce products that are reduced and/or dealkylated; and the reduced NO_2 groups are probably methylated in the oxygenated methanol solution (Marcheterre et al., 1988). In photoirradiation of aryl methylcarbamates Zectran [4-(dimethylamino)-3,5-xylyl methylcarbamate] and Matacil [4-(dimethylamino)-3-cresyl methylcarbamate], the *p*-dimethylamino group had been reported (Abdel-Wahab et al., 1966) to undergo N-dealkylation without other alteration in the molecule and *N*-formamido compounds appeared as intermediates in the dealkylation. Methylamine thus formed upon photolysis of dinoseb can account for the observed reaction with the OPA–MERC reagent.

Phenylamides of the general structure C_6H_5NHCOR are encountered in a large number of pesticide compounds. Important subclasses of this class of chemicals include the phenylcarbamates and acylamides. Photodegradation of these compounds proceeds by cleavage of the amide or ester linkage to yield aniline, CO_2, and alcohol in the case of the phenylcarbamates and aniline and aliphatic acid in the case of acylamides. In the UV irradiation of aryl methylcarbamates, the *p*-dimethylamino group undergoes N-dealkylation without other alteration in the molecule and *N*-formamido compounds appear as intermediates in the dealkylation (Abdel-Wahab et al., 1966). Photolysis of phenylamide, phenylcarbamate, and phenylureas produced hydroxy-substituted anilines that had native fluorescence. Several experiments had indicated that these fluorescent species were hydroxy-substituted anilines, and mass spectra were consistent with this identification (Miles and Moye, 1988). UV photolysis of most of the *N*-methylcarbamates, carbamoyl oximes, carbamothioic acids, dithiocarbamates, phenylureas, and several pesticides tested (e.g., daminozide, molinate, thiobencarb, EPTC, methomyl, etc.) produced primary amines that formed highly fluorescent products upon reaction with the OPA–MERC reagent (see Figure 2). Although most of the analytes produced methylamine, carbamothioic acids produced primary amines corresponding to the alkyl groups attached to the nitrogen atom.

UV photolysis followed by the OPA–MERC reagent derivatization resulted in highly fluorescent products, and very high fluorescence improvements were obtained here for a large number of pesticides through formation of primary amines upon UV irradiation. The previous observation of amine formation upon photolysis of benzamides (Miles and Moye, 1987; Patel et al., 1989) continued in the present work also. Figure 2 summarizes some of the UV photolysis reactions we have observed in this study for several of the pesticides. The UV photolysis fluorescence approach to the generation of primary amines from pesticides, many of which are secondary and tertiary amines, has wide applicability and warrants further investigations.

Photoconductivity Detection. The photoconductivity detector signal responses of the photodegradation products obtained upon UV photolysis followed by the OPA–MERC derivatization reaction of several pesticides were compared to similar measurements with the fluorescence detector. Fourteen solutions of pesticides measured in 1:1 water/methanol mobile phase by the two detectors simultaneously showed that the fluorescence detection approach provided very sensitive and selective detection of these analytes. However, with the photoconductivity detector, the background noise level was so high that it was impossible to measure the analyte signals at the limits of detection generally obtainable with the UV photolysis alone. The very high background and unsteady base line resulted from a large contribution of ionic species from the reagents and buffers used in preparation of the OPA–MERC reagent. The photoconductivity detector response with UV photolysis followed by the OPA–MERC reagent reaction led to very high, noisy background levels and was therefore unsuitable for sensitive detection of pesticides in this mode.

Analytical Figures of Merit. As demonstrated from the foregoing results on the UV photolysis followed by OPA–MERC reagent derivatization approach for fluorescence detection of the pesticide compounds, a large number of these compounds responded to the photolysis–fluorescence detection technique. The optimal experimental conditions for each compound varied greatly though, depending upon its structure. The selectivity and specificity of detection of a large number of pesticide chemicals by HPLC using postcolumn UV photolysis fluorescence detection can be greatly enhanced com-

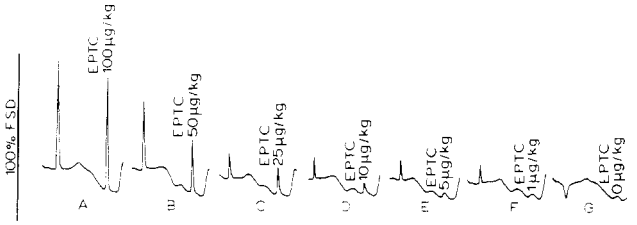

Figure 3. Chromatograms of single pesticide in water using reversed-phase HPLC and postcolumn UV photolysis—OPA-MERC reaction fluorescence detector. Conditions: Perkin-Elmer 3 × 3 C_{18} column (room temperature); solvent program, 1 mL/min, linear gradient of 95:5 water/acetonitrile to 50:50 water/acetonitrile in 10 min, hold at 50:50 for 5 min, step gradient to 95:5 water/acetonitrile, and equilibrate for 8 min; sample injection volume, 0.4 mL. EPTC concentrations: A, 100 µg/kg; B, 50 µg/kg; C, 25 µg/kg; D, 10 µg/kg; E, 5 µg/kg; F, 1 µg/kg; G, 0 µg/kg control.

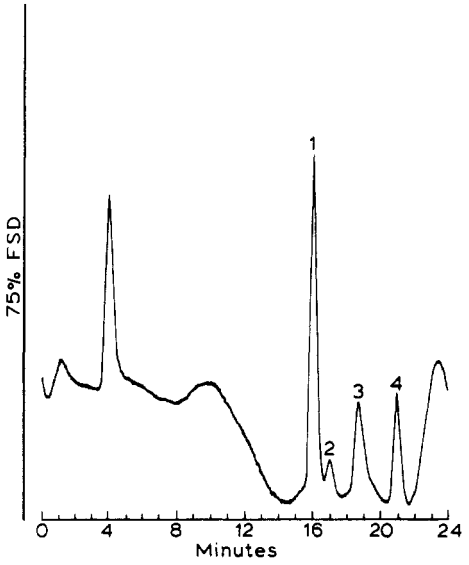

Figure 4. Chromatograms of multicomponent pesticides in water analyzed under conditions mentioned in Figure 3. Pesticides: 1, diphenamid, 20 µg/kg, RT, 15.85 min; 2, molinate, 25 µg/kg, RT, 16.83 min; 3, EPTC, 12.5 µg/kg, RT, 18.52 min; 4, thiobencarb, 20 µg/kg, RT, 20.72 min.

pared to HPLC with UV detection alone. This can be achieved with use of the OPA-MERC reagent derivatization approach following the UV irradiation and phototransformation. This approach was employed for evaluating the analytical figures of merit for several of the more responsive compounds. Each analyte was evaluated using the solvent system providing the greatest fluorescence response, either with or without the photosensitizer. Fluorescence responses were measured for various concentrations following UV photolysis and OPA-MERC reaction.

Relative fluorescence values were measured with methylamine standards, and the photolysis efficiency (percent relative fluorescence) for the analyte was calculated. The useful analytical range for the analyte was determined by measuring the lowest concentration and the highest concentration providing linear analytical curves. The limit of detection for the analyte was evaluated from several fluorescence measurements of lower concentrations. Fluorescence signal peak heights, background noise (peak to peak), and blank measurements were carried out, and detection limits were calculated for a signal-to-noise ratio of 3. Analytical figures of merit for several of the more responsive nitrogenous pesticide analytes obtained in this manner are shown in Table III. As seen from the data, these analytes respond very well (3–4× better) to the present detector compared to the old fluorescence detector FS970, have very good to excellent photolysis efficiencies (7–101%), and have very low limits of detection, generally in the fractional to lower nanogram amounts. Typical analytical curves for several of the analytes were linear over analyte concentrations varying between 2 and 3 orders of magnitude with the low end of the curves in the range of fractional to lower nanogram amounts.

Analytical Applications. Analytical applications of the HPLC postcolumn photolysis—OPA-MERC derivatization fluorescence detection technique were demonstrated by the determination of several classes of pesticides in water. Distilled deionized water samples were fortified with the pesticides diphenamid, EPTC, molinate, and thiobencarb at several concentrations of single- and multiple-pesticide mixtures. HPLC separations of the analytes were achieved in 23 min by using a water/acetonitrile gradient on a Perkin-Elmer 3 × 3 C_{18} column at room temperature. HPLC chromatograms are shown in Figures 3 and 4 for single- and multiple-component injections, respectively. The peak appearing at 4 min is due to the solvent methanol used for preparing the analyte solution. Typical analytical calibration curves for the HPLC-UV photolysis—OPA-MERC derivatization fluorescence detection of several pesticides in water were linear from 0.4 to 40 ng (1–100 µg/kg in water). Analytical figures of merit data for the chromatographic experiments are shown in Table IV. The analytical method limits of detection calculated from signal peak height and background peak-to-peak noise (for signal/noise = 3) from five to eight measurements for each analyte sample range from 0.5 to 7.4 µg/kg. Recoveries of pesticides from fortified water samples obtained by the HPLC-UV photolysis—OPA-MERC derivatization fluorescence detection technique for four pesticides are shown in Table V. These recoveries range from 90% to 110% at the lower 2–25 µg/kg concentrations.

The flow injection analysis as well as the HPLC separation and fluorescence detection techniques following postcolumn photolysis and reaction with the OPA-MERC reagent are sensitive and selective methods for analysis of many pesticides at microgram per kilogram concentrations. Many pesticides produce intense fluorescence upon UV photolysis through formation of fluo-

Table IV. Analytical Figures of Merit for the HPLC-UV Photolysis OPA-MERC Derivatization Fluorescence Detection of Several Pesticides in Water

pesticide name[a]	range of analyte amt studied, ng	analyte concn range, µg/kg	S/B ratio[b] signal peak height/ bkgd noise at 10 µg/kg	lim of detection,[c] µg/kg
diphenamid (1)	0.4–40	1–100	53	0.5
EPTC (2)	1–40	2.5–100	24	1.3
molinate (2)	4–80	10–200	8[d]	7.4
thiobencarb (2)	0.8–40	2–100	22.5	1.3

[a] Class: (1) amide; (2) carbamate. [b] Number of measurements, n = 5. [c] Limit of detection from signal peak height, background peak-to-peak noise for S/N = 3. [d] Concentration 20 µg/kg.

Table V. Recoveries of Several Pesticides from Fortified Water Samples

pesticide name[a]	concn, μg/kg expected	concn, μg/kg estimated	% rec
diphenamid (1)	10	10	100
	20	18	90
	40	38	95
EPTC (2)	12.5	13.3	106.4
	25	25	100
molinate (2)	25	26	104
	50	50	100
thiobencarb (2)	2	2.2	110
	10	10	100
	40	39	97.5

[a] Class: (1) amide; (2) carbamate.

rescent species or primary amines that then undergo OPA–MERC derivatization reaction. Most of the analytes studied can be transformed into fluorescing products upon UV irradiation or UV irradiation followed by OPA–MERC derivatization. The photolytic solvent type seems to play an important role as does the presence of photosensitizers. Acetone greatly enhances the fluorescence of several compounds. Analytical figures of merit for several of the more promising candidates selected from among the nitrogenous pesticides for the UV photolysis followed by OPA–MERC derivatization approach show that these compounds can be detected at very low levels (subnanogram to nanogram). The fluorescence photolysis efficiency ranges from 0.4 to 101% compared to methylamine standard, and the analytical curves are linear over 2 orders of magnitude in the nanogram range. Analytical applications of several pesticides in water after HPLC separation and postcolumn photolysis OPA–MERC derivatization fluorescence detection permit detection of these analytes at 0.5–7.4 μg/kg levels with quantitative recoveries in the range 90–110%. Further, UV photolysis studies with programmed wavelength excitation and emission measurements at characteristic selected wavelengths and use of other photosensitizers such as benzophenone and semiconductors such as TiO_2 are needed that might improve and extend fluorescence responses. Various HPLC separation schemes with suitable solvent systems and column conditions have to be optimized and coupled with the optimized UV photolysis fluorescence detection and UV photolysis—OPA–MERC derivatization fluorescence detection systems. This approach could provide very sensitive and selective multiresidue methods for analysis of many pesticides at the nanogram levels in variety of samples.

Registry No. DNBP, 88-85-7; EPTC, 759-94-4; enide, 957-51-7; eptam, 759-94-4; bolero, 28249-77-6; ordram, 2212-67-1; gramoxone, 1910-42-5; bladex, 21725-46-2; lexone, 21087-64-9; alar, 1596-84-5; dursban, 2921-88-2; temik, 116-06-3; knockmate, 14484-64-1; 1,1-dimethylhydrazine, 57-14-7; lannate, 16752-77-5; *N*-methylformamide, 123-39-7; trifluralin, 1582-09-8; carbendazim, 10605-21-7; carbetamide, 16118-49-3; carboxin, 5234-68-4; alachlor, 15972-60-8; butachlor, 23184-66-9; avermectin, 73989-17-0; paraquat, 4685-14-7; chloramben, 133-90-4; fenamiphos, 22224-92-6; metolachlor, 51218-45-2; metribuzin, 21087-64-9; cyanazine, 21725-46-2; terbacil, 5902-51-2; cyromazine, 66215-27-8; dinoseb, 88-85-7; daminozide, 1596-84-5; molinate, 2212-67-1; thiobencarb, 28249-77-6; diphenamid, 957-51-7; 1,2-benzenedicarboxaldehyde, 643-79-8; 2-mercaptoethanol, 60-24-2.

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Isolation and Characterization of Pentacyclic Triterpene Ovipositional Stimulant for the Sweet Potato Weevil from *Ipomoea batatas* (L.) Lam.

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A methylene chloride extract from the surface of sweet potato storage roots (cultivar Centennial) was fractionated on silicic acid and eluted with hexane, 1:3 methylene chloride–hexane, and 1:1 methylene chloride–hexane. The 1:3 methylene chloride–hexane fraction yielded compound I: >96% purity, the most dominant compound present; mp 238–239.5 °C; IR, 1730, 1250 cm^{-1}. The mass spectrum of compound I displayed a molecular ion at m/z 468 with a molecular formula of $C_{32}H_{52}O_2$. The fragmentation pattern was indicative of a pentacyclic triterpene with isopropyl and acetate moieties. Compound II, which could be produced by the hydrolysis of compound I, was present in the 1:1 methylene chloride–hexane fraction: mp 214–215.5 °C; IR, 3400 cm^{-1}; molecular ion, m/z 426; molecular formula, $C_{30}H_{50}O$. The alcohol of compound I (compound II) was identified as boehmerol. Based on ^{13}C NMR and GC–MS data and physical evidence, the structure of compound I was established and tentatively named boehmeryl acetate. Boehmeryl acetate extracted from the surface of sweet potato storage roots appears to act as an ovipositional stimulant for the sweet potato weevil, *Cylas formicarius elegantulus* Summers.

Sweet potato [*Ipomoea batatas* (L.) Lam.] is a major international staple crop, grown extensively throughout the tropical and temperate zones for its edible storage roots (Pardales and Cerna, 1987). A constraint to sweet potato production in both tropical and temperature growing areas is the sweet potato weevil, *Cylas spp.* (Edmonds, 1971; Schalk and Jones, 1985), which feeds on all parts of the plant and lays its eggs in the storage roots (Reinhard, 1923; Cockerham et al., 1954). The development of insect-resistant sweet potato lines is seen as an essential component in the management of this pest (Martin and Jones, 1986). Son et al. (1989) demonstrated that there are significant differences in surface components of sweet potato storage roots between susceptible lines and those displaying a moderate level of resistance to the weevil [resistance estimates based on field evaluation (Mullen et al., 1981; Mullen et al., 1985)]. Wilson et al. (1988) demonstrated that a methylene chloride surface extract of the periderm of storage roots of the susceptible line Centennial stimulated oviposition of the weevil, *Cylas formicarius elegantulus* (Summers). Nottingham et al. (1987) also showed that ovipositional stimulant resided in the root periderm, not in the core of the storage root. In addition, it has been established that the major component (compound I) of the surface extract was an ovipositional stimulant of female weevils (Wilson et al., 1989).

In this paper, the isolation of two pentacyclic triterpenoids from the storage root of Centennial and the spectral evidence leading to the elucidation of their structures are presented.

MATERIALS AND METHODS

Materials. Sweet potato cultivar Centennial was grown at the University of Georgia Horticulture Farm during 1986 and 1987. After harvesting, the storage roots were washed, air-dried, and cured for 7 days at 29 °C and 90% RH and stored at 13 °C and 85% RH. Storage roots used for analysis were

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