Comparison of Solvent Systems for the Extraction of Atrazine, Benzoylprop, Flamprop, and Trifluralin from Weathered Field Soils

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The extraction of atrazine, benzoylprop-ethyl, flamprop-methyl, and trifluralin from three field soils that had received treatments of the individual herbicides 12 months previously was compared by using different solvent systems. The highest recoveries of atrazine were achieved by using 30% aqueous acetonitrile, at a pH of 9.0, as the extractant. Acetonitrile containing 30% water and 2.5% glacial acetic acid proved satisfactory for the extraction of benzoylprop-ethyl and flamprop-methyl, together with benzoylprop acid and flamprop acid, their respective soil-hydrolysis products. This extraction solvent, methanol, and 10% aqueous acetonitrile were all suitable for the extraction of trifluralin from treated soils. Prewetting of the soils for 18 h before extraction did not result in significantly greater recoveries of benzoylprop-ethyl, benzoylprop acid, flamprop-methyl, flamprop acid, or trifluralin than could be obtained by direct extraction.

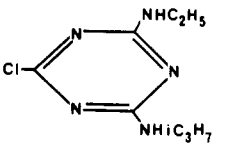
In most laboratories the recovery of pesticide residues from soils is determined by fortifying soil samples with known amounts of the various chemicals. The treated soils are then allowed to equilibrate for time intervals varying from a few hours to several weeks prior to extraction. Under such conditions, a particular procedure may indicate that over 90% of a specific residue is being recovered; however, this may not necessarily mean that the same extraction efficiency will be achieved from field samples treated several months previously (Hamaker et al., 1966; Chiba and Morley, 1968; Saha et al., 1969).

When a pesticide residue remains in contact with field soils for extended periods, a phenomenon known as aging, or weathering, occurs which renders the pesticide residue more resistant to solvent extraction (Hamaker et al., 1966; Chiba and Morley, 1968; Chiba, 1969; Saha et al., 1969; Mattson et al., 1970). This resistance to extraction has been considered to result from an increased adsorption to soil colloids and a diffusion into the interior of humic acid particles (Hamaker et al., 1966; Chiba, 1969; Adams, 1973; Khan, 1973).

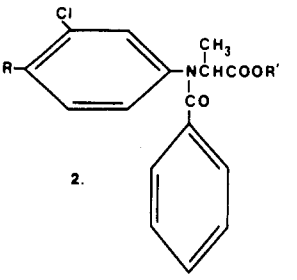
This resistance to extraction can present difficulties for those whose responsibility it is to monitor pesticide residues in field soils, since erroneous persistence data may be obtained. Also, unextracted residues could be considered as being bound to the soil [cf. Kearney (1976)] when, in fact, they are merely being inefficiently extracted.

A practical approach to this problem has been to take samples from field soils that have received prior treatments with pesticides and compare various extraction systems, selecting for routine laboratory analysis that procedure which recovers the greatest amounts of a particular residue (Mattson et al., 1970; Johnsen and Starr, 1970, 1972; Khan et al., 1975, Smith, 1978; Cotterill, 1980).

In the work to be described, field plots were separately treated with the commonly used herbicides atrazine [1; 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], benzoylprop-ethyl [2; R = Cl, R′ = C_2H_5; ethyl (±)-2-[*N*-(3,4-dichlorophenyl)benzamido]propionate], flamprop-methyl [2; R = F, R′ = CH_3; methyl (±)-2-[*N*-(3-chloro-4-fluorophenyl)benzamido]propionate], and trifluralin [3; α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine], all of which are known to persist for more than 1 year under western Canadian conditions (Smith and Hayden, 1976, Smith, 1979a). Following natural weathering in the field for 12 months, the soils from the plots were sampled and extracted by using a variety of solvent systems to determine which procedure resulted in the highest herbicide recoveries. Since the herbicidal esters benzoylprop-ethyl (2; R = Cl, R′ = C_2H_5) and flamprop-methyl (2; R = F, R′ = CH_3) undergo hydrolysis in soils to benzoylprop acid (2; R = Cl, R′ = H) and flamprop acid (2; R = F, R′ = H), respectively (Beynon et al., 1974; Roberts, 1977), the extraction of these acids from the weathered field soils was also compared.



1.



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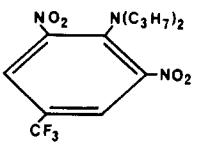

3.

MATERIALS AND METHODS

Soils. The composition and physical characteristics of the soils used in this study are summarized in Table I.

Field Treatments. Atrazine, benzoylprop-ethyl, and flamprop-methyl were applied as unincorporated treatments of 1 kg/ha to the surface of fallow plots at all three locations during the second week of May 1978. At the same time, and at the same locations, applications of 1 kg/ha trifluralin were incorporated to a depth of 5 cm into fallow plots.

Representative soil samples were taken from the top 5 cm of all treated plots during the second week of May 1979. The soils were air-dried at the laboratory temperature, ground, and mixed for 20 min in a laboratory soil mixer to ensure even distribution of the various herbicides throughout the soils.

Extraction Procedures. *Atrazine.* The solvent systems and procedures used are displayed in Table II. For the extractions involving simple mechanical shaking, duplicate soil samples (20 g) were weighed into 150-mL

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Table I. Composition and Physical Characteristics of Soils

soil	composition, % clay	silt	sand	organic content	field capacity moisture, %	pH[a]
heavy clay	70	25	5	4.2	40	7.7
sandy loam	10	25	65	4.0	20	7.6
clay loam	30	40	30	11.7	35	6.0

[a] Soil/water ratio, 1:1.

glass-stoppered flasks and shaken with the respective solvent system (50 mL) on a wrist-action shaker for the required time. Following centrifugation at 3000 rpm for 5 min, supernatant (25 mL, equivalent to 10 g of soil) was evaporated to dryness at 40 °C by using a rotary evaporator. Each flask was then shaken with methylene chloride (50 mL) and water (50 mL), and the contents were transferred to a separatory funnel when the organic layer was separated from the aqueous phase. The evaporation flask was rinsed with a further portion of methylene chloride (25 mL), which was transferred to the separatory funnel and used to further extract the aqueous phase. Combined methylene chloride extracts were evaporated to dryness, and the residue was dissolved in isooctane (5 mL). The isooctane solution was decanted into a 10-mL glass-stoppered tube, and aliquots (5 μL) were examined gas chromatographically by using an instrument equipped with a nitrogen-specific detector.

For extractions requiring reflux conditions, duplicate soil samples (20 g) were weighed into 250-mL flasks and heated with the appropriate solvent (100 mL) for the necessary time. In one case (Table II) the soils were wetted with water (10 mL) for 18 h before methanol (90 mL) was added and the mixture heated under reflux. In a second instance, the soils were pretreated with methanol (25 mL) for 18 h prior to addition of ethyl acetate (75 mL). After being cooled, the soil–solvent mixtures were filtered under suction and the soil cakes washed with a further portion (50 mL) of the extraction solvent. The total volume of filtrate was measured (usually ~140 mL), and exactly half this volume (equivalent to 10 g of soil) evaporated to dryness. The soil residue was extracted with methylene chloride and water as described above, and the final extract taken up as before in isooctane (5 mL) for gas chromatographic analysis.

Soxhlet extractions (in duplicate) were conducted by continuously extracting soil samples (20 g) with the desired solvent system (200 mL) under reflux conditions. After the specified times (Table II), the volume of solvent remaining in the flask was measured and half of this solvent volume (equivalent to 10 g of soil) evaporated and worked

Table II. Comparison of Extraction Procedures for Recovery of Atrazine Residues from Weathered Field Soils

solvent	conditions	atrazine recovered, μg/g[a] HvC[b]	SL[b]	CL[b]
methanol + water (90 + 10)	shake 1.5 h	0.16	0.15	0.17
methanol + water (90 + 10)	shake 18 h	0.18	0.18	0.26
methanol + water (90 + 10)	reflux 2 h	0.22	0.24	0.41
methanol + water (90 + 10)	wet soil with water 18 h; methanol reflux 2 h	0.24	0.24	0.39
methanol + water (90 + 10)	soxhlet 24 h	0.23	0.25	0.38
acetonitrile + water (90 + 10)	shake 1 h	0.16	0.15	0.19
acetonitrile + water (90 + 10)	reflux 2 h	0.21	0.17	0.28
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 1 h	0.20	0.17	0.33
acetonitrile + water + acetic acid (70 + 30 + 2.5)	reflux 2 h	0.24	0.22	0.42
acetonitrile + ammonium hydroxide at pH 9 (70 + 30)	shake 0.5 h; stand 18 h; shake 0.5 h	0.30	0.29	0.43
ethyl acetate + methanol (75 + 25)	wet soil with methanol 18 h; ethyl acetate reflux 2 h	0.16	0.18	0.20

[a] Average from duplicate extractions. [b] HvC, heavy clay; SL, sandy loam; CL, clay loam.

Table III. Comparison of Extraction Procedures for Recovery of Benzoylprop and Flamprop Residues from Weathered Field Soils

solvent	conditions	benzoylprop recovered, μg/g[a] HvC ester	HvC acid	SL ester	SL acid	CL ester	CL acid	flamprop recovered, μg/g[a] HvC ester	HvC acid	SL ester	SL acid	CL ester	CL acid
acetonitrile + water (70 + 30)	shake 2 h	0.37	0.18	0.33	0.13	0.59	0.30	0.26	0.11	0.20	0.15	0.53	0.51
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 1 h	0.35	0.23	0.32	0.21	0.62	0.34	0.26	0.11	0.21	0.15	0.51	0.51
acetonitrile + water + acetic acid (70 + 30 + 2.5)	wet soil with water 18 h; shake with acidic acetonitrile 1 h	0.37	0.24	0.39	0.19	0.74	0.35	–	–	–	–	–	–
acetonitrile + water (70 + 30)	wet soil with water 18 h; shake with acetonitrile 2 h	–[b]	–	–	–	–	–	0.25	0.13	0.21	0.14	0.53	0.51
water + methanol + ethyl acetate + acetic acid (40 + 40 + 20 + 1)	shake 2 h	0.37	0.20	0.36	0.18	0.60	0.32	0.24	0.09	0.21	0.13	0.52	0.48

[a] Average from duplicate extractions. [b] Not determined.

Table IV. Comparison of Extraction Procedures for Recovery of Trifluralin Residues from Weathered Field Soils

solvent	conditions	trifluralin recovered, μg/g[a] HvC	SL	CL
benzene + 2-propanol (67 + 33)	shake 0.5 h	0.17	0.05	0.49
acetone + *n*-hexane (75 + 25)	shake 1 h	0.37	0.15	0.84
methanol	shake 2 h	0.45	0.25	1.44
acetonitrile + water (90 + 10)	shake 1 h	0.48	0.24	1.34
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 1 h	0.48	0.25	1.44
acetonitrile + water + acetic acid (70 + 30 + 2.5)	wet soil with water 18 h; shake with acidic acetonitrile 2 h	0.50	0.29	1.67

[a] Average from duplicate extractions.

up exactly as described above for the extractions conducted under reflux conditions. The final residue was dissolved in isooctane (5 mL) for gas chromatographic examination.

Benzoylprop and Flamprop (Esters and Acids). The solvent systems compared are shown in Table III. Duplicate soil samples (20 g) were weighed into 150-mL glass-stoppered flasks and shaken with extraction solvent (50 mL) on a wrist-action shaker for the required time. In two cases (Table III), the aqueous component of the extractant was added to the soils 18 h prior to addition of acidic acetonitrile and the shaking. After centrifugation at 3000 rpm for 5 min, the supernatant (25 mL, equivalent to 10 g of soil) was added to 5% (w/v) aqueous sodium carbonate solution (100 mL) in a separatory funnel and shaken with *n*-hexane (25 mL). The organic layer was run into a glass-stoppered flask, and aliquots (5 μL) were analyzed gas chromatographically for benzoylprop-ethyl or flamprop-methyl.

The aqueous phase, containing the benzoylprop acid or flamprop acid residues, was acidified with concentrated hydrochloric acid (15 mL) and ether extracted (2 × 50 mL), and the evaporated extracts were methylated by using ethereal diazomethane. After evaporation of excess reagent and ether, the residue was dissolved in *n*-hexane (25 mL), and aliquots (5 μL) were examined gas chromatographically for benzoylprop-methyl and flamprop-methyl. Full details of these workups and derivatizations have been reported (Smith, 1976).

Trifluralin. The solvent systems compared are displayed in Table IV. Duplicate soil samples (20 g) were placed in 150-mL glass-stoppered flasks and shaken with extraction solvent (50 mL) on a wrist-action shaker for the necessary period. In one instance (Table IV), the water component was added to the soils 18 h before the acidic acetonitrile, and the shaking was commenced. Following shaking, the soil extracts derived from the methanol and acetonitrile solvent systems were centrifuged at 3000 rpm for 5 min, when supernatant (25 mL, equivalent to 10 g of soil) was shaken with 5% (w/v) aqueous sodium carbonate solution (100 mL) and *n*-hexane (50 mL). The aqueous phase was discarded and the organic layer run into a stoppered flask. Aliquots (5 μL) were then analyzed gas chromatographically for trifluralin.

The soil extracts derived from the acetone- and benzene-containing solvents were similarly centrifuged, but no further workup was carried out, and aliquots (5 μL) of the supernatant were analyzed directly for trifluralin.

Gas Chromatographic Analysis. Atrazine samples were analyzed by using a Hewlett-Packard Model 5710A gas chromatograph, equipped with a nitrogen–phosphorus flame ionization detector operated in the nitrogen mode. The glass column (1.5 m × 4 mm i.d.) was packed with 100–120-mesh Ultra-Bond 20M. The column carrier gas was helium at a flow rate of 30 mL/min. Flow rates of hydrogen and oxygen through the detector were maintained at 3 and 50 mL/min, respectively. The detector voltage was operated at 18 V. All samples were injected directly onto the column packing. With a column temperature of 180 °C, the retention time for atrazine was 3.25 min. Chromatographic standards were prepared in isooctane, and the atrazine concentrations recovered from the various soils were calculated by comparing the sample peak heights with those of the appropriate standards.

The remaining compounds were analyzed by using a Hewlett-Packard Model 5713A gas chromatograph equipped with a radioactive nickel electron-capture detector operated at 300 °C. The glass column (1.5 m × 4 mm i.d.) was packed with 100–120 mesh-Ultra-Bond 20M. The carrier gas was argon containing 5% of methane at a flow rate of 40 mL/min. All samples were injected directly onto the column. With a column temperature of 230 °C, the retention times for benzoylprop-ethyl and benzoylprop-methyl were 4.85 and 4.75 min, respectively. On a column at 210 °C, the retention time for flamprop-methyl was 6.10 min; while at 160 °C, the retention time for trifluralin was 2.55 min. All samples and standards were prepared in *n*-hexane, and the concentrations of the various herbicides recovered from the soils were calculated by comparing the sample peak heights with those of the standards.

RESULTS AND DISCUSSION

The amounts of the various compounds recovered from the aged field soils using the different extraction procedures are summarized in Tables II–IV. The data are expressed as micrograms of herbicide recovered per gram of air-dried soil, and in all cases there was less than ±5% variation between each duplicate analysis.

Methanol, or aqueous methanol, has been used by many analysts for the extraction of atrazine residues from fortified soils. Extraction procedures have usually involved either simple mechanical shaking of the soil with the solvent at room temperature (Beynon et al., 1972; Khan and Marriage, 1977) or extraction of the soil with hot solvent under reflux or Soxhlet conditions (McGlamery et al., 1967; Mattson et al., 1970; Ramsteiner et al., 1974; Hill and Stobbe, 1974).

Aqueous acetonitrile has also been used for the recovery of atrazine from treated soils, with the extraction being conducted either at room temperature (Purkayastha and Cochrane, 1973; Sirons et al., 1973; Smith et al., 1975) or under reflux or Soxhlet situations (Mattson et al., 1970; Hörmann et al., 1972).

Methanolic ethyl acetate, under reflux conditions, has similarly been reported for the recovery of atrazine residues from treated soils (Young and Chu, 1973; Green et al., 1977).

The results for the extraction of atrazine from the aged field soils using aqueous methanol, aqueous acetonitrile, and methanolic ethyl acetate solutions in combination with various extraction procedures reported in the literature are summarized in Table II. It can be noted that extraction of atrazine from all three soil types by mechanical shaking with either aqueous methanol or 10% aqueous acetonitrile at room temperature was not as effective as extraction with the hot solvents, a fact previously recorded by Mattson et

al. (1970). Neither hot methanolic ethyl acetate nor hot 10% aqueous acetonitrile extraction of the soils appeared to be as efficient for atrazine recovery as hot aqueous methanol. Prewetting of the field soils with water for 18 h prior to reflux extraction with methanol resulted in essentially the same atrazine recoveries that were obtained by direct reflux extraction with methanol for 2 h or as a result of direct Soxhlet extraction for 24 h with the same solvent (Table II).

Extraction of the field soils at room temperature with 30% aqueous acetonitrile containing 2.5% glacial acetic acid resulted (Table II) in slightly increased recoveries of atrazine than were obtained by shaking with 10% aqueous methanol and 10% aqueous acetonitrile, while treatment of the soils with the aqueous acidic acetonitrile under reflux conditions resulted in atrazine recoveries identical with those obtained by using hot methanolic solutions.

The highest atrazine recoveries from all three soils were achieved by using an extractant and procedure developed by Sirons (1980). For this procedure (Table II), involving 30% aqueous acetonitrile adjusted to a pH value of 9.0 with ammonium hydroxide, the soils were initially shaken with this solvent system for 30 min. After the mixture was allowed to stand overnight, a second shaking of 30 min was given before centrifugation and workup. Recovery of atrazine from the clay loam using this solvent system was similar to that obtained with hot methanol and hot aqueous acidic acetonitrile (Table II).

The mechanical shaking of treated soils with neutral, or acidic, acetonitrile solutions has been shown to be satisfactory for the extraction of the structurally related benzoylprop-ethyl and flamprop-methyl, together with their respective carboxylic acid analogues (Smith, 1976, 1978, 1979a; Roberts and Standen, 1978; Hitchings and Roberts, 1979). For the extraction of benzoylprop-ethyl and benzoylprop acid from soils, a mixture of acetic acid, ethyl acetate, methanol, and water has also been reported (Wright and Mathews, 1976).

The extraction of benzoylprop-ethyl (and acid) and flamprop-methyl (and acid) from aged field soils using the various solvent systems is compared in Table III. Simple shaking of the treated soils with all extractants resulted in almost identical recoveries of the herbicidal esters (Table III). However, the solvent systems containing acetic acid appeared to be more efficient in recovering residues of benzoylprop acid than did the nonacidic extractant. In contrast, acetic acid did not seem to be necessary for the efficient extraction of flamprop acid residues from the soils. Prewetting of the soils for 18 h prior to extraction (Table III) resulted in a slightly increased recovery of benzoylprop-ethyl from the sandy loam and clay loam than had been achieved by direct solvent extraction. Recoveries of benzoylprop acid, flamprop-methyl, and flamprop acid did not appear to be affected by the prior moistening of the soils.

Trifluralin is a volatile herbicide [cf. Helling (1976)] so that most analysts have preferred a simple mechanical solvent extraction procedure to one involving hot solvents that could result in volatility losses. Solvents used for recovering trifluralin residues from treated soils have included methanol (Tepe and Scroggs, 1967; Harrison and Anderson, 1970; Walker et al., 1976), aqueous acetonitrile (Smith, 1974, 1979b), a mixture of benzene and 2-propanol (Smith, 1972; Soderquist et al., 1975), and a mixture of acetone and *n*-hexane (White et al., 1977; Savage, 1978).

The recovery of trifluralin from aged field soils using the reported solvent systems is compared in Table IV. The recoveries of the herbicide from each soil using methanol, aqueous acetonitrile, and aqueous acidic acetonitrile are almost identical (Table IV) and considerably greater than those obtained using the acetone–*n*-hexane and benzene–2-propanol mixtures. These data (Table IV) also indicate that prewetting of the soils for 18 h before extraction does not result in significantly greater recovery of residues from the aged field soils.

The present studies indicate that the recovery of herbicide residues from field soils treated 12 months previously is very much dependent upon the extraction procedure adopted by the analyst. All solvent systems compared (Tables II–IV) have been reported to give almost quantitative recoveries of the various herbicides from fortified soils. Thus, for the extraction of pesticide residues from aged field soils, solvent systems and extraction procedures must be compared and that system yielding the highest pesticide residues selected for laboratory use. Although this approach cannot yield information as to the absolute amounts of a particular pesticide residue present in the soil, for which extensive studies with ^{14}C-labeled pesticides are necessary, it can nevertheless be carried out by most laboratories to determine more efficient extraction solvents.

The recovery of certain pesticides is enhanced by the addition of water to the soils before extraction (Chiba, 1969; Khan et al., 1975). The function of the water is, most probably, to effect desorption of the chemicals adsorbed onto the soil colloids (Bailey and White, 1964). In the present study prewetting of the soils before extraction did not appear to increase significantly the recovery of any of the herbicides investigated, when water was a component of the extraction solvent.

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Isolation, Identification, and Insecticidal Properties of *Piper nigrum* Amides

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Three amides were isolated from *Piper nigrum* L. and identified from their spectral characteristics as (*E,E*)-*N*-(2-methylpropyl)-2,4-decadienamide (I), (*E,E,E*)-13-(1,3-benzodioxol-5-yl)-*N*-(2-methylpropyl)-2,4,12-tridecatrienamide (II), and (*E,E,E*)-11-(1,3-benzodioxol-5-yl)-*N*-(2-methylpropyl)-2,4,10-undecatrienamide (III). The topical LD_{50} values of compounds I, II, and III against *Callosobruchus maculatus* (F.) were 2.18, 0.25, and 0.84 μg/insect for males (weight 3.8–5.7 mg) and 6.70, 1.43, and 3.88 μg/insect for females (weight 5.4–7.9 mg), respectively.

Black pepper, *Piper nigrum* L., has been reported to have contact and oral toxicity against stored-product insects (Lathrop and Keirstead, 1946; Su, 1977, 1978). It has been reported to have biological activity on other insects either as a toxicant (Harvill et al., 1943; Synerholm et al., 1945; Scott and McKibben, 1978) or as a repellent (Freeborn and Wymore, 1929). Piperine [(*E,E*)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine], an alkaloidal amide of oleoresin of pepper, has been shown to be a synergist to pyrethrins (Nakayama, 1950; Ono, 1950; Gersdorff and Piquett, 1957; Matsubara and Tanimura, 1966). However, Su (1977) showed that piperine was not the constituent in black pepper that was responsible for contact toxicity to the insects. In an effort to elucidate the responsible toxic components of black pepper, we isolated and identified three amides that are highly toxic to adult cowpea weevils, *Callosobruchus maculatus* (F.).

MATERIALS AND METHODS

Extraction of Black Pepper. Dry fruits of black pepper (distributed by McCormick & Co., Inc.) were purchased from the local supermarket and ground in a high-speed micromill into fine powder of less than 250 μm. The powder (140 g) in acetone (500 mL) was stirred at 40–50 °C for 30 min and filtered. The residue was extracted 3 more times. The filtrates were combined and concentrated under reduced pressure to a small volume and then lyophilized to give the crude acetone extract.

Chemicals and Reagents. Piperine was purchased from Pfaltz and Bauer, Inc. Pyrethrin (21.5% solution) was obtained from the Pyrethrum Marketing Board, Nakuru, Kenya. High-performance LC methanol (Fisher Scientific Co.) was filtered through a Waters Associates solvent clarification kit with a 0.5-μm Millipore organic filtration system. All other solvents were the reagent grade.

Liquid Chromatographic Fractionation of Acetone Extract. Each 1-g portion of the crude extract was placed on a column (40 × 2.0 cm i.d.) of silica gel (70–230 mesh; EM Reagents) and eluted with carbon tetrachloride–ethyl acetate (10:1 by volume). After the first 800 mL of effluent was discarded, the next 600 mL was collected. This effluent was then concentrated under reduced pressure to obtain the toxic material for further separation of individual components.

Thin-Layer Chromatographic Separation of the Toxic Material. For TLC separation, Brinkman EM reagent, precoated silica gel G F_{254}, 0.25 mm, 20 × 20 cm chromatoplates were used. About 3–4 mg of the material was applied to each plate in a straight line 2.5 cm above the lower edge. A total of 95 plates was prepared. Each plate was developed twice in cyclohexane–ethyl acetate (3:2 by volume) and then examined under UV at 254 nm. Three fractions in bands of R_f 0.55–0.60 (I), 0.52–0.55 (II), and 0.46–0.51 (III) were collected. Each fraction was extracted with acetone, and the extracts were concentrated and lyophilized.

High-Performance Liquid Chromatograph Purification and Analysis. A Waters Associates Model ALC/GPC 244 high-pressure liquid chromatograph with a Model 6000A pump, an U6K injector, a R401 differential

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