Even though the toxicity of M_1 is similar to that of aflatoxin B_1 (Sinnhuber et al., 1974), the presence of M_1 in corn has little practical significance. It is present in levels lower than B_1 and also lower than the error in the determination of B_1 by the AOAC Official Method (Shotwell and Stubblefield, 1972).

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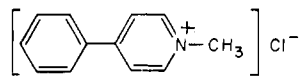
Determination of Cyperquat (1-Methyl-4-phenylpyridinium Chloride) Residues in Soil by Gas–Liquid Chromatography

Catalytic hydrogenation of cyperquat (PtO_2:cyperquat, <2) resulted in the formation of 1-methyl-4-phenylpiperidine and 1-methyl-4-cyclohexylpiperidine. However, hydrogenation with an increasing amount of the catalyst (PtO_2:cyperquat, ≥2) produced only the latter compound. The development of the method for cyperquat residues in soils was based on the formation of 1-methyl-4-cyclohexylpiperidine which gave a single symmetrical gas chromatographic peak. The method involves catalytic hydrogenation of the acid extract of soil, extraction of the material into hexane, and analysis by gas–liquid chromatography. Recoveries of the herbicide added to soil at 0.5- and 1-ppm levels were 77.1 and 85.2%, respectively. The method has been used for the determination of field applied cyperquat.

Cyperquat (I) is a new postemergence herbicide and is reported to give good control of purple and yellow nutsedge in various crops (Gulf Oil Chemical Co., 1975). The compound is available as a chloride salt and is soluble in water. It ionizes completely in aqueous solution into a reactive cation which may quickly disappear from solution



1-methyl-4-phenylpyridinium chloride
I

on contact with soil particles.

With the increasing interest in cyperquat for controlling nutsedge weeds in corn and soybeans (Hamill, 1975), it became of considerable interest to determine the level of the herbicide residues in soil. The possibility exists that the herbicide may remain in soil for some time after spraying the crop. A need was therefore felt to develop a sensitive analytical method for the determination of cyperquat residues in soils. Such a method is reported in this paper. The principle of the method is similar to that recently described for determining paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) and diquat (1,1'-ethylene-2,2'-bipyridinium dibromide) residues in soils (Khan, 1974). The method involves catalytic hydrogenation of the acid extract of soil, extraction of the material into organic solvents, and analysis by gas–liquid chromatography.

MATERIALS AND METHODS

Chemicals. All solvents were pesticide grade and used as received. Platinum oxide (Adam's catalyst) was purchased from Matheson Coleman and Bell Inc., Norwood, Ohio. An analytically pure sample of cyperquat was supplied by Gulf Oil Chemicals Co., Merriam, Kan.

Hydrogenation of Cyperquat. A simple apparatus similar to that described by Vogel (1966) was used for hydrogenation. Ten milligrams of cyperquat dissolved in about 30 ml of methanol was taken in a hydrogenation flask containing 20 mg of platinum oxide (PtO_2). Hydrogenation was carried out at room temperature for 2 h.

The solution was filtered, concentrated to about 5 ml, and made alkaline (pH ~10) with 1 N NaOH. The mixture was extracted with hexane (3 × 10 ml). Hexane was removed in a stream of dry nitrogen and the weight of the hydrogenated material recorded.

The reference standard was made by dissolving the hydrogenated material in hexane (5.84 μg/ml).

Determination of Residues in Soil Fortified with Cyperquat. The soil (sandy loam) was collected from an experimental plot which had not been previously treated with cyperquat. The soil was air-dried at room temperature, pulverized, screened through a 20-mesh screen, and mixed thoroughly. Soil samples (20 g) were fortified with an aqueous solution of cyperquat at 1- and 0.5-ppm levels and allowed to dry at room temperature for 24 h. The sample was then mixed with 80 ml of 18 N H_2SO_4 in a boiling flask and heated under reflux for 5 h. The extract was filtered under suction through an acid-resistant filter paper and diluted to 250 ml with distilled water. A 75-ml aliquot (= 6 g of soil) was introduced into a hydrogenation flask containing 75 mg of PtO_2. Hydrogenation was carried out for 2 h. The solution was made alkaline (pH ~10) with 10 N NaOH, transferred into a separatory funnel, and extracted with three 50-ml portions of methylene chloride. The combined extracts were treated with 5 ml of 1 N HCl and evaporated on a rotary evaporator to remove the organic solvent. The remaining aqueous portion was transferred into a 100-ml test tube, the flask was rinsed several times with a small volume of 0.01 N HCl, and the rinse collected in the test tube. The solution was made alkaline with NaOH and the mixture was shaken with hexane (3 × 10 ml). An aliquot of the hexane layer was injected into the gas chromatograph.

All samples were analyzed in duplicate and average values are reported.

Determination of Residues in Soil Collected from the Field. Surface soil samples (sandy loam) were taken at random from the experimental plot which had received cyperquat (4 lb/acre). The soil (1 kg) was air-dried at room temperature, pulverized, screened through a 20-mesh screen, and mixed thoroughly. Residue(s) of cyperquat from a 20-g subsample was determined as described above. Residues in soil are reported on an oven-dry basis.

Gas Chromatography. The gas chromatograph used was a Pye Series 104, Model 124, fitted with an alkali flame ionization detector having a RbCl Annulus. The chromatographic column was 1.5 m × 0.6 cm glass tube packed with 3% Carbowax 20M + 1% KOH coated on 80–100 mesh Chromosorb WHP. Column, detector, and injector temperatures were 140, 285, and 140 °C, respectively. The carrier gas (nitrogen) flow rate was 40 ml/min.

The concentration of the herbicide in the soil extracts was determined by comparing the peak heights with those of the hydrogenated reference standards and correcting the value for the change in molecular weight on hydrogenation. Identity of the desired peak was proved by comparing its retention time and mass spectrum with those of the reference hydrogenated cyperquat and by cochromatography with the latter.

Spectroscopic Methods. For a mass spectrum determination, an aliquot of the above solution containing approximately 1 μg of the hydrogenated material was injected into a gas chromatograph with a hydrogen flame detector coupled to a Finnigan Model 31000 mass spectrometer interfaced with a Model 6100 computer controlled data acquisition system. Mass spectra were also determined on an Associated Electrical Industries MS-9 double beam focusing high-resolution mass spectrometer. The ionization energy was 70 eV.

Ultraviolet spectra were obtained with a Unicam SP 800 spectrometer. The sample was dissolved in spectrophotometric grade hexane. Infrared spectra were obtained with a Beckman IR 12 spectrometer as smears between NaCl plates. The NMR spectra were obtained on a Varian A 60 spectrometer. The sample was dissolved in spectrophotometric grade deuterated chloroform-d_1.

RESULTS AND DISCUSSION

Hydrogenation of 10 mg of cyperquat (PtO_2:cyperquat, 2:1) yielded a material weighing 6.8 mg (77% yield). Preliminary experiments indicated that hydrogenation longer than 2 h did not result in an increased yield of the product. Furthermore, an increase in the weight of the catalyst (PtO_2:cyperquat, >2) did not change the product yield. Hydrogenation of cyperquat in acidic methanol (6 N H_2SO_4) had no effect on the yield of hydrogenated material. Gas chromatography of the hydrogenated material resulted in a single symmetrical peak with a retention time of 2.8 min. Hydrogenation of cyperquat with lesser amounts of the catalyst (PtO_2:cyperquat, <2) produced a product which resulted in two gas chromatographic (GC) peaks with retention times of 2.8 and 6.3 min, respectively. Under the experimental conditions described the yield of this product was not reproducible. Further hydrogenation of the material resulted in a product which gave a single GC peak with a retention time of 2.8 min.

The ultraviolet spectrum of the product (showing two GC peaks) exhibited characteristic structures of monosubstituted benzene in the 245–265 mμ region. The infrared spectrum of this material showed bands at 700 (monosubstituted benzene), 1495 and 1605 (aromatic C=C), and multiple bands in the 3000–3080-cm^{-1} (=C—H) region. None of these absorption bands were present in the ultraviolet and infrared spectra of the perhydro product showing only one GC peak with a retention time of 2.8 min.

The mass spectrum (GC–MS) of the material represented by the GC peak with a retention time of 2.8 min (compound II) had a molecular ion peak at m/e 181 with a most abundant ion at m/e 98 attributed to $C_6H_{12}N^+$. A GC–MS of the peak with a retention time of 6.8 min (compound III) showed a molecular ion at m/e 175 and other ions at m/e 98 and 77 attributed to $C_6H_{12}N^+$ and $C_6H_5^+$, respectively. Each of the hydrogenated products (compounds II and III) was separated by preparative gas chromatography and reexamined on a high-resolution mass spectrometer. The molecular weights (mass spectra) for the products II and III were 181.1768 (calculated for $C_{12}H_{23}N$, 181.1764) and 175.1291 (calculated for $C_{12}H_{17}N$, 175.1296), respectively.

In view of the foregoing it is apparent that compound III was formed due to the hydrogenation of only the pyridine moiety of cyperquat (I) and the benzene ring of the molecule was unaffected [NMR δ 0.97–1.98 (br multiplet, 8 H, $-CH_2$'s), 2.35 (singlet, 3 H, N–CH_3), 2.92–3.02 (multiplet, 1 H, methine), and 7.3 (singlet, 5 H, aromatic)]. Thus, compound III was identified as 1-methyl-4-phenylpiperidine. Compound II was formed due to complete hydrogenation of both pyridine and benzene rings present in the cyperquat molecule [NMR δ 0.76–2.01 (br multiplet, 18 H, $-CH_2$'s), 2.20 (singlet, 3 H, N–CH_3), and 2.67–3.01 (multiplet, 2 H, methine)]. This compound was identified as 1-methyl-4-cyclohexylpiperidine. The development of the method for cyperquat residues in soils was based on the formation of compound II which gave a single reproducible GC peak with a retention time of 2.8

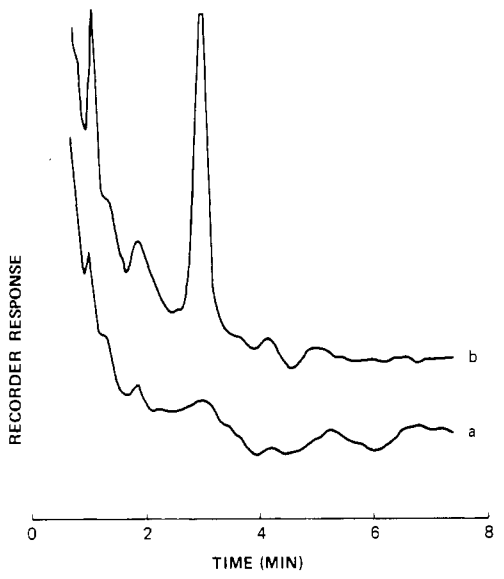
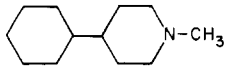
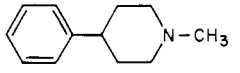


Figure 1. Gas chromatograms from determination of cyperquat in soil: (a) check soil, (b) treated soil. Final volume 6.2 ml, 3 μl injected.



1-methyl-4-cyclohexylpiperidine
II



1-methyl-4-phenylpiperidine
III

min. For a 50% full-scale deflection, a sample of 10 ng was required. Under the GC conditions described, the response in the concentration range used (0.5–15 ng) was linear on the thermionic detector.

Recoveries of cyperquat residue from the fortified untreated soil samples at 0.5- and 1-ppm levels (based on 6 g of soil) were 77.1 and 85.2%, respectively. Under the experimental conditions described the method has a sensitivity of 0.5 ppm. However, concentration of the final solution five times may result in an improved sensitivity of about 0.1 ppm without any significant interferences from soil background. The chromatographic tracings of the check and cyperquat treated soil samples taken from the experimental plots are shown in Figure 1. Interference from coextractives was negligible. The peak (retention time, 2.8 min; Figure 1b) gave a mass spectral molecular ion peak at m/e 181. The fragmentation pattern was similar to that of 1-methyl-4-cyclohexylpiperidine. The soil sample contained 1.9 ± 0.14 ppm of cyperquat residue. Twelve milligrams of the catalyst per g of soil was adequate for the maximum recovery of cyperquat residue. A further increase in the weight of the catalyst did not increase the recovery of cyperquat (Figure 2). Under these conditions the hydrogenation of soil extracts was complete in 2 h as a further increase in time of hydrogenation did not improve the recovery (Figure 3). The maximum recovery of the residue was not affected by changing the concentration of the acid (6 and 18 N) used for extracting the herbicide from soil.

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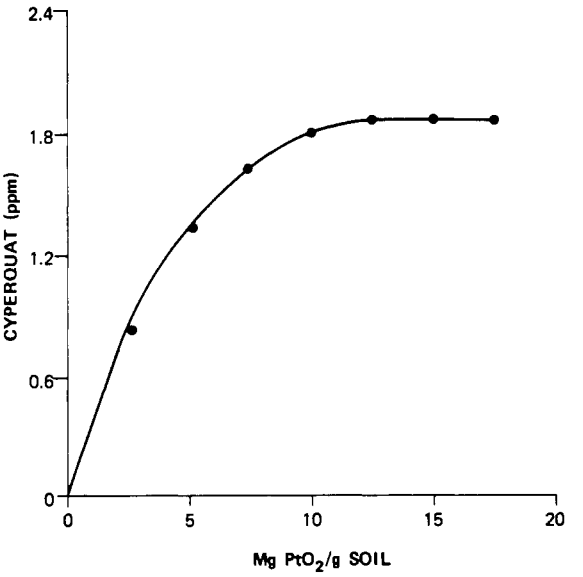

Figure 2. Effect of the amount of PtO_2 used in hydrogenation of soil extract on the recovery of cyperquat (2 h hydrogenation).

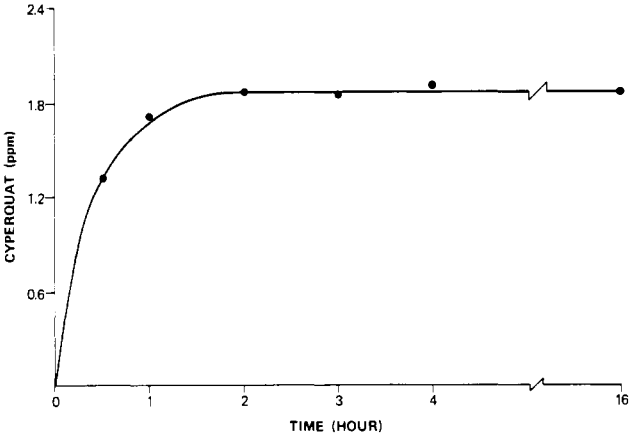

Figure 3. Effect of time of hydrogenation on the recovery of cyperquat (PtO_2 = 12.5 mg/g of soil).

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