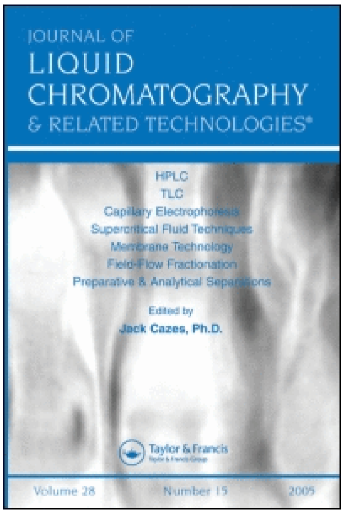

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SEPARATION OF PESTICIDES AND IMPURITIES BY COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

Countercurrent chromatography (CCC) was chosen for study as an isolation technique for pesticides and the impurities found with them, because its high capacity makes possible comparatively large batches of purified materials and because its mild operating conditions minimize the danger of degradation during the separation. Technical grade fenthion, an organophosphorus insecticide, showed traces of six impurities. The principal one found in an analytical standard fenthion was identified as 3-methyl-4-

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(methylthio)anisole, well separated from the fenthion. The *n*-octyl ester of the herbicide 2,4,5-T was easily separated from the methyl ester impurity.

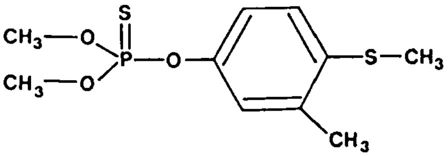
INTRODUCTION

The objective of this work was to evaluate countercurrent chromatography (CCC) as a method for the isolation of pure pesticides and associated materials for photochemical studies. In photochemistry, many experiments are done to measure the initial rate of formation of a product or the initial rate of degradation of the starting material, with both the concentration and the extent of reaction held to low values. The smaller the extent of degradation, the greater are the uncertainties associated with the presence of impurities. The questions raised include not only whether minor components are degraded, but also whether sensitization or quenching occurs through the interaction of energy states of the desired reagent and an unknown or undesired species. The organophosphorus pesticides are important partly because of their environmental degradability, but that same reactivity contributes to the difficulty of obtaining high purity materials and of preserving them in storage.

Countercurrent chromatography routinely handles much larger quantities of materials than either gas chromatography (GC) or high performance liquid chromatography (HPLC). That is a distinct advantage whenever a relatively large quantity of a purified material is needed for an experimental program, because of the general principle that it is desirable to use portions of a single uniform batch of reagent in a series of experiments. Equally important is the gentleness of countercurrent chromatography as a technique for handling reactive chemicals or biological materials. The CCC system consists of two preequilibrated phases contained by inert teflon walls. The sample in solution is partitioned between the two liquids. The absence of an adsorbing solid phase eliminates the loss of sample components by irreversible adsorption. It minimizes any denaturation of biochemicals by reactions associated with the adsorption-desorption process. The degradation of the sample is reduced by the absence of heated apparatus from the CCC system. Where thermally-activated reactions occur at room temperature, they should be further reducible by operation at a lower temperature in a thermostatted room. Oxidative degradation of sensitive materials could be reduced by initial purging of solvent phases during preequilibration and by operation of the system in an inert atmosphere.

Fenthion (I) was developed by Schrader and Schlegk (1,2). It is a contact and stomach insecticide and has been used against a variety of household, garden, and crop pests and against animal parasites (2,3). Its IUPAC name is O,O-dimethyl O-4-methylthio-*m*-tolylphosphorothioate. In the *Chemical Abstracts* system, it is called O,O-dimethyl O-[3-methyl-4-(methylthio)phenyl]phosphorothioate. The literature contains many code numbers and trade marks (2). References to various analytical procedures are listed by Ibrahim and Cavagnol (3), who developed a method based on the ultraviolet (UV) absorption by fenthion at 252 nM, after chromatography on a Florisil column with heptane.

A number of compounds are sometimes found in association with fenthion as original impurities or as alteration products. They include the S-methyl isomer; the oxon, in which the sulfur bonded to the phosphorus is replaced by an oxygen atom; 4-(methylthio)-*m*-cresol; 3-methyl-4-(methylthio)anisole (II); O,O-dimethyl chloridophosphorothioate; and O,O,O,O-tetramethyl dithiopyrophosphate



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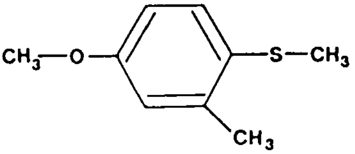
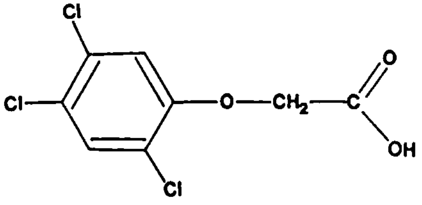


II

(3). The sulfoxides and sulfones of fenthion, of its S-methyl isomer, and of its oxon would be expected to be formed by oxidation of the thioethers (4,5).

The history of World War II research on the phenoxy herbicides and of their introduction as commercial weedkillers after the war has been reviewed by Loos (6). The compounds are especially valued for their selective action against broad-leaved weeds but not against grasses. The commercial "isooctyl" esters are mixtures of the esters of many alcohols, mainly branched octanols. Their photodegradation products become correspondingly more complex mixtures. In order to simplify the photoproduct analysis in the case of 2,4,5-trichlorophenoxyacetic acid (III), called 2,4,5-T, the _n_-octyl ester was prepared (7). Countercurrent chromatography was applied for the purification of the ester.



III

MATERIALS AND METHODS

Materials

Technical grade fenthion (96%) was obtained from the Environmental Protection Agency's laboratory in Beltsville, and analytical standard fenthion from the Agricultural Chemicals Division of the Mobay Chemical Corporation, Kansas City, Missouri. The 3-methyl-4-(methylthio)anisole was ENT-70081 from the Entomology Collection of the Beltsville Agricultural Research Center, made by S. C. Johnson and Son. The 4-(methylthio)-_m_-cresol was ENT-28074, made by the Chemical Products Division of Crown Zellerbach. The fenthion oxon, fenthion oxon sulfoxide, and fenthion oxon sulfone were EPA Technical Materials Nos. 568, 569, and 570, respectively. The fenthion sulfoxide and fenthion sulfone were Chemagro materials. The n-octyl ester of 2,4,5-T was prepared in the Organic Chemical Synthesis Laboratory at Beltsville.

Countercurrent Chromatography

All of the CCC separations were done with the new horizontal flow-through coil planet centrifuge (8). The column consisted of 10 helical units wound from #10 PTFE tubing (2.6 mM I.D.) and connected in series, as described in detail by Ito and Bowman (9). It had a total of about 1000 helical turns and a capacity of about 260 mL. It was mounted on the gear side holder at $\beta = 0.23$ (10). The speed of rotation was 400 rpm. The solvent flow rate from the Chromatronix metering pump was 24 mL/hour. The monitor was an LKB Uvicord S. An LKB recorder, operated at a chart speed of 1 section per hour, and an LKB fraction collector were used.

The solvent system for fenthion was prepared by equilbrating hexane and methanol (2:1 by volume). The upper phase was mobile. The partition coefficient C_u/C_l, where C_u and C_l are the concentrations in the upper and lower phases, respectively, was 0.56. The sample was 20 μL fenthion, mixed with 5 mL of the upper phase for charging. The fraction collector was operated at 6 mL/tube (15 minutes/tube).

For processing the 2,4,5-T n-octyl ester, a hexane-methanol-water system was investigated. In hexane-methanol, without water, the partition coefficient C_u/C_l was 0.60. For 100 mL hexane, 90 mL methanol, and 10 mL water, the coefficient was calculated to be 2.43. For 100 mL hexane, 80 mL methanol, and 20 mL water, the partition coefficient was 8.40. The hexane-methanol system, without water, was judged to be satisfactory and was used in the separation. The upper phase was mobile. For the countercurrent chromatogram in Figure 1(C), the sample consisted of 250 μL of the ester mixed with 5 mL of the upper phase and 5 mL of the lower phase, which gave a homogeneous, single-phase sample for injection.

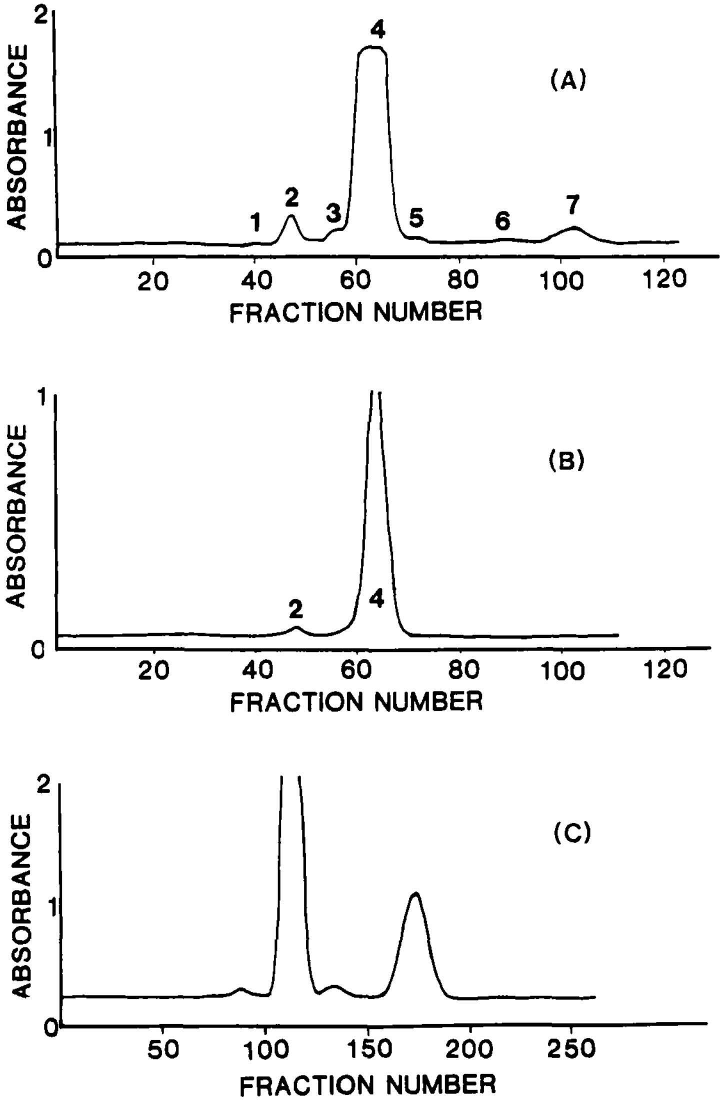


Figure 1. Countercurrent chromatograms. (A) Technical fenthion (96%). (B) Fenthion analytical standard (97.2%). (C) The _n_-octyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). (Numerical peak labels as defined in the discussion.)

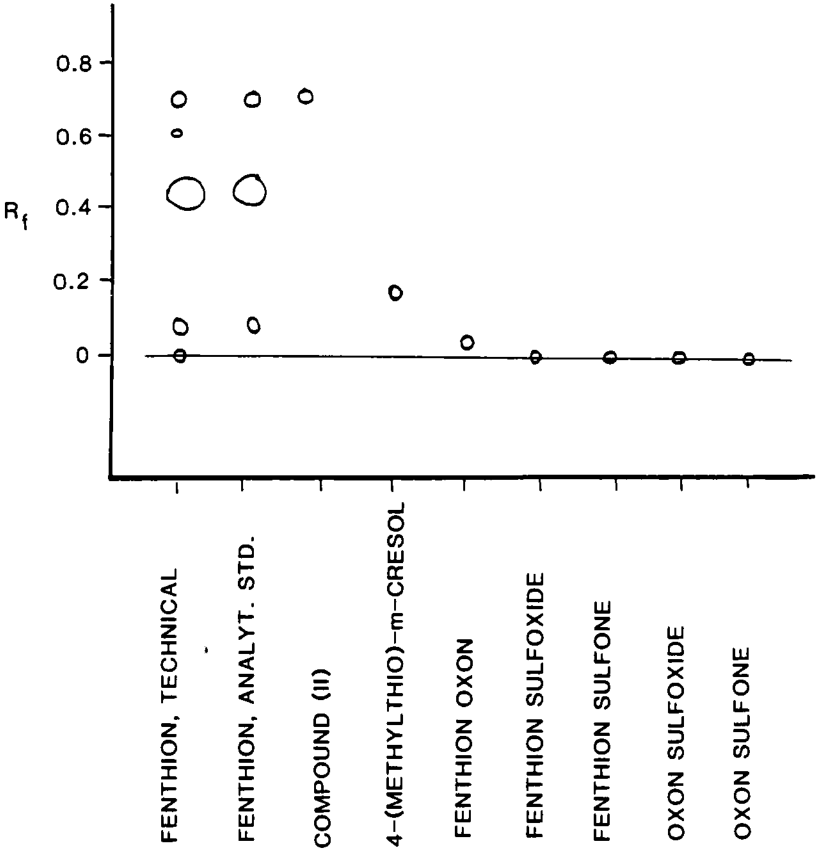


Figure 2. Results of thin layer chromatographgic tests of fenthion and related compounds. R_f = (distance traveled by spot)/(distance traveled by solvent front).

A similar run with one-fifth of this sample size had given similar results.

Thin Layer Chromatography

Thin layer chromatography was done according to the procedure described by Ibrahim and Cavagnol (3). The plates used were Applied Science Labs Safety-Kotes. Fenthion, technical 96% and analytical standard 97.2%, was used without dilution (1 µL/spot).

The other compounds were each dissolved in redistilled acetone for spotting (10 μL of 1 g/L solution/spot). The plates were developed with a mixture of heptane and redistilled acetone (7:1 by voline), sprayed with DCQ reagent (2,6-dibromo-N-chloro-p-quinoneimine, 1% in acetone), and heated in the oven at 110 C for 10 min.

MASS Spectrometry

Gas chromatography/mass spectrometry of fenthion and related compounds was done with an OV-17 column, an RMU6 mass spectrometer, and an INCOS data system, except for the compound II standard. For that one, an OV-101 column, a Finnigan 3200 mass spectrometer, and the INCOS data system were used. The 2,4,5-T esters were analyzed with a 60-M SE30 capillary column, a Finnigan 4021 mass spectrometer, and a Finnigan INCOS data system.

RESULTS AND DISCUSSION

The countercurrent chromatogram of the technical fenthion is shown in Figure 1(A) and that of the analytical standard fenthion, in Figure 1(B). The technical grade insecticide yielded traces of six impurities, in addition to the fenthion, which is represented by CCC peak 4. The fenthion analytical standard showed only the fenthion and one impurity, CCC peak 2. Thin layer chromatography (Figure 2) did not find all of the impurities of the technical fenthion that are clearly seen on the CCC chart. On the other hand, it did show a weak spot corresponding to the slow-moving component of R_f 0.08 found also in the technical grade material. This may represent a trace of the component recorded by CCC peak 7 in Figure 1(A) for the technical grade sample, widely separated from the fenthion.

The mass spectrum (Figure 3(A)) from fraction 47, CCC peak 2, the main impurity in the analytical standard, has as its most significant ions m/z 168(RI 100%), 153(80%), 138(50%), 109(40%), 79 (26%), and 77(27%). The corresponding peaks in the mass spectrum

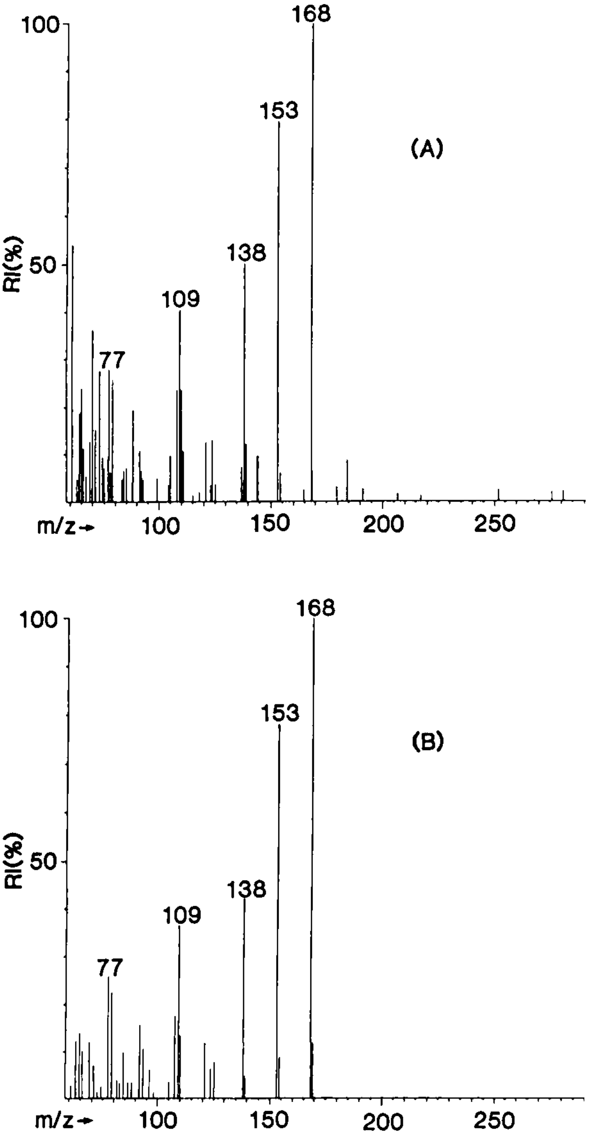


Figure 3. Mass spectra of 3-methyl-4-(methylthio)anisole, with intensity relative to the base peak, RI(%), versus the mass/charge ratio, m/z. (A) CCC Fraction 47, Figure 1(A). (B) 3-methyl-4-(methylthio)anisole.

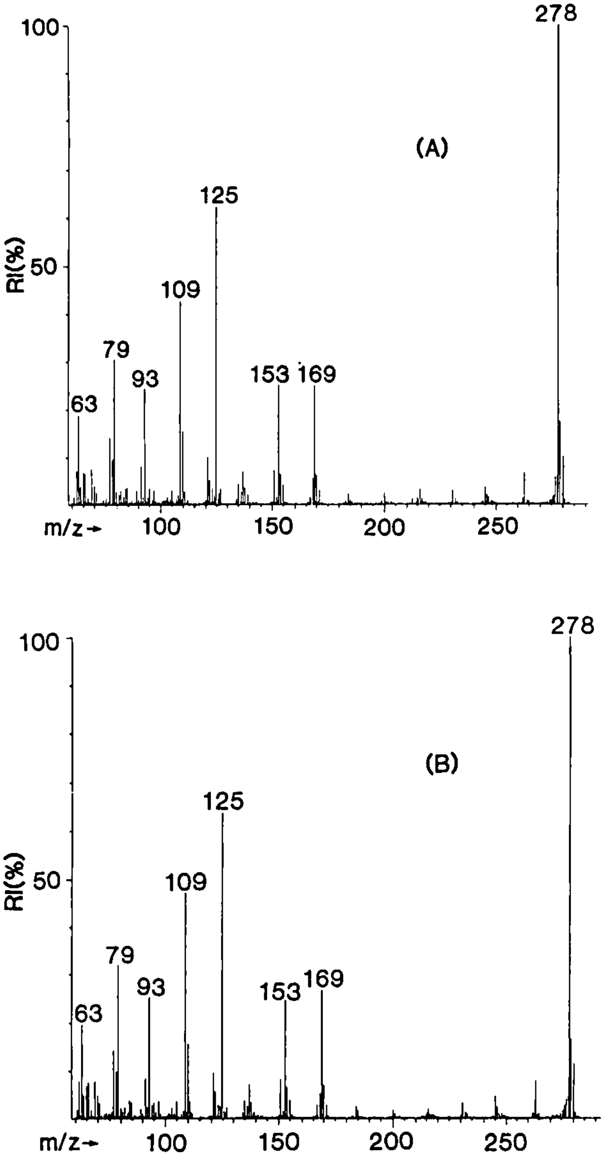


Figure 4. Mass spectra of fenthion, with intensity relative to the base peak, RI(%), versus the mass/charge ratio, m/z. (A) CCC fraction 63, Figure 1(A). (B) Fenthion.

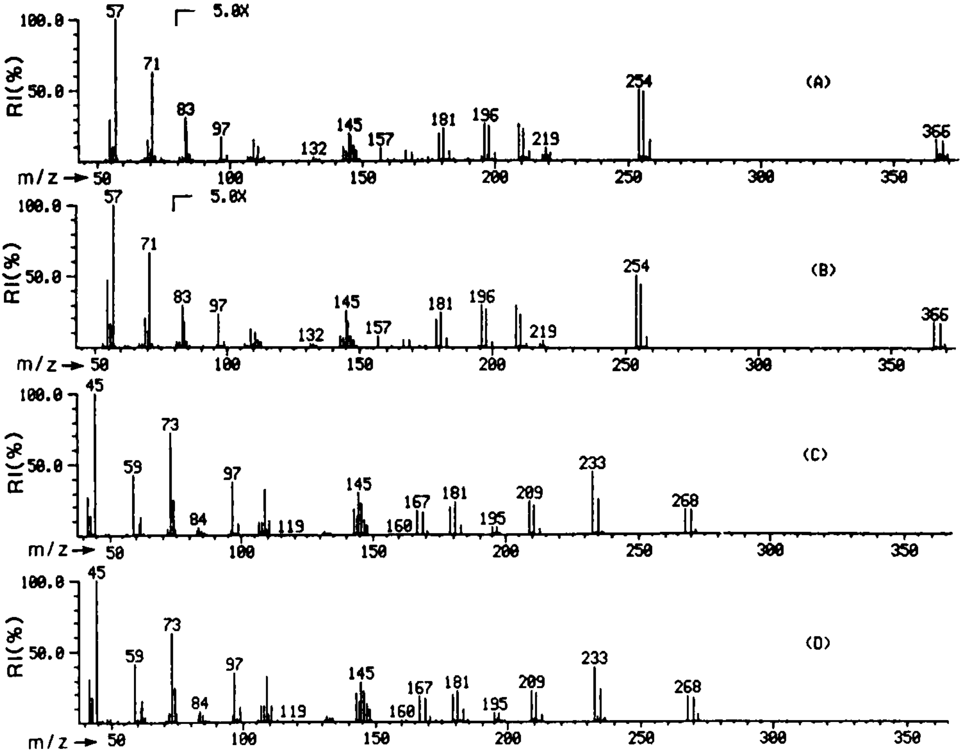


Figure 5. Mass spectra of the 2,4,5-T esters, with intensity relative to the base peak, RI(%), versus the mass/charge ratio, m/z. (A) The component in CCC fractions 105-123. (B) The n-octyl ester of 2,4,5-T. (C) The component in CCC fractions 160-190. (D) The methyl ester of 2,4,5-T.

(Figure 3(B)) of 3-methyl-4-(methylthio)anisole (II) are m/z 168 (RI 100%), 153(78%), 138(42%), 109(36%), 79(22%), and 77(26%), confirming the thin layer identification of CCC peak 2 as this compound. Considering the small size of the GC peak obtained from CCC fraction 47 (about 3 times the height of the background) and the overlap of its trailing side with a small phthalate peak, the agreement is very good. The phthalate plasticizer is a common contaminant in laboratory procedures, probably introduced in the analytical manipulation of the fraction.

Fraction 63, CCC peak 4 yielded a mass spectrum (Figure 4(A)) with its most intense ion peaks at m/z 278(RI 100%), 169(25%), 153 (25%), 125(62%), 109(43%), 93(24%), 79(31%), and 63(18%). In the mass spectrum (Figure 4(B)) of fenthion (I), they are m/z 278(RI 100%), 169(28%), 153(24%), 125(62%), 109(47%), 93(25%), 79(30%), and 63(19%), confirming the identification of CCC peak 4 as the fenthion peak. The fenthion standard was easily purified by countercurrent chromatography.

GC/MS analysis of fractions from the two principal peaks in the CCC separation of the 2,4,5-T esters agreed with an earlier GC/MS analysis of the synthesis mixture, and both sets of analyses were confirmed by computer comparisons of the mass spectra with library spectra. As expected for the ^{35}Cl-^{37}Cl isotopic combinations in a trichloro compound, the material in CCC fractions 105-123 (Figure 5(A)) gave molecular ions at m/z 366(RI 2.1%), 368 (2.0%), and 370(0.4%). The *n*-octyl ester (Figure 5(B)) shows m/z 366 (RI 3.5%), 368(3.6%), and 370(0.6%). The agreement in relative intensities is good, under the difference in ion source conditions.

The compound in CCC fractions 160-190 (Figure 5(C)) gave molecular ions at m/z 268(RI 17%), 270(16%), and 272(2.4%). The methyl ester (Figure 5(D)) showed m/z 268(RI 18%), 270(17%), and 272 (4.7%). The mass spectra identify the material in CCC fractions 105-123 as the *n*-octyl ester of 2,4,5-T and that in fractions 160-190 as the methyl ester of 2,4,5-T.

In both of the examples studied, the organophosphorus insecticide fenthion and the phenoxy herbicide 2,4,5-T, countercurrent chromatography was found to be an excellent technique for the separation of quantities of pure material.

ACKNOWLEDGMENTS

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10. The constant β is the ratio r/R, in which r is the radius of rotation and R is the radius of revolution of the helical column, as described in Reference (9) above.