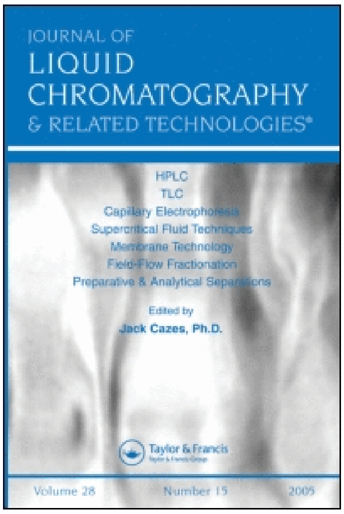

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PREPARATIVE SEPARATION OF DEMETON ISOMERS WITH ITO MULTI-LAYER COIL COUNTERCURRENT CHROMATOGRAPHY

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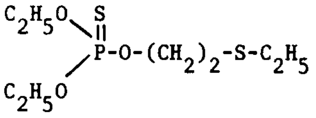
ABSTRACT

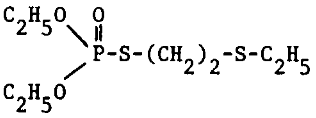
The active constituents of the insecticide Demeton, consisting of isomeric O,O-diethyl (O,S)-ethylmercaptoethyl thiophosphates, have been conveniently separated on a preparative scale with an Ito multi-layer coil countercurrent system.

INTRODUCTION

The active constituent of the insecticide Demeton (1,2,3) is stated to be an isomeric mixture of demeton-O (O,O-diethyl-O-ethylmercaptoethyl thiophosphate) 1 and demeton-S (O,O-diethyl-S-ethylmercaptoethyl thiophosphate) 2. Another name for demeton-O is Systox and for demeton-S is Isosystox (4).



1, demeton-O



2, demeton-S

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Many compounds of the S=P-O type are known to be thermally unstable and isomerization (S=P-O- $\rightleftarrows$ O=P-S-) appears to be fairly general (3,5,6). Therefore, it is difficult to prepare quantities of the isomers in a state of high purity.

The first successful separation of demeton isomers was reported by Gardner and Heath (4) with a silica gel partition chromatography employing iso-octane/methanol as the solvent system. Small amounts (∿ mg) of the individual demeton isomers were obtained. Separation was confirmed by coelution with radioactive samples. Toxicological study (4) with the purified isomers revealed that demeton-S is about ten times more toxic to insects and mammals than demeton-O isomer. This adequately explains some discrepancies of the early work on the toxicity and cholinesterase-inhibiting activities of the isomeric mixture (7,8).

A prerequisite for further toxicological evaluation is the development of a preparative method of separating the demeton isomers. This paper describes a convenient method of separating the demeton isomers on a preparative scale with an Ito multi-layer coil countercurrent system. The advantages of countercurrent chromatography were attested in various aspects of this work.

EXPERIMENTAL

Reagents

The demeton isomers and authentic demeton-S and demeton-O were provided by Ms. Doris Smith of Research Triangle Institute and Ms. Patricia Wiley of Northrop, Inc., Research Triangle Park, North Carolina. n-Pentane used in the two phase solvent system was HPLC grade from Fisher Scientific. Absolute ethanol was obtained from AAPER Alcohol and Chemical Co.

Method

Countercurrent chromatography was performed with an Ito multi-layer coil countercurrent system (for details see references

9,10). In this work, a coiled column made of 1.6 mm (ID) PTFE tubing with a total capacity of 360 mL was used.

The two phase solvent system n-pentane:ethanol:H_2O (6:5:1) was thoroughly equilibrated in a separatory funnel at room temperature and separated before use. The coiled column was first filled with the upper phase solvent at a flow rate of 15 mL/min. The mixture of demeton isomers (600 mg) was dissolved in 3.0 mL of the two phase solvent (1:1) and injected through the inlet (head) of the column. Following sample addition, the mobile phase (lower phase) was pumped through the inlet with an FMI pump at a flow rate of 2.5 mL/min, and the system was rotated at 800 rpm. Column effluent was collected every 2 min, and each fraction was monitored by silica gel thin layer chromatography with 5% acetone in CH_2Cl_2 as the developing solvent system. Solvent was removed from the fractions by evaporation at 25°C under reduced pressure.

RESULTS AND DISCUSSION

The profile obtained from countercurrent chromatographic separation of demeton isomers is illustrated in Figure 1. Thin layer chromatography of aliquots from the CCC fractions together with the authentic isomers revealed that the demeton-S isomer $\underline{2}$ (R_f 0.13) was eluted first and found in Fractions 15, 16 and 17. The less polar demeton-O isomer $\underline{1}$ (R_f 0.45) was eluted much later and spread between Fractions 53 and 59. Total amounts of 293 mg of demeton-S ($\underline{2}$) and 190 mg of demeton-O ($\underline{1}$) were obtained from 600 mg of mixture. The structural assignments were unanbiguously confirmed by ^1H NMR analyses. The ^1H NMR (60 MHz) spectrum of the demeton-O isomer shows a 6-proton multiplet centered at 4.1 ppm for oxygen bonded methylenes and a 4 proton multiplet centered at 2.8 ppm for sulfur bonded methylenes, while the spectrum of demeton-S isomer shows a 4 proton multiplet centered at 4.1 ppm for oxygen bonded methylenes and a 6 proton multiplet centered at 2.7 ppm for the sulfur bonded methylenes. The entire separation including column equilibration took less than 3 h. The wide baseline area between the two peaks suggests that baseline separation could still be obtained at much greater sample loads.

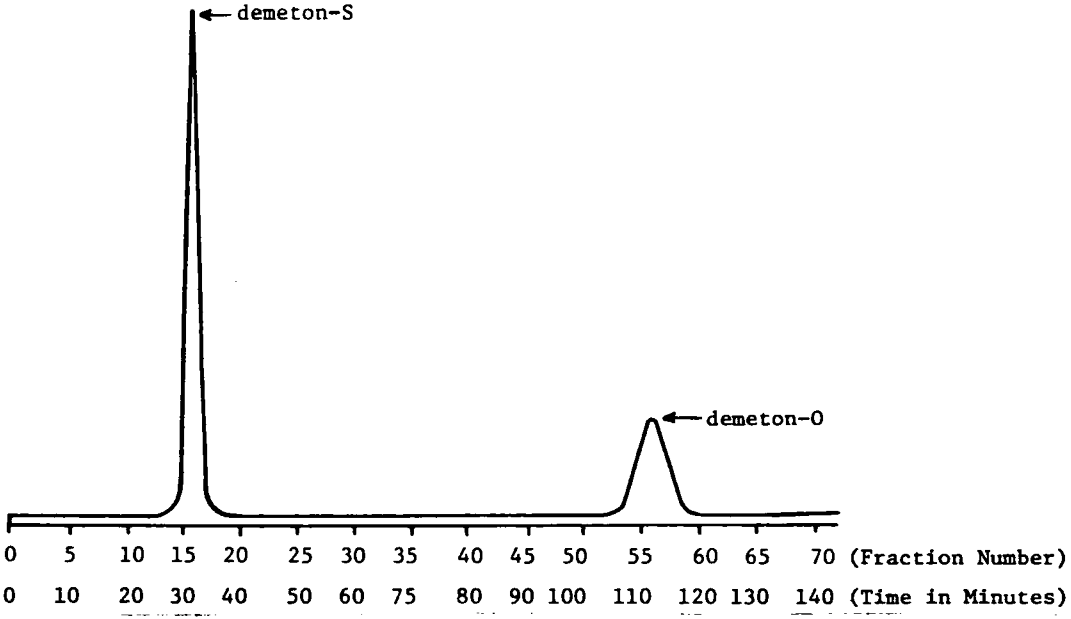

FIGURE 1. Plot of countercurrent chromatogram of Demeton-O and Demeton-S derived from TLC monitoring by assuming Gaussian distribution of weight in the fractions containing the isomers. Fractions 15-17: demeton-S; 53-59: demeton-O. Revolution Speed: 800 rpm; Flow Rate: 2.5 mL/min; Fraction Volume: 5 mL/tube.

The relatively small volumes in which the separated samples are obtained illustrates a unique advantage of counter-current chromatography--that it is devoid of the band broadening problems commonly observed in liquid-solid chromatography (9).

The two phase solvent system chosen (n-pentane:ethanol:H_2O, 6:5:1) has been proved to be excellent for nonpolar as well as medium polarity compounds (11). With this particular solvent system, 85-90% of the coil volume is occupied by the stationary phase at equilibrium. This high retention of stationary phase has the effect of increasing the resolution of countercurrent chromatography and thus plays a major role in the excellent results obtained.

CONCLUSIONS

Countercurrent chromatography has proved a mild and efficient technique for the preparative separation of the thermally labile S=P-O- and O=P-S isomers found in the insecticide Demeton. The two-phase solvent system from n-pentane:ethanol:water (6:5:1) used for this separation results in the retention of 85-90% of stationary phase in the coil. This provides excellent resolution for the countercurrent chromatography. The solvent system is applicable to compounds of intermediate or low polarity and is being applied in the routine purification of natural products and synthetic intermediates.

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REFERENCES

1. Deichmann, W. B., Brown, T. and Downing, D., Science, 116, 221 (1952).

2. Dubois, K. P. and Coon, J. M., Arch. Ind. Hyg. Occupational Med., 6, 1 (1952).

3. Schrader, G., "Die Entwicklung neuer Insektizide USW" Berlin, Verlag Chemie, 1952.

4. Gardner, K. and Heath, D. F., Anal. Chem., 25, 1849 (1953).

5. Topley, B., Chemistry & Industry, 5859 (1950).

6. Emmett, W. A. and Jones, H. O., J. Chem. Soc., 99, 713 (1911).

7. Aldridge, W. N. and Davison, A. N., Biochem. J., 52, 663 (1952).

8. Diggle, W. H. and Gage, J. C., Biochem. J., 49, 491 (1952).

9. Ito, Y., Countercurrent Chromatography, J. Biochem. Biophys. Method, 5, 105 (1981).

10. Ito, Y. and Sandlin, J. L., J. Liq. Chromatogr., 7 (2), 323 (1984).

11. The n-pentane:ethanol:H_2O (6:5:1) solvent system has been applied in the separation of many synthetic steroid derivatives as well as fairly nonpolar natural products.