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Note

Sequential thin-layer chromatography of O-ethyl O-4-nitrophenyl phenylphosphonothioate and related compounds

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Although the insecticide and acaricide O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN) has been marketed for over a quarter of a century, it has not been used on a large scale in the U.S.A. Recently, interest has been renewed in the use of EPN, largely because of the ban on most chlorinated hydrocarbon pesticides. This compound is relatively persistent in the environment[1,2]; *e.g.*, half-life values of EPN were found to be 80, 10 and 5 days for citrus fruits, peach fruits and peach leaves, respectively. Small oral doses of EPN can build up to cause delayed neurotoxicity in hens[3].

Various analytical procedures have been used to determine EPN[4–8]. Gas–liquid chromatographic (GLC) analysis, using microcoulometric titration[9–11], sodium thermionic[12] and electron capture detectors[13] has been utilized. However, GLC is invariably less sensitive to the oxygen analogue than to its parent compound. Furthermore, other possible degradation products of EPN were not successfully analyzed, because of their instability. Recently, sequential thin-layer chromatographic (STLC) methods have been developed to analyze the insecticide leptophos[14] [O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate] and the herbicide paraquat[15] (1,1′-dimethyl-4,4′-bipyridinium) and their metabolites.

This communication reports a rapid STLC method for the fast and convenient analyses of EPN and six possible degradation products.

EXPERIMENTAL

Chemicals

EPN, its related compounds and the radioactive EPN used in this experiment were provided by DuPont (Wilmington, Del., U.S.A.). The following analytical grade compounds were investigated: EPN, EPN oxon (O-ethyl O-4-nitrophenyl phenylphosphonate), EPN amino (O-ethyl O-4-aminophenyl phenylphosphonothioate), EPPTA (O-ethyl phenylphosphonothioic acid), EPPA (O-ethyl phenylphosphonic acid), PPA (phenylphosphonic acid), PNP (*p*-nitrophenol). Two radioactive chemicals were used: [^{14}C]EPN, O-ethyl O-4-nitrophenyl [^{14}C]phenylphosphonothioate (specific activity 1.64 μCi/mmol) (New England Nuclear, Boston, Mass., U.S.A.). [^{14}C]EPN

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oxon, O-ethyl O-4-nitrophenyl [^{14}C]phenylphosphonate (specific activity 1.64 μCi/mmol), prepared by oxidation of [^{14}C]phenyl EPN with excess of concentrated nitric acid[16].

Thin-layer plates

Gelman type SA, ITLC, silicic acid-impregnated glass fiber sheets (Gelman, Ann Arbor, Mich., U.S.A.) were used.

Solvents

The solvents used were: primary solvent A, acetonitrile–water–ammonia (40:9:1) and secondary solvent B, *n*-hexane–benzene–acetic acid (5:5:1).

Procedure

Aliquots (10 μl) of acetone solution (1 mg/ml) of EPN and related compounds were spotted on the ITLC sheets. Chromatograms were developed in a lined, pre-equilibrated tank up to 10 cm with the primary solvent A, then to 16 cm with the secondary solvent B (35 min). The respective regions were identified by visualization in an iodine chamber.

The radiochemical purities of [^{14}C]EPN and [^{14}C]EPN oxon were evaluated using the same STLC system. For the characterization of impurities, a mixture of EPN and structurally related compounds was added to each of the [^{14}C]EPN and [^{14}C]EPN oxon solutions and the chromatography of each solution was subsequently determined. The standards were detected by their color in iodine vapor. The sheets were cut into 5-mm strips, placed in scintillation vials, and vigorously mixed with a scintillation medium. The scintillation solvent was composed of one volume of ethylene glycol monomethyl ether and two volumes of toluene containing 5 g of 2,5-diphenyloxazole (PPO) and 200 mg of 1,4-bis[2(5-phenyloxazolyl)benzene] (POPOP) per litre. Radioactivity was determined using a Beckman Model LS-100 liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Although EPN was first introduced over 25 years ago, the information available on its metabolism is only meager. Since it is possible to attribute the neurotoxic effect of technical EPN to contaminants or metabolites[3], it is important to develop a simple accurate method, for the separation, isolation and identification of EPN and its degradation products in biological and non-biological systems.

The R_F values (average of three developments) of EPN and related compounds in two single and one sequential solvent system are listed in Table I. These compounds could be classified into two groups according to their separation on ITLC: non-polar compounds, EPN and EPN oxon and polar compounds, EPPTA, EPPA and PPA. *p*-Nitrophenol showed a behavior intermediate between non-polar and polar. The compound EPN amino, behaved as a non-polar compound in alkaline solvents and as a polar compound in acidic solvents.

The best solvent system for the separation of polar compounds from non-polar compounds was the primary solvent A. In this system all non-polar compounds (including EPN amino) moved together with the solvent front (R_F 0.83–0.86) and

TABLE I

R_F VALUES FOR EPN AND RELATED COMPOUNDS ON ITLC SHEETS USING SINGLE AND SEQUENTIAL SOLVENT SYSTEMS

Solvents: A, acetonitrile–water–ammonia (40:9:1); B, *n*-hexane–benzene–acetic acid (5:5:1); sequential solvent system, primary solvent A for 10 cm and secondary solvent B for 16 cm.

Compound	*Solvent A*	*Solvent B*	*Solvent A followed by solvent B*
EPN	0.83	0.76	0.89
EPN oxon	0.83	0.37	0.71
EPN amino	0.86	0.09	0.58
EPPTA	0.69	0.41	0.44
EPPA	0.59	0.30	0.35
PPA	0.15	0.03	0.08
PNP	0.73	0.26	0.51

too quickly to interfere with the polar compounds. The latter substances moved in good separable distances from each other with the following R_F values: EPPTA 0.69, EPPA 0.59, and PPA 0.15. The intermediate *p*-nitrophenol had an R_F value of 0.73. On the other hand, when the secondary solvent B was used, EPN separated well from the other compounds with an R_F value of 0.76. The compounds EPN oxon, EPPTA, EPPA and PNP, moved too close together to be separated, with R_F values ranging between 0.26 and 0.41. In this system, EPN amino and PPA moved slightly from the origin with R_F values of 0.09 and 0.03, respectively.

By employing a two-solvent sequential TLC system, which developed the ITLC sheets first with the primary solvent A for 10 cm followed by the secondary solvent B for 16 cm, excellent resolution of all the compounds tested was obtained (Table I).

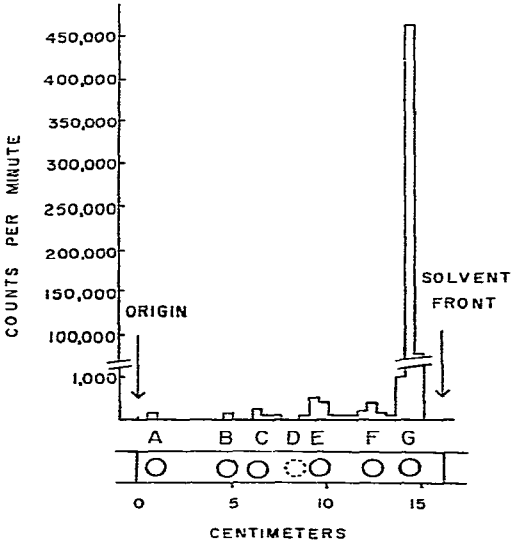

Fig. 1. Chromatogram and scan for [^{14}C]EPN, using Gelman type SA, ITLC silicic acid-impregnated glass fiber sheets, following sequential elution with primary solvent A, for 10 cm and secondary solvent B for 16 cm. A = PPA; B = EPPA; C = EPPTA; D = PNP; E = EPN amino; F = EPN oxon; G = EPN.

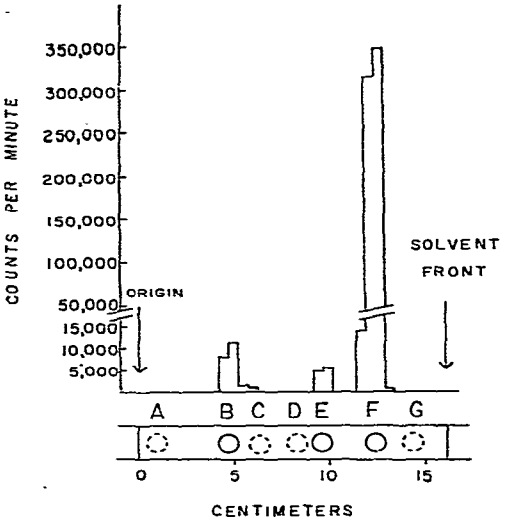

Fig. 2. Chromatogram and scan for [^{14}C]EPN oxon using Gelman type SA, ITLC silicic acid-impregnated glass fiber sheets, as described in legend of Fig. 1.

The radiochemical purity of ^{14}C-labeled EPN and EPN oxon was evaluated using the two-solvent sequential TLC system. [^{14}C]EPN was found to be 99.58% pure (Fig. 1). The impurities were identified by STLC to be PPA (0.03%), EPPA (0.03%), EPPTA (0.03%), EPN amino (0.19%) and EPN oxon (0.14%). The purity of [^{14}C]EPN oxon was determined to be 99.94% with 0.03% each of EPPA and EPN amino as impurities (Fig. 2).

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REFERENCES

1 F. A. Gunther and R. C. Blinn, *Analysis of Insecticides and Acaricides*, Interscience, New York, 1955, p. 139.
2 M. H. Brunson and L. Koblitsky, *J. Econ. Entomol.*, 45 (1952) 963.
3 M. B. Abou-Donia and D. G. Graham, *Toxicol. Appl. Pharmacol.*, (1978) in press.
4 P. R. Averell and M. V. Norris, *Anal. Chem.*, 22 (1948) 753.
5 D. E. Coffin and W. P. McKinley, *J. Ass. Offic. Agr. Chem.*, 46 (1963) 223.
6 D. E. Coffin and G. Savary, *J. Ass. Offic. Agr. Chem.*, 47 (1964) 875.
7 R. J. Gejan, *J. Ass. Offic. Agr. Chem.*, 46 (1963) 216.
8 D. A. George, *J. Ass. Offic. Agr. Chem.*, 46 (1963) 960.
9 H. P. Burchfield, J. W. Rhoades and R. J. Wheeler, *J. Agr. Food Chem.*, 13 (1965) 511.
10 J. Burke and W. Holswade, *J. Ass. Offic. Agr. Chem.*, 48 (1964) 845.
11 R. C. Nelson, *J. Ass. Offic. Agr. Chem.*, 47 (1964) 289.
12 L. Giuffrida, *J. Ass. Offic. Agr. Chem.*, 47 (1964) 289.
13 J. J. Kirkland and H. L. Pease, *J. Agr. Food Chem.*, 15 (1967) 187.
14 M. B. Abou-Donia, *J. Chromatogr.*, 150 (1978) 238.
15 M. B. Abou-Donia and A. A. Komeil, *J. Chromatogr.* 152 (1978) 585.
16 M. B. Abou-Donia and M. M. Ashry, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 37 (1978) 504.