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Separation of Methyl parathion and Fenitrothion Metabolites by Liquid Chromatography

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Parathion and fenitrothion are widely used as pesticides. The metabolism of these pesticides in insect and mammalian systems, incruding human, has been extensively studied. The formation of oxons from these chemicals by oxidation in the environment has focused our urgent attention on their toxity (SPEAR 1977a). Parathion incorporated into the body is metabolized to paraoxon by tissue oxygenases (DAVIS 1977b). Paraoxon irreversibly inhibits the activity of acetylcholine esterase, and especially strongly affects neural tissues (OBERSTEINER 1978). Further degradation of the paraoxon is carried out by esterases and gives p-nitrophenol and phosphoric acid (MENDOZA 1976). On the other hand, methylparathion is demethylated by a reaction catalyzed by glutathion-S-transferase to produce desmethyl-methylparathion (FUKAMI 1966). Fenitrothion is metabolized to fenitrooxon, 4-nitrocresol, phosphoric acid and desmethylfenitrothion. Until now the identification of these metabolic compounds has been performed using gas chromatography, either directly or after treatment of trifluoroacetic acid (GREENHALGH 1975a and KLUS,1975b). The analysis of these compounds by high pressure liquid chromatography (HPLC) except for S-methylfenitrothion (GREENHALGH 1975a), has not been reported.

In this paper, we would like to report the analysis of a mixture of parathion metabolites, such as desmethyl-methylparathion, desmethylfenitrothion, p-nitrophenol, 4-nitrocresol, methylparaoxon, fenitrooxon, methylparathion and fenitrothion in the picomole range by the HPLC.

MATERIALS AND METHODS

The structures of the compounds which were used in this study are shown in Fig.1. Methylparathion and fenitrothion (Sumithion) were gifts from Sumitomo Chemical Co., LTD. Methylparaoxon and fenitrooxon were synthesized by the DCC method (T.Abe et al, in preparation).

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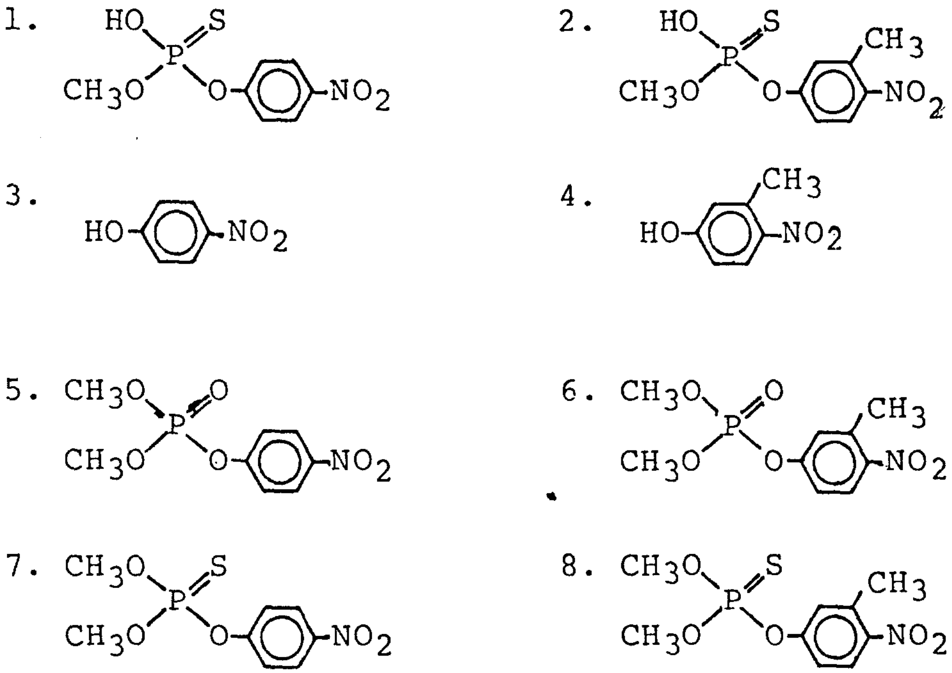

Fig.1. Structures of the parathion and fenitrothion metabolites: 1. desmethyl-methylparathion, 2. desmethyl fenitrothion, 3. p-nitrophenol, 4. 4-nitrocresol, 5. methylparaoxon, 6. fenitrooxon, 7. parathion, 8. fenitrothion.

Desmehthyl-methylparathion and desmethylfenitrothion were synthesized from methylparathion and fenitrothion, respectively, using the potassium dithiocarbamate method (OHKAWA 1977). p-Nitrophenol and 4-nitrocresol were purchased from Tokyo Kasei Chemical Co., LTD.

The HPLC equipment routinely used was the Hitachi Model 635 packed with Hitachi 3010 (Φ 2.6 X 500 mm) and 3011 (Φ 2.6 X 500 mm) gels. The compounds were detected by optical density at 260 nm by a Hitachi wave length tunable UV monitor in the picomole range. Through out all the experiments, the flow rate of the solvent was kept at 1 ml per min., and the pressure at 150 p.s.i. at room temperature.

RESULTS AND DISCUSSION

Fenitrothion and its metabolites such as desmethylfenitrothion, 4-nitrocresol, and fenitrooxon were separated using a solvent system of iso-propanol : methanol

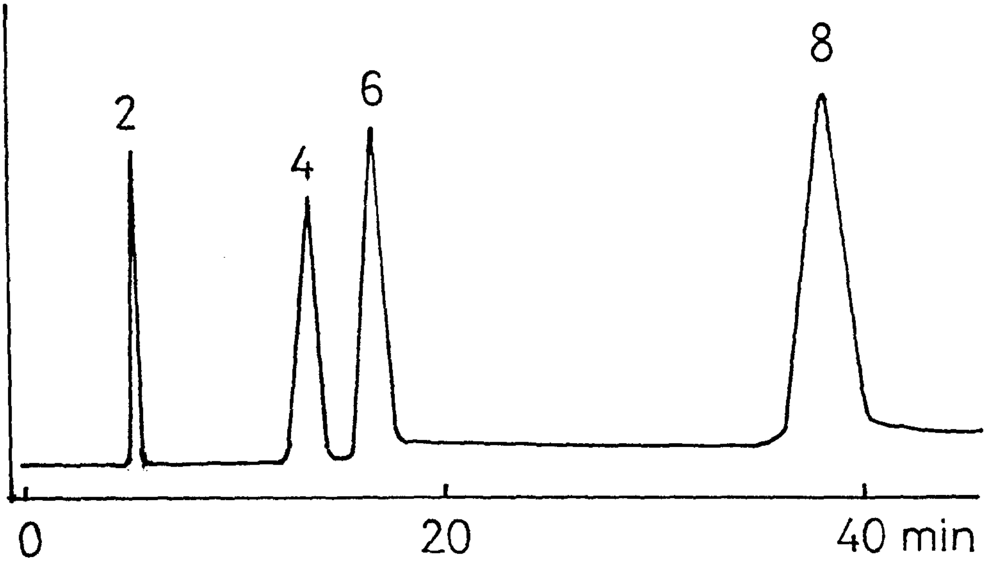

Fig.2. HPLC of a mixture of desmethylfenitrothion (2), 4-nitrocresol (4), fenitrooxon (6) and fenitrothion (8) using the solvent system of iso-propanol : methanol : water = 5 : 10 : 0.75 (v/v/v).

: water = 5 : 10 : 0.75 (v/v/v) as shown in Fig.2. The related compounds from methylparathion gave similar results to those fenitrothion. In this solvent system,

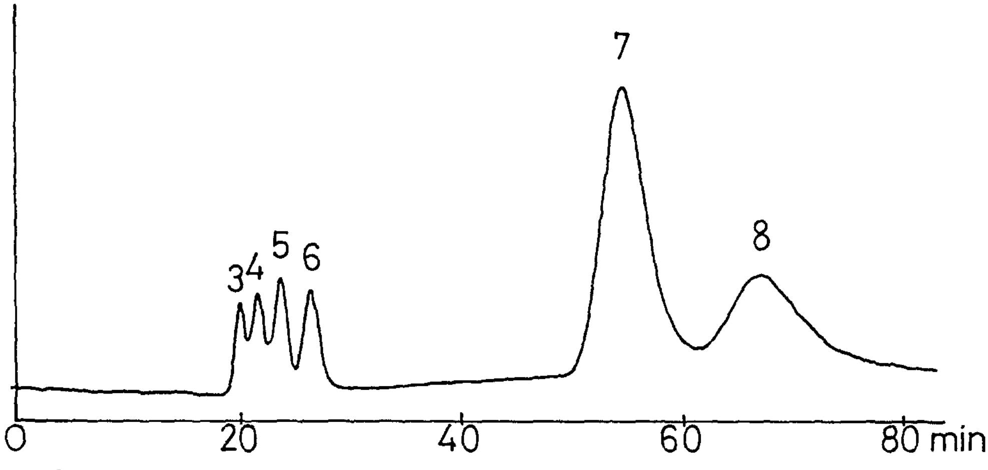

Fig.3. HPLC of a mixture of p-nitrophenol (3), 4-nitrocresol (4), methylparaoxon (5), fenitrooxon (6), methyl parathion (7) and fenitrothion (8) using the solvent system of tert-butanol : methanol : water = 30 : 65 : 5 (v/v/v).

the percentage of water did not have much influence on the separation of each compound. However increasing the water tended to extend the elution time.

Chromatography of the mixture of p-nitrophenol, 4-nitrocresol, methylparaoxon, fenitrooxon, methylparathion and fenitrothion was done using a different solvent system consisting of tert-butanol : methanol : water = 30 : 65 : 5 (v/v /v) as shown in Fig. 3. Each of these compounds was separated by this solvent, except for desmethylmethylparathion and desmethylfenitrothion, which came out together at about 10 min. Separation of the first four compounds was delicately influenced by the amount of water. When the water content was less than 5 %, 4-nitrocresol eluted early and p-nitrophenol later. At over 5 % water, the 4-nitrocresol moved to the methylparaoxon area. When the iso-propanol solvent system was used on the same sample, there were more serious overlapping situations, depending on the water content, than with the tert-butanol solvent system.

For the analysis of desmethylmethylparathion and desmethylfenitrothion, when methanol was used as a solvent (Fig.4), the separations of p-nitrophenol and 4-nitrocresol, and methylparaoxon and fenitrooxon were imcomplete.

These results can be useful for the microanalysis of these pesticides as environmental contaminants and their metabolites. Combined with mass spectrometry this method will give a more accurate identification of the metabolites.

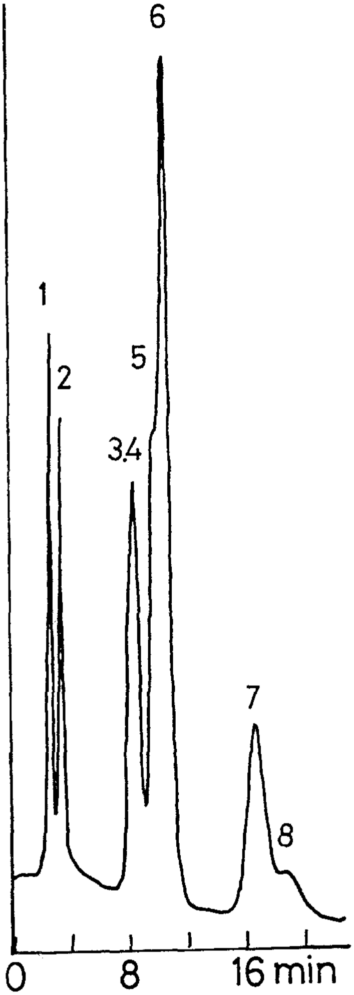

Fig.4. HPLC of a mixtire of desmethylmethylparathion (1) and desmethylfenitrothion (2) using methanol. Number 3 to 8 crrespond to the compounds in Fig.1.

ACKNOWLEDGMENT

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REFERENCES

DAVIS,J.E. and T.J.MENDE : J.Phalmacol.Exp.Ther.201,490 (1977b).

FUKAMI,J and T.SHISHIDO : J.Econ. Entom.59,1338(1966).

GREENHALGH,R. and W.D.MARSHALL andJ.KOVACICOVA : Bull. Environ.Contam. Toxico.13,291(1975a).

KLUS,H. and H.KUHN : Mikrochimi.Acta 1975b I, 405.

MENDOZA,C.E.,J.B.SHIELDS and G.W.LAVER : Toxicol.Appl. Pharmacol.38,499(1976).

OBERSTEINER,E.J. and R.P.SHARMA : Can.J.Comp.Med.42,80 (1978).

OHKAWA,H.,N.MIKAMI andJ.MIYAMOTO : Agric.Biol.Chem.41, 369(1977).

SPEAR,R.C.,W.J.POPENDORF,W.F.SPENCER and T.H.MILBY : J.Occup.Med.19,411(1977a).