Analytical Studies on Pesticides by Infrared Spectroscopy. (I)*¹
Identification of Microgram Quantities of Some Organophosphorus
Insecticides by Combination of Thin-layer Chromatography
and Infrared Spectrophotometry.

Naobumi Ōi and Hiroshi Takeda

(*Research Department, Osaka Works, Sumitomo Chemical Co., Ltd.*²*)

A combination of thin-layer chromatography and infrared spectrophotometry technique was used for identification of trace amounts of some organophosphorus insecticides (dimethoate and a mixture of methylparathion-sumithion) extracted from plant materials. The advantages of this method are the relative simplicity, the rapidity, the reliability of identification in one or mixture of insecticides, and the possible application to another insecticides.

(Received December 17, 1963)

Widespread use of organophosphorus insecticides has created problems for the analyst who is engaged in the detection of microgram quantities of insecticide residues. Previous investigations of these problems have dealt almost exclusively with paper chromatography,^{1)~7)} paper electrophoresis,⁷⁾ thin-layer chromatography,^{8)~10)} and chromogenic reagents for making the pesticides visible.^{11)~13)}

Although thin-layer chromatography is very useful as a clean up tool because of its ease of operation, rapidity and high resolving power, R_f values and specific color reactions are not sufficient for positive identification of unknown materials. So, it is rather difficult to differentiate insecticides which have similar chemical structures by thin-layer chromatography alone.

On the other hand, identification of an insecticide by use of infrared absorption in the "fingerprint" region gives a highly desirable degree of specificity,^{14),15)} but direct analysis by infrared spectrophotometry may not be possible if there are interfering substances, such as vegetable oil, natural dye and detergent present in the plant material.

Therefore the present investigation was undertaken to establish a useful method for reliable identification of trace amounts of organophosphorus insecticides by combination of thin-layer chromatography and infrared spectrophotometry.

This method makes possible the reliable identification of organophosphorus insecticides in plants containing as little as about 1 μ g by use of infrared beam condensing system and potassium bromide micro pellet technique,^{16)~18)} and as little as about 20 μ g by use of potassium bromide semimicro disk technique^{19)~21)} without beam condensing accessories.

Moreover this method is effective for identification of mixture of insecticides which are difficult to be resolved chromatographically.

*1 A part of this work was presented at the 17th General Meeting of the Pharmaceutical Society of Japan, April, 1963.

*2 Kasugade-cho, Konohana-ku, Osaka (大井尚文, 竹田宏; 大阪市此花区春日出町, 住友化学大阪製造所研究部).

Experimental

Apparatus—Infrared spectra were recorded by using a Hitachi model EPI-2, EPI-S and a Perkin-Elmer model 21 double beam infrared spectrophotometer equipped with a sodium chloride prism. The microspectroscopic measurements were made by use of Hitachi model IM-2P infrared microspectroscopic attachment.

Materials—All chemicals and solvents (analytical reagent grade) were used without further purification. Standard samples of the organophosphorus insecticides available were listed in Table I.

Procedure

Thin-layer chromatography—A thin-layer, 250 μ in thickness, was applied to a glass plate with Silica gel G (E. Merck. Co.) and activated prior to use by drying at 110° for 30 min. The activity is tested by the R_f value of *p*-dimethylamino-azobenzene (solvent system: n-hexane-acetone (4+1)). Each sample (CHCl_3 solution) was spotted with a micropipette at the position of 1.5 cm from the lower end of the plate. The following 3 kinds of solvent systems were used for development.

- 1) n-hexane-acetone (4+1)
- 2) n-hexane-ethylacetate (9+1)
- 3) benzene

When the solvent front reached 10 cm from the starting point, the plate was taken out of the chromatographic chamber. The time required for development was usually about 30 min. After solvent was dried, the plate was submitted to detection by color reaction. The insecticides were detected by the following 3 kinds of reagents.

- 1) 0.5 % solution of PdCl_2 in diluted HCl ⁸⁾
- 2) 0.5 % solution of 2,6-dibromo-N-chloro-*p*-quinoneimine¹³⁾ in EtOH
- 3) iodine vapor¹⁰⁾

Infrared spectrophotometry—An equivalent area of silica gel at the same R_f level for insecticide, left untreated with reagents, ^{*3} was recovered and extracted with chloroform. To disperse the sample in potassium bromide, a freeze drying technique^{17), 22), 23)} or the following procedure was adopted. The small test tube containing the aqueous solution of potassium bromide was swirled and heated so that the potassium bromide was applied in a thin layer on the sides of the tube. A sample solution was added in this tube and evacuated and mixed by conventional grindings. For micro work, with a infrared microscope attachment, a 1 mg micro pellet, 1.5 mm in diameter, is prepared by using a Perkin-Elmer micro die. For semimicro work, without any beam condensing accessory, the following procedure is used. The disk holder is a circular piece of thick filter paper, 13 mm in diameter by about 1 mm thick, with a rectangular 3 x 9 mm aperture in its center. In preparing the disks, the disk holder is placed on the lower plunger of the die assembly. The sample, which is dispersed in potassium bromide by conventional grinding, is transferred to the aperture. The disk holder containing the sample is pressed in a vacuum as usual. The sample fused in potassium bromide is concentrated into the aperture.

Results and Discussion

In Table I are shown R_f values of six organophosphorus insecticides in thin-layer chromatography, using 3 solvent systems. R_f values of methylparathion and sumithion were very near.

^{*3}. When iodine vapor is used as a reagent of detection, direct extraction may be possible¹⁰⁾.

Table I Rf Values of Some Organophosphorus Insecticides in Thin-Layer Chromatography

	{n-hexane acetone	4 1	{n-hexane ethylacetate	9 1	benzene
methylparathion		0.37		0.37	0.70
ethylparathion		0.49		0.52	0.76
sumithion		0.38		0.38	0.73
EPN		0.42		0.42	0.82
dimethoate		0.08		0.00	0.00
malathion		0.35		0.23	0.29

At the top of Fig. 1 is shown the result with a micro pellet containing only 1 μ g of sumithion in 1 mg potassium bromide using infrared microscope. In the middle is shown the spectrum obtained with a semimicro disk containing 20 μ g of sumithion in 30 mg

Fig. 1 Infrared Spectra of Sumithion

- A 1 μ g sample in 1 mg KBr using micro technique
- B 20 μ g sample in 30 mg KBr using semi-micro technique
- C 200 μ g sample in 200 mg KBr using macro technique

Fig. 2 Infrared Spectra of Some Organophosphorus Insecticides

- A methylparathion
- B ethylparathion
- C sumithion
- D EPN
- E dimethoate
- F malathion

potassium bromide and this was run without extensive instrument modification or expensive beam condensing accessories. Shown at the bottom, for comparison, is usual macro run on 200 μg of sumithion contained in a 200 mg potassium bromide disk 13 mm in diameter.

The result presented in Fig. 1 clearly demonstrate that the infrared microscope and potassium bromide micro pellet technique are well suited to the identification when only a few microgram amounts of material available from micro separation technique. Semi-micro technique is practically useful because of its simplicity.

Typical spectra of six organophosphorus insecticides obtained from about 5 μg samples in microtechnique are illustrated in Fig. 2. Each of the pesticides gave unique spectrum and could be readily differentiated.

Fig. 3. Infrared Spectra of Dimethoate
A isolated from spinach
B reference spectrum

Fig. 4 Infrared Spectra of Methylparathion and Sumithion in Mixture

A isolated from lettuce
B reference spectrum of methylparathion
C reference spectrum of sumithion

The combination technique thin-layer chromatography and infrared spectrophotometry was being applied to the identification of dimethoate from spinach. For extraction and purification the following procedure is used. Cut the spinach into small pieces, macerate and grind with chloroform in a blender, and filter the extract through a filter paper disk in a glass filter. If the chloroform and aqueous layers do not separate readily, centrifugation may be used. And if the chloroform layer contains large amounts of plant pigments, shake with charcoal and filter again. Evaporate the chloroform carefully with slight vacuum at room temperature. The concentrated solution was spotted and separated by thin-layer chromatography using a solvent system n-hexane-acetone (1 + 1), and the developed chromatogram was thoroughly dried and was placed in a chamber over iodine crystals. Vegetable oil and dye were separated from dimethoate and dimethoate made visible by this treatment may be outlined. Then the iodine allowed to vaporize, the outlined area of silica gel was recovered and eluted with chloroform. Potassium bromide disk was prepared with this extract. The spectra of dimethoate separated from spinach and standard dimethoate are shown in Fig. 3. Both spectra were identical and exhibited characteristic peaks at 1660, 1550, 1420, 1175, 1015, 820 cm^{-1} .

If two insecticides in mixture were not resolved chromatographically, it might even be possible to analyze them by infrared spectrophotometry as a multi component mixture.

For example, a typical infrared spectrum of the mixture of methylparathion and sumithion separated from lettuce and identified by this technique is shown in Fig. 4. Spectrum A exhibited characteristic peaks at 1115, 930, 865, 770 cm^{-1} for methylparathion, at 1275, 975, 890, 720 cm^{-1} for sumithion.

The technique described here may be applied to any other insecticides and should find wide application in analytical studies of residue of insecticides. Further work is in progress on the quantitative analyses and will be reported at a latter date.

The authors thank Sumitomo Chemical Co., Ltd. for permission to publish this work. They are indebted to Mr. I. Umeda for his technical assistance.

References

- 1) J. W. Cook : J. Assoc. Offic. Agr. Chemists, **37**, 987 (1954).
- 2) R. B. March, R. L. Metcalf, T. R. Fukuto : J. Agr. Food Chem., **2**, 732 (1954).
- 3) W. P. McKinley, J. H. Mahon : J. Assoc. Offic. Agr. Chemists, **42**, 725 (1954).
- 4) L. C. Mitchell : Ibid., **43**, 810 (1960).
- 5) H. F. MacRae, W. P. McKinley : Ibid., **44**, 207 (1961).
- 6) H. F. MacRae, W. P. McKinley : J. Agr. Food Chem., **11**, 174 (1963).
- 7) T. Ukita, M. Irie, S. Ishihara : J. Hyg. Chem. (Japan), **8**, 1 (1960).
- 8) J. Bäumler, S. Rippstein : Helv. Chim. Acta, **44**, 1162 (1961).
- 9) M. Uchiyama, S. Okui : J. Food Hyg. Soc Japan, **3**, 277 (1962).
- 10) K. C. Walker, M. Beroza : J. Assoc. Offic. Agr. Chemists, **46**, 250 (1963).
- 11) H. E. Wade, D. M. Morgan : Nature, **171**, 529 (1953).
- 12) J. W. Cook : J. Assoc. Offic. Agr. Chemists, **37**, 984 (1954).

- 13) J. J. Menn, W. R. Erwin, H. T. Gordon : J. Agr. Food Chem., **5**, 601 (1957).
- 14) W. Fischer, U. Uhlich : Z. Anal. Chem., **172**, 175 (1960).
- 15) D. F. McCaulley, J. W. Cook : J. Assoc. Offic. Agr. Chemists, **43**, 710 (1960).
- 16) D. H. Anderson, N. B. Woodall : Anal. Chem., **25**, 1906 (1953).
- 17) W. B. Mason : The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, (1958).
- 18) M. Sparagana, W. B. Mason : Anal. Chem., **34**, 242 (1962).
- 19) F. E. Resnik, L. S. Harrow, J. C. Holmes, M. E. Bill, F. L. Greene : Ibid., **29**, 1874 (1957).
- 20) F. Bissett, A. L. Bluhm, L. Long. Jr. : Ibid., **31**, 1927 (1959).
- 21) Z. Tamura, M. Ninomiya, F. Suzuki, M. Chiku : Bunseki Kagaku, **12**, 372 (1963).
- 22) T. Y. Toribara, V. D. Stefano : Anal. Chem., **26**, 1519 (1954).
- 23) H. P. Schwarz, R. C. Childs, L. Dreisbach, S. V. Mastrangelo, A. Kleschick : Appl. Spectroscopy, **12**, 35 (1958).