Technical Papers

Reversed Phase Paper Chromatography of Parathion and Related Phosphate Esters

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In our studies of the mode of action of parathion and related insecticides it became necessary to separate parathion, its S-phenyl and S-ethyl isomerides, and para-oxon (*1, 2*). After trials with paper chromatographic techniques using a variety of solvents and stationary phases, it was found that the separation could be accomplished by impregnating Whatman No. 1 filter paper with silicone 550[1] from a 5% hexane solution,[2] allowing it to dry, applying the phosphate esters in 5–10 µl acetone solution, and employing ascending chromatography, using as solvent the upper phase from a mixture of 10 parts chloroform, 10 parts absolute ethyl alcohol, and 6 parts water. A similar technique was developed by Kritchevsky and Tiselius (*3*) for the separation of steroids. After chromatography, the position of the *p*-nitrophenyl esters was determined by converting them to the intensely yellow *p*-nitrophenate ion by spraying the paper with 5% alcoholic potassium hydroxide and heating in the oven for a few minutes at 105° C. By this means spots containing as little as 0.1 µg of *p*-nitrophenol could be detected. The relative lengths of time required for the appearance of the yellow color are characteristic of the various phosphate esters and are determined by the alkaline hydrolysis constants as shown in Table 1. In general, the S-alkyl isomerides appear first, then the phosphates; heating is necessary to demonstrate the thionophosphates. Therefore, this characteristic can be used in determining the identity of unknown spots.

The described technique was applied to a variety of *p*-nitrophenyl dialkyl phosphate and thionophosphate esters and the R_F values shown in Table 1 were obtained. When the separations were carried out at a constant temperature with carefully impregnated paper, the R_F values were satisfactorily reproducible, as indicated by the standard errors for replicate determinations. In this connection it is important to impregnate the papers as evenly as possible, using a constant concentration of silicone.

When the chromatographic technique was applied to the separation of the constituents of technical parathion, three spots were consistently obtained with average R_F values of 0.04, 0.47, and 0.78. These appear to represent parathion, its S-ethyl isomeride, and *p*-nitrophenol, in ascending order. The percentages of the various constituents present can be estimated by cutting pieces of equal area around the spots, and then dividing these into tiny pieces, which are eluted for 24 hr in 0.1 *N* NaOH in a 10-ml volumetric flask. After centrifugation, the *p*-nitrophenate ion in each sample is determined from the percentage transmission at 400 mµ as compared to a blank of filter paper and alkali. The method has also proved of value in demonstrating *p*-nitrophenol or other impurities in samples of the various aryl dialkyl phosphates or thionophosphates. The free *p*-nitrophenol can be distinguished by its yellow color, which is visible before treatment with alkali.

TABLE 1

R_F VALUES AND HYDROLYSIS CONSTANTS FOR PARATHION AND RELATED MATERIALS

Compound	R_F 26° C	First order K hyd min^{-1} 1*M* NaOH at 37° C
p-Nitrophenyl dimethyl phosphate	0.84 ± 0.03*	7.7
p-Nitrophenyl diethyl phosphate	.74 ± .06	1.7
o-Nitrophenyl diethyl phosphate	.74 ± .09	1.7
p-Nitrophenyl di-2-chloroethyl phosphate	.68 ± .03	
2,4-Dinitrophenyl diethyl phosphate	.64 ± .03	
p-Nitrophenyl 0,S-dimethyl thiophosphate†	.58 ± .03	
p-Nitrophenyl diisopropyl phosphate	.47 ± .06	1.4
p-Nitrophenyl 0,S-diethyl thiophosphate†	.47 ± .03	68
p-Nitrophenyl dimethyl thionophosphate	.14 ± .01	0.69
p-Nitrophenyl diethyl thionophosphate	.04 ± .01	0.23
p-Nitrophenyl ethyl thionobenzene phosphonate	.017 ± 0.002	13.8
bis-(*p*-Nitrophenyl) ethyl thionophosphate	0.00	1.2

* Standard deviation.
† Determined from mixture.

With this chromatographic method, it may be of value to determine the spots which contain phosphorus. The perchloric acid-ammonium molybdate technique of Hanes and Isherwood (*4*) is satisfactory for this purpose and is especially valuable with phosphate esters which contain no *p*-nitrophenyl group for identification. With this technique it is necessary to hydrolyze the esters on the paper for periods of 2–24 hr in a moist chamber at 85° C before developing the molybdenum blue color.

Two other techniques have been found useful to characterize the compounds resolved from complex mixtures. One consists in eluting the spots in acetone solution and determining the anticholinesterase activi-

[1] Dow Corning Corp.
[2] Skellysolve B.

ties of the compounds manometrically (*5*). In the other, the material is applied to the paper along 8 cm of the base line rather than as a spot and, after resolution, areas 8 × 5 cm containing the various compounds are cut from the paper and rolled in shell vials. Ten anesthetized houseflies are then introduced into each vial, and the toxicity of the compounds is characterized by rate of knockdown and 24-hr mortality.

The paper chromatographic method is useful in studying the metabolism of phosphorus insecticides in plants, mammals, and insects. With it, for example, we have been able to demonstrate the conversion of parathion and its methyl analog to the corresponding phosphates by an enzyme system found in *Periplaneta americana* (L.) (*2*). Further studies are in progress. The method has also been of value in studying the action of heat on purified parathion and methyl parathion and in isolating the compounds formed and in studying their biological properties (*1*).

References

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A Production of Amino Acids Under Possible Primitive Earth Conditions

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The idea that the organic compounds that serve as the basis of life were formed when the earth had an atmosphere of methane, ammonia, water, and hydrogen instead of carbon dioxide, nitrogen, oxygen, and water was suggested by Oparin (*1*) and has been given emphasis recently by Urey (*2*) and Bernal (*3*).

In order to test this hypothesis, an apparatus was built to circulate CH_4, NH_3, H_2O, and H_2 past an electric discharge. The resulting mixture has been tested for amino acids by paper chromatography. Electrical discharge was used to form free radicals instead of ultraviolet light, because quartz absorbs wavelengths short enough to cause photo-dissociation of the gases. Electrical discharge may have played a significant role in the formation of compounds in the primitive atmosphere.

The apparatus used is shown in Fig. 1. Water is boiled in the flask, mixes with the gases in the 5-l flask, circulates past the electrodes, condenses and empties back into the boiling flask. The U-tube prevents circulation in the opposite direction. The acids and amino acids formed in the discharge, not being volatile, accumulate in the water phase. The circulation of the gases is quite slow, but this seems to be an asset, because production was less in a different apparatus with an aspirator arrangement to promote circulation. The discharge, a small corona, was provided by an induction coil designed for detection of leaks in vacuum apparatus.

The experimental procedure was to seal off the opening in the boiling flask after adding 200 ml of water, evacuate the air, add 10 cm pressure of H_2, 20 cm of CH_4, and 20 cm of NH_3. The water in the flask was boiled, and the discharge was run continuously for a week.

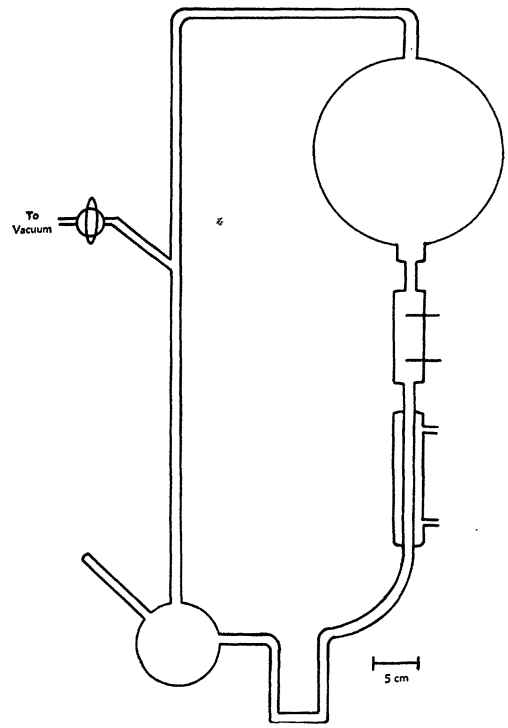

FIG. 1.

During the run the water in the flask became noticeably pink after the first day, and by the end of the week the solution was deep red and turbid. Most of the turbidity was due to colloidal silica from the glass. The red color is due to organic compounds adsorbed on the silica. Also present are yellow organic compounds, of which only a small fraction can be extracted with ether, and which form a continuous streak tapering off at the bottom on a one-dimensional chromatogram run in butanol-acetic acid. These substances are being investigated further.

At the end of the run the solution in the boiling flask was removed and 1 ml of saturated $HgCl_2$ was added to prevent the growth of living organisms. The ampholytes were separated from the rest of the constituents by adding $Ba(OH)_2$ and evaporating *in vacuo* to remove amines, adding H_2SO_4 and evaporat-

[1] National Science Foundation Fellow, 1952–53.

[2] Thanks are due Harold C. Urey for many helpful suggestions and guidance in the course of this investigation.