Metabolic Fate of Malathion and Methyl Parathion in Rice Plant

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The development of organic insecticides has increased and improved agricultural production, but residues of some organic insecticides in edible plant materials have induced the problem concerning mammalian toxicity. (Barnes, 1957; Waites & Van Middellem, 1955) The persistence of insecticides on and in plant, however, is a factor which is desirable for insect control. Residues of insecticides sprayed on plants generally tend to disappear or otherwise lose their analytical identity with time, although the rate of disappearance is different with insecticide concentration (Gunther & Blinn, 1956). Very little attention has been paid to the establishment of either the nature or the fate of aged insecticide residues within plant tissue. It is necessary from both the standpoints of public health and insect control to elucidate the fate of insecticides within plant tissue. This study was designed to examine the nature of the metabolites of 35S-labeled malathion and methyl parathion in rice plant.

MATERIALS AND METHODS

Application of Radioactive Insecticides to Rice Plant: In order to examine the metabolites of malathion and methyl parathion in rice plant, 35S-labeled compounds were applied because of the possibility of more sensitive detection than by chemical analysis. 35S-labeled malathion was synthesized by a slight modification of the description of March et al. (1956), starting from 35S-labeled sulfuric acid. The synthesis of 35S-labeled methyl parathion was described previously (Sato & Tomizawa, 1960). Specific activities of 35S-labeled malathion and methyl parathion were 282 and 5786 cpm per µg, respectively, when assayed on a GM counter.

Emulsifiable concentrate of radioactive insecticide, consisting of insecticide 50 parts, emulsifier (Sorpol 213)1 30 parts and xylene 20 parts, was prepared. A water emulsion of insecticide was prepared by diluting the emulsifiable concentrate of the insecticide with five hundred times of water by weight, and 15 ml of the water emulsion were sprayed by means of a small glass spray nozzle on two pots in which two hills of rice plant were planted. Rice plants, variety Norin No. 1, at heading stage were used in both experiments with radioactive malathion and methyl parathion, and the heads of the plants were covered with bags made of polyethylene sheets in order to avoid the direct deposition of the radioactive insecticides on the heads of the plants. After spraying, the pots were kept in a greenhouse, and after the elapse of one week the rice grains of the heads were collected for radioassay. Rice grains were collected just at milky stage in both cases of malathion and methyl parathion. The growth period of the plants used in experiment with malathion was from January to May, 1960, and that with methyl parathion was from May to September, 1960. The application of radioactive insecticides was carried out during the last month of each growth period. The growth condition of the plant was, therefore, quite different between malathion and methyl parathion. The heads of the plants used in experiment with methyl parathion were seen to be normal in growth condition, while the heads of the malathion treated plants were rather immature because of inadequate conditions for growth of the rice plant.

Separation of Water Extractable Metabolites of Malathion and Methyl Parathion: An ion
exchange chromatography for the separation of water extractable metabolites of malathion and methyl parathion was carried out according to the description of PLAPP & CASIDA (1958a). The size of chromatography system was reduced to one-fifth from the original one. The elution gradients for the separation of malathion metabolites were modified as described by KRUEGER & O'BRIEN (1959).

Rice grains were macerated in a waring blender with 20ml of chloroform and 20ml of 10% trichloroacetic acid aqueous solution. After filtering the macerate through three folds of gauze, chloroform and water phases were separated by centrifugation, and the radioactivity of each phase was determined per unit volume. In the case of malathion metabolites, 10ml of the chloroform phase were re-partitioned against 10ml of water containing enough KOH to give a pH of 8 after partitioning, and 5ml of the water phase were combined with 5ml of the first water phase which contained trichloroacetic acid aqueous solution. After adjusting the pH of the water phase around 3.5 by 20% KOH, a volume which corresponds to 5ml of the water phase was pipetted into the column of the chromatography. The top of the column was washed with 5ml of distilled water, and the chromatography was carried out. The column was placed on a fraction collector, and 5ml eluate per fraction was collected. One ml aliquot of each fraction was transferred to a planchet for radioassay, and after adding 1ml of 10% alcoholic kali to the planchet, the content of the planchet was dried up under the mild heat by an infrared lamp. Radioactivity of 85S was assayed on a gas flow counter. Identity of eluted metabolites was based on the comparison with the result of an ion exchange chromatography carried out with known compounds. In order to assist the identity of the metabolites, the following paper chromatography systems were used.

1) Filter paper impregnated with 5% Silicone 550 in hexane, mobile solvent, the upper phase from a mixture of ethyl alcohol 10 parts, chloroform 10 parts and water 6 parts by volume (METCALF & MARCH, 1953).

2) Filter paper uniformly deposited with silicic acid, mobile solvent, the upper phase from a mixture of hexane 10 parts, acetic acid 2 parts, and water 1 part (ASAKAWA & SUWANAI, 1957).

3) Filter paper without any treatment, mobile solvent, a mixture of isopropyl alcohol 75 parts and concentrated ammonium hydroxide 25 parts (PLAPP & CASIDA, 1958a).

The insecticides and their derivatives were located on the developed chromatographs by spraying with ammoniacal silver nitrate or molybdate reagent (HANES & ISHERWOOD, 1949). In the case of the radioactive metabolites, the paper chromatography was carried out with the concentrates of the fractions which were separated by ion exchange chromatography, and the detection of the spots was done by autoradiography of paper chromatograms. The Rf values of the compounds used in the experiment are shown in Table 1. The major parts of these compounds were prepared according to the description of MARCH et al. (1956) and KOSOLAPOFF (1950).

RESULTS AND DISCUSSION

When malathion or methyl parathion was sprayed on rice plant, it was found that the insecticide and its metabolites penetrated rather quickly into the leaf tissues of the plants and were translocated to the plant parts where had not been exposed to the insecticides, especially to the growing tissues of the plant (TOMIZAWA et al., 1960). When these insecticides were sprayed at the heading stage of the plant, some metabolites of the insecticides moved towards the heads resulting in gradual accumulation of those metabolites with the elapse of days. Since the heads of the plants sprayed with these insecticides contained comparatively high amount of the insecticide metabolites, it is easy to examine the fate of the insecticides.

Partition of Radioactive Metabolites in the Rice Grains between Chloroform and Water: The water phase after partition between chloroform and trichloroacetic acid aqueous solution, should contain several hydrolysis pro-
Table 1. Rf values for malathion, methyl parathion and their derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>(1)*</th>
<th>(2)*</th>
<th>(3)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃O │ S – O – NO₂</td>
<td>0.11</td>
<td>0.65</td>
<td>0.93</td>
</tr>
<tr>
<td>CH₂O │ S – O – NO₂</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃S │ S – O – NO₂</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HO │ S – O – NO₂</td>
<td>0.88</td>
<td>0.85</td>
<td></td>
</tr>
</tbody>
</table>

* Solvent systems were shown in the text.

ducts (O'BRIEN, 1960). Since the partition between chloroform and alkaline aqueous solution was carried out in the case of malathion, it was presumed that the hydrolysis products were almost transferred into the water phase. On the other hand, the partition between chloroform and alkaline aqueous solution was omitted in the case of methyl parathion, and some parts of the hydrolysis products might remain in chloroform phase. From the consideration of the chemical structure of methyl parathion, O-methyl O-\(p\)-nitrophenyl thiophosphoric acid (desmethyl parathion) and O,O-dimethyl thiophosphoric acid were presumed to be the primary metabolites of methyl parathion in biological system (O'BRIEN, 1960; PLAPP & CASIDA, 1958b), and it is therefore important to examine the partition ratio of these compounds between chloroform and trichloroacetic acid aqueous solution. It is possible that the partition of those metabolites between chloroform and trichloroacetic acid aqueous solution is affected by pK value of hydrolysis products (O'BRIEN, 1960). In order to examine partition ratio of hydrolysis products, desmethyl parathion was partitioned between chloroform and trichloroacetic acid aqueous solution in the same manner as that of the plant metabolites. The partition ratio of desmethyl parathion was found to favor water in the ratio of about 9:1. O,O-dimethyl thiophosphoric acid gave a partition ratio of 95 or more to 5 in favor of water. As shown in Table 2, the chloroform phase had still radioactivity although malathion or methyl parathion had not been detected by the autoradiography of paper chromatogram which was made with concentrated chloroform phase, and this low activity might be due to \(^{35}\)S of the hydrolysis products partitioned in chloroform phase. The centrifugation was needed for complete separation of both phases after the partition between chloroform and trichloroacetic acid aqueous solution.

Table 2. Partition of the metabolites in the rice grains of rice plant sprayed with \(^{35}\)S-labeled malathion or methyl parathion between chloroform and trichloroacetic acid aqueous solution.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Metabolites in µg. per gm. fresh weight of the rice grains (calculated as malathion or methyl parathion)</th>
<th>Total</th>
<th>Chloroform extractable</th>
<th>Water extractable</th>
<th>C/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>Methyl parathion</td>
<td></td>
<td>14.8</td>
<td>0.3</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.5</td>
<td>0.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Separation of Water Extractable Metabolites by Ion Exchange Chromatography: After the partition of the insecticide metabolites between chloroform and trichloroacetic acid aqueous
solution, the water phase was subjected to ion exchange chromatography using Dowex 1-X8 (PLAPP & CASIDA, 1958a). As shown in Fig. 1, two big peaks (a and b) were found in the case of malathion, and they corresponded to those of thiophosphoric acid and O,O-dimethyl S-(1,2-bis-carboxy)-ethyl dithiophosphate (dicarboxylic acid of malathion), respectively. The peak of dicarboxylic acid was followed by another small peak (c) which corresponded to O,O-dimethyl S-1-carboxy-2-carbethoxy)-ethyl dithiophosphate (mono-carboxylic acid of malathion). After concentrating the eluates which corresponded to dicarboxylic acid and monocarboxylic acid of malathion, the purity of the compounds in each eluate was examined by paper chromatography using isopropyl alcohol-ammonium hydroxide as developing solvent, (PLAPP & CASIDA, 1958a). The result showed that some fractions between peaks b and c contained both carboxylic acids of malathion. Anyway, it was obvious that the proportion of dicarboxylic acid of malathion is dominant. Since the radioactivities of several peaks which appeared following the carboxylic acids of malathion were relatively low, further examinations of those metabolites were not carried out.

As shown in Fig. 2, only three peaks of the metabolites were found in the case of methyl parathion, and they agreed with those of thiophosphoric acid, O-methyl O-p-nitrophenyl thiophosphoric acid (desmethyl parathion), respectively.

Monodemethylation of methyl parathion has been already found in insect by PLAPP and CASIDA (1958b). Monodemethylation of phosphoric esters in plant was also observed with dimethoate (DAUTERMAN et al., 1960) and phosdrin acid (SPENCER & ROBINSON, 1960), and it was found in the case of dimethoate that the percentage of desmethyl dimethoate production is different with kind of plants. Over all metabolism of phosphoric esters in plants may be different with growth stages and plant parts. Since 35S-labeled insecticide was used as a tracer, it was impossible to detect the presence of methyl paraoxon and their hydrolysis products because of the release of sulfur atom.

In the case of malathion, only two main metabolites were detected, which were thiophosphoric acid and dicarboxylic acid of malathion. In the previous experiment with 32P-labeled malathion, the presence of O,O-dimethyl dithiophosphoric acid was suggested as the rice plant metabolites from the result of paper chromatography (TOMIZAWA et al., 1960), but in the present experiment the presence of O,O-dimethyl dithiophosphoric acid was obscure. If O,O-dimethyl dithiophosphoric acid existed in the water extract of the rice plant, it should be eluted in the latter part of elution gradient (IV) which was shown
in Fig. 1. Since the solution of elution gradient (IV) was strongly acidic, it was probable that O,O-dimethyl dithiophosphoric acid was degraded during the elution process.

As shown in Figs. 1 and 2, the hydrolysis of organophosphoric acid was obvious, although the extent of the hydrolysis was different in the two insecticides. Since the rice grains used in the present experiment were collected just after the heading stage of the plant, it is expected that the hydrolysis of the insecticides to thiophosphoric acid in the plant tissues progresses further towards the maturing stage of the plant. It has been confirmed from the result of radiometric and chemical analyses, (GOTO et al., 1959; TOMIZAWA et al., 1960) that the rice grains of the plant which was sprayed with malathion, methyl parathion or ethyl parathion, did not contain any residues of these insecticides. As shown in Table 2, it is also an useful indication that chloroform extractable metabolites tend to be hazardous compounds, whereas the water extractable metabolites are usually entirely harmless (METCALF et al., 1955; O'BRIEN, 1960). Since the metabolites such as carboxylic acids of malathion and desmethyl parathion, which are quite close to the parent insecticides in their chemical structures, already lost their toxicity against not only insects but also against mammals in physiological concentrations, the residue problem of malathion and methyl parathion in harvested rice grains may be negligible.

SUMMARY

The metabolic fate of ³⁸S-labeled malathion and methyl parathion which were sprayed on rice plants was examined. When the metabolic rate of the insecticides was examined by the partition ratio of the insecticide metabolites between chloroform and trichloroacetic acid aqueous solution, it was found that the greater part of the insecticide metabolites contained in rice grains was water extractable even one week after spraying insecticides. The water extracts of the insecticide metabolites in rice grains were subjected to ion exchange chromatography, and the existence of several hydrolysis products of the insecticides was confirmed in both cases of malathion and methyl parathion. The main metabolites in the case of malathion were thiophosphoric acid and carboxy derivatives of malathion, while those of methyl parathion were thiophosphoric acid, O,O-dimethyl thiophosphoric acid and O-⁴-nitrophenyl thiophosphoric acid.

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摘 要

水稲におけるマラチオンおよびメチルパラチオンの代謝生成物

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出穂期の水稲に散布した 35S-malathion および methyl parathion の穂稲部分における分解生成物の性質を調べた。散布一週間後に穂稲部分の 35S 含有物質をクロロホルム〜水間に分配させると、大部分は水稲に移行する加水分解生成物に変化していることを認めた。イオン交換クロマトグラフィーにより分解生成物の分離を行ない、主要な 35S 含有物質として malathion の場合 thiophosphoric acid, 0,0-dimethyl S-(1,2-bis-carboxy)-ethyl dithiophosphate, 0,0-dimethyl S-(1-carboxy-2-carboethoxy)-ethyl dithiophosphate, methyl parathion の場合、thiophosphoric acid, 0,0-dimethyl thiophosphoric acid, 0-methyl 0-p-nitrophenyl thiophosphoric acid を検出した。

未精製砂糖上でのノコギリヒラタムシの発育と産卵


被害物（主に米）から採集したノコギリヒラタムシ Oryzaephilus surinamensis (L.) とオオメノノコギリヒラタムシ O. mercator (Fabric.) を室内で飼育し、これから得られた幼虫を 1 個体ずつ未精製の砂糖（粗糖と未精製）で飼育した。そして成虫になってからは雌雄 1 対ずつを再び未精製の砂糖上で産卵させ、産卵数と幼虫の活性を観察した。

両種ともなる上糖の発育の産卵数は粗糖中のそれに比べて約 1.5 倍多く、また卵の発育も粗糖上の方が高かった。一方同じ飼育上の産卵数はオオメノノコギリヒラタムシの方がノコギリヒラタムシより 3〜4 倍多かった。

さらに幼虫の発育では、粗糖と未精製で飼育した 20 頭のうちオオメノノコギリヒラタムシの 13 頭、ノコギリヒラタムシの 3 頭がそれぞれ成虫になった。また幼虫から成虫までの発育見ると、粗糖で飼育したものも黄砂糖で飼育したものより 8〜16日遅縮している。

これらのことからノコギリヒラタムシとオオメノノコギリヒラタムシは未精製の砂糖で発育を完了することがわかった。しかし産卵数と卵の活力および幼虫の発育という点の観点からするとオオメノノコギリヒラタムシの方が優れており、飼料としては粗糖の方が黄色ラメよりも好ましいことがわかった。

砂糖の被害の発生から見ると、ノコギリヒラタムシは未精製砂糖上では産卵数は少なく、幼虫の発育も非常に悪いが、オオメノノコギリヒラタムシでは砂糖上のものは穀物上上のものより産卵数が少ないが、砂糖上で孵化した幼虫の 2 期が成虫になるので増殖率は低いが次個体数を増加していくことが予想される。（牧野大農 武田 孝）