Fate of *O,O*-Dimethyl *O*-(3-methyl-4-methylmercaptophenyl) Thiophosphate Sprayed on Rice Plant

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INTRODUCTION

O,O-Dimethyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphate (Baycid) is one of the organophosphorus insecticides which are characterized by low order mammalian toxicity and high resistance to alkaline hydrolysis. Although the chemical structure of Baycid is close to that of methyl parathion, it has been shown that the physico-chemical properties of Baycid are rather different from those of methyl parathion or metasystox (SCHRADER, 1960). It is necessary for establishing the use of Baycid against agricultural purposes to elucidate the fate of Baycid in plants. The present experiments were undertaken to examine the natures of the metabolites of Baycid sprayed on rice plant for the control of rice plant pests, using ^{32}P -labeled tracer.

MATERIALS AND METHODS

Test insecticide: The emulsifiable concentrate consisted of ^{32}P -labeled Baycid 55 parts, xylene 20 parts, and emulsifier¹⁾ 25 parts. ^{32}P -labeled Baycid was obtained from Farben-fabrick-Bayer through the courtesy of Nihon Tokushu Nôyaku Seizô Company. An initial activity of ^{32}P -labeled Baycid was 3.4 mc per gram.

Scale of Experiment: Three kinds of rice plants which were transplanted at different time, respectively, were cultured in a tent covered with vinyl sheet. The varieties of the rice plant used in the experiments were as follows; Norin No. 18 at 3 weeks and 5 weeks

after transplantation, and Gaisenmochi at a few days before heading. The tent had a space of 5.4×1.8 meters and a height of 1.8 m, in which a space of 3×1.8 meters were in a similar condition of paddy field. Seventy rice plants were kept under the tent.

Application of radioactive Baycid to rice plants: The nozzle-boom of 1.7 m, which had 5 nozzles at intervals of 0.3 m in length, was moved horizontally in the vinyl tent, and the water emulsion of the insecticide was uniformly sprayed on rice plants under the pressure of 3.5 kg per cm^2 . Sprayed volume corresponded to 12.5 liters per acre, and the concentration of Baycid in the water emulsion was 0.07 per cent. The top and side of the vinyl tent were remained to cover closely with vinyl sheets for about 1 hour after the application of Baycid until the mist of the sprayed emulsion in the tent completely fell. Therefore, the temperature in the tent must have been considerably risen in this period as compared with that of the outside. Baycid was applied on 15th of August in 1960. Fine weather. Temperature, 28.2°C .

Sampling: At various intervals, whole plants or stems of rice plants were sampled at random, and leaf blade and leaf sheath were separated, and each portion was sliced finely by scissors. The first ear of the Gaisenmochi variety developed 3 days after the application, and the ears were sampled 3, 7 and 12 days after the application. Rice grains from the ears were also sampled 14 and 29 days after

1) Sorpol, manufactured by Tôhō Chemical Co., Ltd.

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the application in order to examine the distribution of radioactive metabolites in rice grains.

Radioassay: An aliquot of sliced sample was wet ashed with a mixture of concentrated sulfuric acid and nitric acid (5:1). After combustion, a precipitate of ammonium phosphomolybdate containing ^{32}P was made by a conventional manner. The radioactivity per gram fresh weight of sample was determined by a GM counter. On the other hand, an aliquot of sample was macerated with equal volume of chloroform and 10 per cent trichloroacetic acid aqueous solution (including moisture of sample) in a waring blender, and centrifuged. The radioactivities of chloroform and aqueous phases were determined per unit volume, and the amounts of radioactive materials which were partitioned in chloroform and aqueous phases, were calculated.

Separation of chloroform extractable metabolites: The chloroform extract after the partition between chloroform and trichloroacetic acid aqueous solution was concentrated in vacuo after drying over anhydrous sodium sulfate, and the metabolites in the concentrate were separated by the paper chromatography with the following solvent systems.

a) Stationary phase, filter paper impregnated with 50 per cent propylene glycol in ethanol; mobile phase, toluene 7 parts and hexane 3 parts by volume (BENJAMINI *et al.*, 1959).

b) Stationary phase, untreated filter paper; mobile phase, isopropanol 75 parts and concentrated ammonium hydroxide solution 25 parts by volume (PLAPP and CASIDA, 1958).

c) Stationary phase, filter paper impregnated with 5 per cent Silicone 550 in hexane; mobile phase, the upper phase from a mixture of ethanol 10 parts, chloroform 10 parts, and water 6 parts by volume (METCALF and MARCH, 1953).

System a) was mainly used, because of the better separation of Baycid metabolites on chromatograms. Two chromatograms were made from each sample, and one of them was used for making radioautogram of paper chromatogram, and the other was cut in each 1 cm width from origin to solvent front, and the radioactivity of each section was determined by a GM counter. From the both results

of radioautogram and radioassay, the percentage of radiotactivity corresponding to each metabolite was calculated.

Separation of water extractable metabolites: After 14 days from the application, the aqueous phase of the rice grains after the partition between chloroform and trichloroacetic acid aqueous solution was subjected to ion exchange chromatography using Dowex 1-X8. The size of chromatography column was reduced to one-fifth of the original one described by PLAPP and CASIDA (1958). The pH of the aqueous phase was adjusted to about 3.5 by 20 per cent KOH. The volume which corresponded to 5 ml of the aqueous phase was placed onto the column of the chromatography, and after washing the sample into the resin with 5 ml of distilled water, the chromatography developed. The column was placed on a fraction collector, and 5 ml of eluate per fraction was collected. One ml aliquot of each fraction was transferred to a planchet for radioassay, and after adding 1 ml of 10 per cent alcoholic kali solution to the planchet, the content in the planchet was dried up under mild heating with an infrared lamp. Radioactivity of ^{32}P was assayed on a gas flow counter because of its low radioactivity. Identification of eluted metabolites was based on comparison with the result of ion exchange chromatography which was carried out with known compounds. The major part of the compounds which were presumed to be the Baycid metabolites, were prepared according to the description of KOSOLAPOFF (1950). In order to assist the identification of the water extractable metabolites, the paper chromatography which was mentioned above, was also applied.

RESULTS AND DISCUSSION

Change of radioactive materials on and in rice plant: The partition ratio of radioactive materials, $(c/c+w) \times 100$, in which c shows radioactivity in chloroform phase and w in aqueous phase, was an useful indication for showing the extent of the degradation of radioactive insecticide on and in plant. The partition ratio of the radioactive materials in rice plant between chloroform and aqueous

Fig. 1. Change of radioactive materials after the application of ^{32}P -labeled Baycid on rice plants.

phases is shown in Fig. 1. The results were expressed as percentage of radioactivity per gram fresh weight of leaf blade or leaf sheath to initial radioactivity after the application. The data obtained from the experiment with Gaisenmochi at its heading stage were omitted, because of the tendency similar to the results shown in Fig. 1. ^{32}P -labeled Baycid used in the experiments was partitioned 99.8 per cent in chloroform phase when partitioned between chloroform and trichloroacetic acid aqueous solution.

The deposit just after the application is shown in Table 1, and it was heavier deposit than in usual spray program for the control of rice plant insects. Chloroform extractable metabolites decreased rapidly within 6 hours after the application. This decrease might be due mainly to the evaporation of Baycid from the surface of rice plant before penetration into plant tissues, judging from the vapor

pressure of this compound and the degree of appearance of hydrolysis products. In addition to this presumption, the decrease might be affected by a shower of 11.2 mm which happened 3 hours after the application. The feature of the decrease was scarcely affected by the difference in the growth stage of the rice plants, and it was observed on all of the rice plants that the radioactive metabolites partitioned in aqueous phase were found in leaf sheath more than in leaf blade. This fact would show either that chloroform extractable metabolites

disappeared more rapidly in leaf sheath than in leaf blade, or that these were more mobile from leaf sheath to leaf blade than water extractable metabolites. It could not be, however, concluded only from the result of the present experiment, because the deposit of Baycid on leaf blade was five to eight times of that on leaf sheath just after the application. In any case, the metabolic rate of Baycid in rice plant seemed to be rather low as compared with that of parathion and malathion (TOMIZAWA and SATO, 1962).

Separation of chloroform extractable metabolites in rice plant: Chloroform extractable metabolites of each sample were separated by the paper chromatography. The R_f values of Baycid (PS-sulfide) and its oxidation products in the paper chromatography with the solvent system a) were shown in Table 2. The chromatographic result of ^{32}P -labeled Baycid used in the present experiment, was also

Table 1. Deposit of Baycid on rice plants just after the application of Baycid.

Portion	Growth stage of rice plant		
	3 weeks after transplantation	5 weeks after transplantation	Few days before heading
Leaf blade	121.0*	152.7	109.8
Leaf sheath	27.6	18.5	—

* Expressed as p. p. m. of Baycid.

Table 2. Rf values of Baycid and its oxidation products on propylene glycol impregnated paper with the solvent system, toluene and hexane (7 : 3).

Rf value	Compound	Constitution of ^{32}P -labeled Baycid (%)
0.00	derivatives of phosphate ester (PO-form)	2.0
0.46	S-methyl isomer?	3.6
0.64	PS-sulfoxide	0.0
0.76	PS-sulfone	0.0
0.86	PS-sulfide	94.4

shown in Table 2. The oxidation products of PO-form and all of the hydrolysis products were located at the origin of paper chromatogram, and the product which was located on the Rf value of 0.46, was suggested to be S-methyl isomer of Baycid by a comparison with the heat decomposition products of Baycid. The Rf values shown in Table 2 were obtained from the result of paper chromatography with pure compounds. However, when the pure compounds were chromatographed with plant extract, their Rf values on the chromatograms were generally lowered by about 0.1 in comparison with those of the pure compounds and the chromatograms sometimes showed long tailing spots. The percentage of radioactivity of each metabolites to total radioactivity in chloroform extract

was calculated on basis of the results of paper chromatogram and its radioautogram (Fig. 2). Baycid (PS-sulfide) disappeared rapidly after the application, and only about 10 per cent of PS-sulfide remained after 6 hours. The major part of the metabolites was constituted by PS-sulfoxide and PS-sulfone, and the percentage of radioactivity in the spot which was located at the origin of paper chromatogram, and the spot which was regarded as S-methyl isomer was comparatively low. The over-all metabolism of Baycid in plants was rather similar to that of Bayer 25141 or systox (BENJAMINI *et al.*, 1959 b; METCALF *et al.*, 1955), but quite different from that of malathion or methyl parathion (TOMIZAWA *et al.*, 1960) in the fact that Baycid contains the conversion of sulfide to sulfoxide and sulfone in mercapto-methyl group. It was probable that the oxidation ratio of thionophosphate ester is relatively low in rice plant. As shown in Fig. 2, PS-sulfide in leaf blade or leaf sheath persisted for longer period in younger plant than older one. It seemed that PS-sulfide remained in leaf sheath more than in leaf blade at every stage of rice plants.

The female larvae of the rice stem borer (*Chilo suppressalis* WALKER) reared on artificial medium for about 25 days were topically applied with Baycid or its oxidation

products, and LD₅₀ based on mortality after 48 hours, was determined. As shown in Table 3, LD₅₀ of PS-sulfoxide and PS-sulfone was about two-third of PS-sulfide.

Accumulation of Baycid metabolites in ears and grains:

Since it has been found that considerable amounts of malathion or methyl parathion metabolites were accumulated in the grains of the rice plants which were sprayed with the insecticides before heading stage (TOMIZAWA *et al.*, 1960), the accumulation of Baycid metabolites to ears

Fig. 2. Metabolism of ^{32}P -labeled Baycid on and in rice plants.

Table 3. Toxicity of Baycid and its oxidation products against the larvae of the rice stem borer.*

Compound	LD 50 ($\mu\text{g/g}$)
PS-sulfide	1.26
PS-sulfoxide	0.77
PS-sulfone	0.81
PO-sulfide	0.47
PO-sulfoxide	0.76
PO-sulfone	0.47
Methyl parathion	0.74

* Average body weight, 59.6 mg. Temperature, 25°C.

Fig. 3. Accumulation of Baycid metabolites in ears of rice plants.

and grains was examined on Gaisenmochi variety. As shown in Fig. 3, the content of the radioactive metabolites in the ears 29 days after the application (dough stage) did not increase as compared with that after 12 days (milk ripe stage). It was presumed, however, that the absolute amount of the radioactive metabolites considerably increased, because the ears grow progressively toward the maturing stage of the rice plant. On the other hand, the partition ratio, $(c/c+w) \times 100$, in which c shows radioactivity in chloroform phase and w in aqueous phase, decreased gradually after the application. The chloroform extractable

Table 4. Paper chromatographic separation of chloroform extractable metabolites in the ears of the rice plant 12 days after the application.

Probable metabolites	Per cent ^{32}P
PS-sulfide	0.0
PS-sulfoxide	45.1
PS-sulfone	18.3
S-methyl isomer ?	19.5
Rf 0	17.1

metabolites in the ears after 12 days from the application were examined by the paper chromatography, and the results were shown in Table 4. PS-sulfide was not detected, but about half of the metabolites presented PS-sulfoxide like the case in leaf tissues. The percentage of PS-sulfone was not so high as in leaf tissues, and the percentage of the radioactivities in the spot which was located at Rf value of 0, and in another spot which was regarded as S-methyl isomer was comparatively high. After 29 days from the application, the rice grains were divided into three portions of husk, bran and polished rice, and the partition ratio of the metabolites in each portion between chloroform and trichloroacetic acid aqueous solution was examined in the same manner with that of whole grains. The result was shown in Table 5. It was interesting from the standpoint of plant physiology that the radioactive metabolites were concentrated in bran but not in polished rice and husk. The distribution percentage of the absolute amount of the radioactive materials which were accumulated in three portions of the rice grains was as follows; husk, 6.5%, bran, 48.8%, and polished rice, 14.7%.

The sum of the radioactive metabolites which were contained in chloroform and aqueous

Table 5. Distribution of Baycid metabolites in the rice grains 29 days after the application.

Portion of rice grains	Dry weight	Metabolites expressed as p.p.m. of Baycid			
		Total	Chloroform extractable (c)	Water extractable (w)	c/w
Husk	1.7	2.42	0	0.78	0
Bran	0.5	60.04	0.88	30.38	0.029
Polished rice	5.0	5.71	0.09	2.04	0.044

Fig. 4. Ion exchange separation on Dowex 1-X8 of water extractable metabolites of Baycid in rice grains.

- I. Elution gradient, HCl pH 2 to HCl pH 1; 200 ml.
- II. Elution gradient, HCl pH 1 + methanol (1+3) to HCl 1 N + methanol (1+3); 100 ml.
- III. Elution gradient, HCl 1 N + methanol (1+3) to HCl conc. + H₂O + methanol (1+1+6); 100 ml.

phases was always about half or less of the total metabolites. This fact would show that some portion of the metabolites existed as chloroform and water unextractable form. The existence of the unextractable form was possibly due to the incorporation of ³²P into cellular structure of the rice grains.

The water extractable metabolites in the rice grains after 14 days from the application were separated by ion exchange chromatography. As shown in Fig. 4, five peaks of the metabolites were found, and four of them coincided with phosphoric acid and thiophosphoric acid (peak A), *O,O*-dimethyl phosphoric acid (peak B), *O,O*-dimethyl thiophosphoric acid (peak C), and *O*-methyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphoric acid (peak E), respectively. Peak A probably contained the both of phosphoric acid and thiophosphoric acid, but they would be inseparable due to the insufficient length of the chromatography column (TOMIZAWA and SATO, 1962). When the both of phosphoric acid and thiophosphoric acid were subjected to ion exchange chromatography, the former compound eluted ahead of the latter, but some part of the eluted fractions of the two compounds was overlapped. The compound which corresponded

to peak D was not identified, and it might be the hydrolysis products of phosphate derivative of Baycid.

When the metabolites of ³²P-labeled Baycid were compared with those of ³⁵S-labeled methyl parathion, it was remarkable that the proportion of monodemethylated compound is higher in Baycid than in methyl parathion (TOMIZAWA and SATO, 1962). Like the cases of malathion and methyl parathion (TOMIZAWA *et al.*, 1960), the conversion to inorganic phosphoric acid of organophosphorus ester was confirmed. Although the extent of the conversion was different among the three organophosphorus esters, it was possible that the conversion progresses further toward the maturing stage of the rice grains.

SUMMARY

³²P-labeled Baycid was sprayed on the three kinds of rice plant which were different in growth stage, and the behavior of the insecticide on and in rice plant was examined.

1. The hydrolysis rate of Baycid in rice plant seemed to be slower than that of methyl parathion or malathion. Baycid disappeared rapidly after the application in the three kinds of rice plant, and only about 10 per cent of chloroform extractable metabolites remained as Baycid 6 hours after the application.

2. Chloroform extractable metabolites of Baycid in rice plant which were detected throughout the experimental period, consisted mainly of PS-sulfoxide and PS-sulfone, and the appearance of the oxidation products as PO-form seemed to be a small even if existed.

3. When Baycid was sprayed on the rice plant at a few days before heading stage, the Baycid metabolites tended to be accumulated to ears and grains. The content of the metabolites detected in the rice grains 29 days after the application was about 7 p.p.m., as expressed as Baycid. The metabolites in the rice grains were concentrated in bran but not in polished rice and husk.

4. The water extractable metabolites in the rice grains 14 days after the application were separated by ion exchange chromatography, and the existence of phosphoric acid, thiophos-

phoric acid, *O,O*-dimethyl phosphoric acid, *O,O*-dimethyl thiophosphoric acid, *O*-methyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphoric acid and one unknown metabolite were found. It was remarkable that the proportion of monodemethylated compound of Baycid is relatively high.

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REFERENCES

- BENJAMINI, E., R. L. METCALF, and T. R. FUKUTO (1959a) J. Econ. Ent. **52**: 94~98.
 BENJAMINI, E., R. L. METCALF, and T. R. FUKUTO (1959b) J. Econ. Ent. **52**: 99~102.
 KOSOLAPOFF, G.M. (1950) Organophosphorus Compounds, Wiley, New York.
 METCALF, R. L. and R. B. MARCH (1952) Science **117**: 527~528.
 METCALF, R. L., R. B. MARCH, T. R. FUKUTO, and M. G. MAXON (1955) J. Econ. Ent. **48**: 364~369.
 PLAPP, F. W. and J. E. CASIDA. (1958) Analyt. Chem. **30**: 1622~1623.
 SCHRADER, G. (1960) Höfchen-Briefe, Bayer **13**: 1~13.
 TOMIZAWA, C., T. SATO, H. YAMASHINA, and H. KUBO (1960) Botyu-Kagaku **25**: 99~105.
 TOMIZAWA, C. and T. SATO. (1962) Japanese Jour. Appl. Ent. Zool. **6**: 70~75.

摘 要

水稻に散布した *O,O*-dimethyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphate の消長

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生育程度を変えて栽培した3種類の水稲に ^{32}P -Baycid乳剤を散布し、その変化を追跡した。稲体においてBaycidが加水分解される速度はmethyl parathionなどに比較してかなり遅いと考えられる結果を得たが、Baycidの形(PS-sulfide)は散布後急速に消失した。Baycidの酸化物としてはPS-sulfoxide PS-sulfoneが大部分を占め、thiophosphate(PS-型)のphosphate(PO-型)への酸化はきわめて少ないと考えられる。出穂数日前に散布した場合、Baycidの代謝物質は出穂後の穂に

移行し、主として“ぬか”に集積する傾向が認められた。乳熟期の“もみ”における水溶性代謝物質をイオン交換樹脂により分離した結果、Phosphoric acid, thiophosphoric acid, *O,O*-dimethyl phosphoric acid, *O,O*-dimethyl thiophosphoric acid, *O*-methyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphoric acid および一個の未同定物質を検出したが、特にBaycidの脱メチル化合物の割合が高かった。